Theor Appl Genet (1992) 84:97–105

Effect of gene *Lr34* in the enhancement of resistance to leaf rust of wheat*

S. E. German[1,***] **and J. A. Kolmer****

[1] Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2
[2] Agriculture Canada Research Station, Winnipeg, Manitoba, Canada R3T 2M9

Received June 24, 1991; Accepted October 17, 1991
Communicated by A. R. Hallauer

Summary. Leaf rust resistance gene *Lr34* is present in many wheat cultivars throughout the world that have shown durable resistance to leaf rust. Fourteen pair-wise combinations of *Lr34* and seedling leaf rust resistance genes were developed by intercrossing near isogenic 'Thatcher' lines. In both seedling and adult plant tests homozygous paired combinations of specific resistance genes with *Lr34* had enhanced resistance relative to either parent to different numbers of isolates that were avirulent to the additional resistance genes. The Tc*Lr34,18* line also expressed enhanced resistance to specific isolates virulent to *Lr18* in seedling and adult plant stages. In rust nursery tests, homozygous lines were more resistant than either parent, if the additional leaf rust gene conditioned an effective of resistance when present singly. The ability of *Lr34* to interact with other genes conditioning effective resistance may contribute to the durability of leaf rust resistance in cultivars with *Lr34*.

Key words: *Triticum* – *Puccinia recondita* – Resistance gene combinations

Introduction

Rust resistance in wheat (*Triticum aestivum* L.) has traditionally been based on the use of specific resistance genes. Resistance to leaf rust (causal organism *Puccinia recondita* Rob ex Desm. f. sp. *tritici*) in common wheat has been particularly short lived. One possible reason for the rapid adaptation of leaf rust populations is that resistance to leaf rust has often been based on the use of single resistance genes (Samborski 1985). Durable resistance to wheat leaf rust has rarely been found, and the basis for the most durable resistance to wheat leaf rust has been combinations of resistance genes *Lr13* + *Lr34*, and probably *Lr12* + *Lr34* (Roelfs 1988b). Dyck et al. (1966) determined the presence of *Lr12* in 'Exchange' and *Lr13* in 'Frontana', cultivars which have been used extensively in breeding programs because of their durable resistance. Both genes required the presence of modifiers to produce the resistance of the original cultivars. One of the modifiers of *Lr13* is probably *Lr34*, which is in 'Frontana' (Dyck and Samborski 1982).

The cvs 'Chris' (*Lr13* + *Lr34*) (Roelfs 1988b) and 'Era' (*Lr13* + *Lr34* + *Lr10*) (Ezzahiri and Roelfs 1989) have been resistant to leaf rust since being released in 1966 and 1971, respectively. The Canadian cv 'Columbus', with *Lr13* and *Lr16*, has had a high level of resistance to leaf rust since being released in 1980 (Kolmer et al. 1991; Samborski and Dyck 1982).

In wheat cultivars that have combinations of rust resistance genes, the genes usually act independently, exhibiting the infection type of the gene that conditions the lower infection type when present singly (Dyck and Kerber 1985; Roelfs 1988a). Interactions between leaf rust resistance genes have been reported. Schafer et al. (1963), Dyck (1977), and Samborski and Dyck (1982) generically defined gene interaction as the combination of two or more genes resulting in higher resistance than that conferred by the individual genes. Dyck and Kerber (1985) considered the possibility that this effect may be additive. Interactions between seedling and adult plant wheat leaf rust resistance genes have been previously noted (Samborski and Dyck 1982).

Resistance gene *Lr34* is involved in gene combinations conferring durable resistance to leaf rust and ex-

* Contribution *1453* Agriculture Canada
** Correspondence to: J. A. Kolmer
*** Present address: Estacion Experimental La Estanzuela Colonia, Uruguay CP 70.000

presses enhanced resistance in combinations with other resistance genes. *Lr34* was initially found in PI58548, where it interacted with *Lr33* to condition enhanced resistance (Dyck 1977), and was located to chromosome 7D (Dyck 1987). It is present in many cultivars and lines of very diverse origins (Dyck and Samborski 1982; Shang et al. 1986). *Lr34* is the only effective resistance gene in the Canadian cv 'Glenlea' (Dyck et al. 1985), which has been resistant to leaf rust since release in 1972, and in the American hard red winter wheat 'Sturdy' (Dyck 1991), which has also shown durable resistance to leaf rust. In the adult plant stage, the 'Chinese Spring' substitution lines with the single genes *Lr10* and *Lr23* were more resistant than the near isogenic 'Thatcher' lines with *Lr10* and *Lr23*, respectively (Sawhney et al. 1989). This enhanced resistance was postulated to be due to the combination of the 'Chinese Spring' adult plant resistance of *Lr34* and *Lr12* (Dyck 1991) and the additional resistance genes. 'Chinese Spring' also has *Lr31*, which interacts with *Lr27*, but it is not known whether this gene interacts with other genes for enhanced resistance.

In the field *Lr34* expresses variable pustule size (M reaction) and low percentage of infection (Dyck 1987). Perez and Roelfs (1987) compared the development of leaf rust epidemics on Tc*Lr34* and 'Thatcher'. Tc*Lr34* exhibited "slow rusting" resistance and had a terminal severity 50% lower than 'Thatcher'. *Lr34* can be detected in the seedling stage under certain temperature and light conditions as a 2 or 2+ infection type (Dyck and Samborski 1982); however it is best expressed in adult plants. The objective of this work was to examine paired combinations of leaf rust resistance genes with *Lr34* in a common 'Thatcher' background for enhanced resistance.

Materials and methods

Plant material

'Thatcher' near isogenic line *Lr34* (RL 6058) was used as the maternal parent and crossed with 'Thatcher' near isogenic lines carrying the seedling resistance genes *Lr1* (RL 6003), *Lr2a* (RL 6016), *Lr3* (RL 6002), *Lr3ka* (RL 6007), *Lr11* (RL 6053), *Lr16* (RL 6005), *Lr17* (RL 6008), *Lr18* (RL 6009), *Lr21* (RL 6043), *Lr26* (RL 6078), and *LrB* (RL 6047) and with the 'Thatcher' isoline carrying the adult plant resistance gene *Lr13* (RL 4031) (Long and Kolmer 1989, McIntosh 1988). Tc*Lr34* was also crossed with the cv 'Columbus', which has *Lr16* and *Lr13* (Samborski and Dyck 1982). Seedlings were grown in 24 × 30 × 7 cm flats, and adult plants in 12- and 15-cm pots, both containing a mixture of 40% soil, 30% peat, and 30% sand and fertilized weekly with a solution of a 20:20:20 NPK formulation.

Leaf rust isolates and inoculation

The isolates used in this study were tested for infection type with 'Thatcher' near isogenic lines in the seedling stage (Table 1). None of the isolates were virulent on adult plants of Tc*Lr34*, although variation in avirulent infection types between isolates was observed.

Seedlings were inoculated with a mixture of urediniospores and talc and placed in a dew chamber overnight (16 h minimum) at approximately 18 °C. Seedling flats were then placed on a

Table 1. Virulence formulas of selected *Puccinia recondita* isolates

Isolate	Virulence formula
Race 1	–
Race 15	3
35	1,3,24
44	1,3
63	1,3,24,26,B
103	1,2c,B,18
107	3,3ka,30,B
118	2c,30,B,18
119	1,2c,3,17
122	1,2c,B,18
125	1,2c,3,17
127	1,2c,3,17
133	1,2a,2c,3
167	1,2a,2c,3,17,18
215	1,2a,2c,3,11,17,18
269	1,3,11
270	2a,2c,3,26
313	1,2a,2c,3,11,18
315	2a,2c,3,11
323	1,2a,2c,3,11
358	1,2c,3,3ka,17
363	1,2c,3,3ka,17,30,B
364	1,2c,3,3ka,11,30,B,18
366	1,2c,3,9,3ka,B,18
369	2c,3,3ka,B,18
378	1,2c,3,3ka,B,18
384	1,2c,11,30,B,18
394	1,2c,3,3ka,11,B,18
Ae16-1	1,2c,3,24,3ka,30,18
Ae41-3	1,2c,3,24,3ka,B,18
U2-1	1,26,11,18

Table 2. Percentage of isolates from Manitoba and Saskatchewan virulent to 12 leaf rust differential lines in 1988 and 1989

Resistance gene	Percent isolates virulent	
	1988[a]	1989[b]
Lr1	94.4	88.5
Lr2a	49.0	63.9
Lr3	100.0	100.0
Lr16	1.2	0.0
Lr26	23.5	25.8
Lr3ka	0.0	0.4
Lr11	11.6	44.3
Lr17	1.6	0.0
Lr30	0.0	0.4
LrB	0.8	0.0
Lr18	2.4	0.4
Lr21	0.0	0.0

[a] Kolmer (1989)
[b] Kolmer (1990)

greenhouse bench at 18°–25°C, with 8–12 h of supplemental fluorescent light.

Seedlings of F_2 families were inoculated with race 1 when the primary leaves were completely expanded (7–8 days old). Adult plants were inoculated at anthesis with a mixture of urediniospores and talc or oil, and placed in a dew chamber overnight (minimum 16 h). After incubation, plants were placed on a greenhouse bench or in a growth cabinet at 20°–25°C/15°–20°C with a 16 h/8 h light/dark cycle. Adult plants of F_3 families from the cross Tc*Lr34* × Tc*Lr13* were inoculated in a growth cabinet.

A mixture of leaf rust isolates from the 1988 and 1989 virulence surveys of *P. recondita* in the prairie region of Canada (Table 2) (Kolmer 1989, 1990) was used as inoculum for rust nursery evaluations of homozygous lines in 1989 and 1990, respectively. A mixture of susceptible cultivars arranged in rows at right angles with respect to the single row plots was inoculated with a mixture of urediniospores and talc.

F_2 families

From each cross, approximately 200–300 seedlings of two or more F_2 families derived from different crossed heads were inoculated on the first leaf with race 1, which was avirulent to all the seedling genes used in this study (Table 1). Infection types were assessed 10–12 days after inoculation according to the scale used by Stakman et al. (1962). Ten to 16 F_2 plants from each family with the lowest infection type to race 1 were selected (total of 30–32 from each cross) and grown to maturity.

F_3 and F_4 lines

F_3 lines derived from selected single F_2 plants from each cross were tested in the rust nursery as adults to determine which were segregating for *Lr34*. Seventy to 80 seeds from each line were planted in 2-m rows. Rust severity of the F_3 and F_4 lines was evaluated using the modified Cobb scale (Peterson et al. 1948). Response was evaluated according to Stakman et al. (1962). Readings were taken when severity and response on susceptible control 'Thatcher' was 80% susceptible (80S) or higher. The five or six most resistant F_3 lines homozygous for *Lr34* from each cross were harvested and progeny tested in the greenhouse as seedlings (15 plants/F_3 line) to determine which lines were homozygous for the seedling gene. In this manner, F_4 lines homozygous for both resistance genes were obtained. Homozygous F_4 lines were evaluated in four replicates in the 1990 rust nursery.

F_4 seedling tests

To further test resistance of the gene pair combinations, two homozygous F_4 lines per cross, the parental 'Thatcher' lines, and 'Thatcher' were tested as seedlings with the individual leaf rust isolates in Table 1. Six to ten seeds per genotype were planted in clumps in flats. The lines *Lr34,2a*, *Lr34,3ka*, *Lr34,11*, *Lr34,16*, *Lr34,17*, *Lr34,18*, *Lr34,21*, *Lr34,B*, and *Lr34* × 'Columbus' were tested with 26 isolates in four separate tests. Results from the two most consistent tests were presented. F_4 lines of *Lr34,1*, *Lr34,3*, *Lr34,13*, and *Lr34,26* were tested with 10 isolates. Infection types were assessed as decribed for F_2 seedlings.

F_4 greenhouse adult plant test

Homozygous F_4 lines *Lr34,2a*, *Lr34,3ka*, *Lr34,11*, *Lr34,16*, *Lr34,17*, *Lr34,18*, *Lr34,21*, and *Lr34,B* were tested at anthesis with specific isolates of leaf rust. Three to four plants of one F_4 line from each cross, Tc*Lr34*, the seedling resistance gene parents, and 'Thatcher' were grown in 15-cm pots and tested with 4 individual isolates of leaf rust at anthesis, in three different tests. Infection types were assessed as described for seedlings.

TcLr34 × TcLr13

Approximately 150 F_2 plants from Tc*Lr34* × Tc*Lr13* were evaluated for resistance in the 1989 rust nursery. Sixteen plants with lower rust reactions than either Tc*Lr34* or Tc*Lr13* were selected, and progeny tested as adults (12 plants per F_3 line) in a growth cabinet. Thirty-eight F_3 plants were selected, and progeny tested in the field in 1990. In all tests, a higher level of resistance in the paired gene combinations than in either 'Thatcher' parental line was considered to be indication of the expression of interaction between resistance genes.

Results

F_4 seedlings tests

In general, isolates that were avirulent to the seedling genes had lower infection types on the homozygous F_4 lines than on either parent. Infection types of 18 of the 26 tested isolates are presented in Table 3. Isolates avirulent to *Lr1*, *Lr2a*, *Lr3*, *Lr21*, and *Lr26* had lower infection types on the F_4 lines than on the single gene lines (Table 3 and 4). The majority of the isolates avirulent to *Lr3ka*, *Lr11*, and *Lr18* had lower infection types on the F_4 lines than on either parental lines. The F_4 line *Lr34* × 'Columbus' expressed enhanced resistant to selected isolates, and Tc*L34,17* and Tc*Lr34,18* expressed enhanced resistance to few isolates (Table 3). Tc*Lr34,B* and *TcLr34,13* did not express enhanced resistance to any isolate (Tables 3 and 4).

As an example of enhanced resistance conferred by *Lr34*, the 13 *Lr2a* avirulent isolates had lower infection types on the Tc*Lr34,2a* F_4 line (0 to 0;1) than on Tc*Lr2a* (0; to 2) (Table 3). Five *Lr16* avirulent isolates had lower infection types on Tc*Lr34,16* (0;1− to 1−) than on Tc*Lr16* (1; to 1) (Table 3).

Reduced chlorosis, associated with the resistant infection type in the F_4 lines when compared to the seedling gene parent, was observed to many isolates in the Tc*Lr34,1* Tc*Lr34,3*, Tc*Lr34,16*, Tc*Lr34,17*, Tc*Lr34,26*, and Tc*Lr34* × 'Columbus' lines and to a few isolates in the Tc*Lr34,11*, Tc*Lr34,18*, and Tc*Lr34,B* lines.

Reduced infection type or chlorosis in the F_4 lines when compared to the single gene lines was not detected with isolates virulent to the seedling genes, with the exception of *Lr34,18*. Isolates 378, 394, 364, 366, and 369 had virulent infection types on Tc*Lr18* and Tc*Lr34*, and had avirulent infection types on Tc*Lr34,18* in the first test. The same isolates produced virulent infection types on Tc*Lr34,18* and on both parents in the second test when higher temperatures occurred in the greenhouse. The F_4 line-isolate combinations of Tc*Lr34,17*-35, 44; Tc*Lr34,B*-167, and Tc*Lr34* × 'Columbus'-race 15, 103, 119, 323 had higher infection types than the seedling resistant single gene lines (Table 3).

Table 3. Seedling infection type[a] of homozygous 'Thatcher' F_4 lines and isogenic 'Thatcher' wheat lines to 18 leaf rust isolates

Tc Line	Test	Leaf rust isolate																	
		Race 1	Race 15	63	103	107	122	127	133	167	215	269	313	323	358	363	364	366	384
Tc	1	3	3	4	4	4	4	4	4	4	3	4	33+	4	4	4	4	4	3+
Lr34[b]	1	3	3	3	4	3+	3	3	3	4	3+	4	33+	4	3+	4	4	4	3+
Tc	2	4	33+	3+	4	34	34	34	3+4	4	4	3+4	3+	4	4	3+4	4	34	4
Lr34	2	3	3	33+	3	3	33+	3	33+	33+	33+	33+	33+	33+	3	3+	33+	33+	3+
Lr2a	1	0;	0;	;	1	;1	;1	;1−	4	3+	3+	0;	3+	3	;1+	2	2	2	;1
F_4[c]	1	0	0	0	0;1=	0	0;1=	0;	3	4	3	0	3+	4	0;1=	0;	0;1	0;1	0;
Lr3ka	2	;1−	1−;	1−;	1−;	33+	1−;	2−	1−	1−;	1;	1;	1−	1;	4	34	34	4	1;
F_4	2	0;1=	0;1−	;1−	0;1−	3	0;1=	0;1−	1−	0;1−	0;1−	0;1−	0;1−	0;1−	3+	33+	3+4	33+	0;1
Lr11	2	2	2	2	2+3+	1−;	23	1	2	2+3	4	3+	34	4	2	2+	3+	23	2+
F_4	2	;11+	0;1−	12	13−;	0;1=	23	0;1−	;1−[d]	;23	34	33+	34	4	0;1	;12	3+	;2	0;2−
Lr16	2	1	2+3+	1;	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1+
F_4	2	0;1[d]	2+3+	;1[d]	1	1	1	1[d]	1[d]	1[d]	1	1−[d]	1[d]	1[d]	1−;	1−;[d]	1[d]	1[d]	1+[d]
Lr17	2	;1−	1−;	1;	2	;1−	1	34	1	33+	4	;1−	1	1;	4	33+	;1−	1−;	1+
F_4	2	0;1−[d]	0;1−[d]	;1[d]	2[d]	;1−[d]	1−[d]	33+	1[d]	33+	34	;1−[d]	1−;[d]	1;[d]	33+	33+	;1−[d]	1−;	1
Lr18	1	1	;1	;1−	4	;1=	4	;1−	;1−	4	3	1−;	2	2	;1−	;1=	3	33+	33+
F_4	1	0;	0;	0;	3+	0;	4	0;1=	0;	3+	3	0;1−	;1	;1−	;1=[d]	0;	23	22+	33+
Lr21	1	;1	;2	;1+	2	;1	;1	2	;1	2	;2	22+	;1	;1	2	;2	;12	;2	2
F_4	1	0;	0;	0;	0;	0;	0;1−	0;1=	0;	;1=	0;	;1−	0;	0;	0;	0;	0;	0;1−	0;1
LrB	2	2	2	2+	4	33+	3+	2	2	22+	33+	2	2	2	2+	2+3+	4	34	4
F_4	2	2[d]	2	2+	33+	2+3+	34	2	2[d]	2+3+	33+[d]	2[d]	2	2	2+	2+3+	34	33+	4
Columbus	1	;1	1	;1−	;1	;1−	1	1;	;1	1−	;	;1	1−	;	;	;	;1	1	1+
F_4	1	;1=[d]	12[d]	0;	1[d]	;[d]	1[d]	1;[d]	1;[d]	;1−[d]	;1−[d]	0;1=	;1−[d]	;1−[d]	0;	0;	;1[d]	;1[d]	1+[d]

[a] Stakman et al. (1962)
[b] Tc*Lr* line
[c] F_4 line from the cross Tc*Lr34* and the preceding seedling gene 'Thatcher' line
[d] Reduced chlorosis or necrosis compared with the seedling gene parental line

Table 4. Seedling infection type[a] of homozygous 'Thatcher' F_4 lines and isogenic 'Thatcher' wheat lines to 10 isolates of leaf rust

Tc Line	Leaf rust isolate									
	Race 1	35	63	103	107	118	270	Ae16-1	Ae41-3	U2-1
Tc	3+4	3+4	33+	4	3+4	4	3+4	3+4	3+4	3+4
Lr34[b]	23+	24	13	23+	;22+	23	23+	13	23+	2+3+
Lr13	3+4	3+4	33+	4	3+	4	3+4	34	3+4	3+4
F_4[c]	23	23+	23+	23+	2	23	23+	13	23+	2+3+
Lr1	;	3+4	33+	4	;	;	;	34	3+4	3+4
F_4	0;[d]	24	23+	23+	0;[d]	0;[d]	0;[d]	13	23+	2+3+
Lr3	;1−	3+4	33+	;1−	3+4	;1	3+4	3+	3+4	;1−
F_4	0;[d]	24	13	0;1−[d]	;2	0;1−[d]	23+	13	23	0;1−[d]
Lr26	;1−	;	2+3+	;1−	;	;	2	;1−	;1−	3+4
F_4	0;[d]	0;[d]	;13	0;1=[d]	0;[d]	0;[d]	0;1[d]	0;[d]	0;1−[d]	2+3+

[a] Stakman et al. (1962)
[b] Tc*Lr* line
[c] F_4 line from the cross Tc*Lr34* and the preceding seedling gene 'Thatcher' line
[d] Reduced chlorosis or necrosis compared with the seedling gene parental lines

Table 5. Adult plant infection types[a] of near isogenic 'Thatcher' wheat lines and lines with combinations of two leaf rust resistance genes

Tc Line	Leaf rust isolate									
	Test	Race1	Race15	44	125	215	323	363	364	366
Tc	1	3+4	3+4	3+4	3+4					3+4
Lr34[b]	1	0;12−	0;12−	0;1	0;12−	[e]			0;12	
Lr16	1	2c	2+	2c	2+					
F_4[c]	1	0;[d]	0;12−[d]	0;1[d]	0;[d]					
Lr21	1	2	2	2						22+
F_4	1	0;[d]	0;[d]	0;[d]						0;1−
Tc	2	4				4		4	4	4
Lr34	2	0;23				0;12		0;12+	0;23	0;12
Lr3ka	2	0;1						4	4	4
F_4	2	0;						0;12	0;23	0;12+
Lr18	2	2c				4			4	3+
F_4	2	0;1[d]				0;1			0;1	0;1
Tc	3	4			4	4	4	4	4	4
Lr34	3	−			0;13	0;13	0;12	0;1	0;13	;13
Lr2a	3	0;				4	3+	0;1		
F_4	3	0;[d]				0;13	0;12+	0;[d]		
Lr11	3	2c				4	4		4	
F_4	3	0;[d]				0;12	0;1		0;13	
Lr17	3	;1−n			12c	4		;1n		
F_4	3	0;[d]			0;13[d]	;23+		0;12[d]		
LrB	3	2+						4	4	4
F_4	3	0;1[d]						0;12	0;13	;13

[a] Stakman et al. (1962)
[b] Tc*Lr* line
[c] F_4 line homozygous for Tc*Lr34* and the preceding seedling gene
[d] Reduced necrosis or chlorosis compared with the seedling resistance gene parental line
[e] Blanks indicate isolate – line combination was not tested

Adult plant test

Adult plants of Tc*Lr34* expressed variable infection types and percentage of infection between and within tests with different isolates of leaf rust. Tc*Lr34* had a few moderate size pustules at the base of the flag leaf and very few smaller pustules towards the tip of the leaf. Chlorosis was not associated with the pustules. Very faint hypersensitive flecks were observed throughout the leaves. All of the isolates tested were avirulent on adults of Tc*Lr34* (Table 5). Infection type and severity of infection were always higher on the 'Thatcher' susceptible control than on Tc*Lr34*.

Table 6. Leaf rust severity[a] and response[b] of adult 'Thatcher' isogenic wheat lines and homozygous lines with paired combinations of *Lr34* and an additional leaf rust resistance gene in the 1989 and 1990 rust nurseries

Gene	Field severity and response			
	1989		1990	
	Tc line	Homozygous line	Tc line	Homozygous line
Thatcher	80S[c]		90S	
Lr34	2-20M		T-20M	
Lr2a	80S	T-10M	90S	T-20M
Lr3ka	50MR	5VR	50MR	5VR
Lr11	70S	T-5M	90S	T-20M
Lr16	50R	5VR	70MR	5VR
Lr17	50MR	5VR	60MR	5VR
Lr18	5M	5VR	TR	5VR
Lr21	5M	2VR	5R	5VR
LrB	70MS	T-10M	80MS	T-5M
Columbus	5R	5VR	5R	5VR
Lr13	50MR	5VR	60MR	5VR
Lr1			90S	T-30M
Lr3			90S	T-5M
Lr26			70MS	T-5M

[a] Modified Cobb scale (Peterson et al. 1948)
[b] Stakman et al. (1982)
[c] Response: VR, very resistant; hypersensitive flecks with no sporulation; R, resistant; hypersensitive flecks and small uredia with necrosis; MR, moderately resistant; moderate size uredia with necrosis; MS, moderately susceptible; moderate size uredia with chlorosis; S, susceptible; large uredia without necrosis or chlorosis; M, mixture of variable size uredia and hypersensitive flecks; T, trace level of infection

The F_4 line-isolate combinations that expressed lower infection types than either parental line in the seedling stage also expressed lower infection types in the adult tests. The F_4 line-isolate combination Tc*Lr34,16*-125, Tc*Lr34,3ka*-363, Tc*Lr34,18*-215, Tc*Lr34,11*-215, and Tc*Lr34,11*-323 expressed lower infection types than Tc*Lr34* in the adult plant stage, but not in the seedling stage. Reduced chlorosis was also observed in the adult plant test.

As adult plants the F_4 line-isolate combinations of Tc*Lr34,3ka*-366, Tc*Lr34,2a*-323, Tc*Lr34,17*-215, Tc*Lr34,17*-363, and Tc*Lr34,B*-363 had higher infection types than Tc*Lr34*. Isolates 125 and 363 had low infection types to *Lr17* in the adult plant test, and high infection types to *Lr17* in the seedling tests.

Rust nursery tests

Tc*Lr34* had leaf rust ratings of 2-20 M and T-20 M in 1989 and 1990, respectively (Table 6). 'Thatcher' lines with *Lr1*, *Lr2a*, *Lr3*, and *Lr11* were either almost as susceptible or as susceptible as 'Thatcher'. Tc*Lr26* had a high percentage of infection and a moderately susceptible response. The 'Thatcher' lines with genes *Lr3ka*, *Lr13*, *Lr16*, *Lr17*, and *LrB* had varying degrees of moderate resistance in both years. The 'Thatcher' lines with *Lr18*, *Lr21*, and 'Columbus' had a high level of resistance.

Tc*Lr34,3ka*, Tc*Lr34,16*, Tc*Lr34,17*, Tc*Lr34,18*, Tc*Lr34,21*, and Tc*Lr34* × 'Columbus' were more resistant than both parental lines in 1989 and 1990. Tc*Lr34,1*, Tc*Lr34,2a*, Tc*Lr34,3*, Tc*Lr34,11*, Tc*Lr34,26*, and Tc*Lr34,B* had the same level of resistance as Tc*Lr34*.

Discussion

In all tests, *Lr34* interacted for enhanced resistance the most effectively with other genes that conditioned resistance when present singly. In the seedling stage, *Lr34* interacted with seedling genes mostly to isolates that were avirulent to the seedling genes. *Lr1*, *Lr2a*, *Lr3*, *Lr3ka*, *Lr11*, *Lr18*, *Lr21*, and *Lr26* interacted with *Lr34* to more isolates than other genes. In the adult plant stage, isolates avirulent to the seedling genes usually had lower infection types than expected, while isolates virulent to the seedling genes usually had similar infection types on *Lr34* and the homozygous F_4 lines.

Certain F_4 lines displayed enhanced resistance to specific isolates as adults that was not observed in the seedling tests. However, in most cases of an isolate virulent to the seedling gene, the difference in infection type between F_4 lines and Tc*Lr34* was very small and may have been caused by nonuniformity in density and amount of inoculum applied or the variability of infection types observed in genotypes with *Lr34*. The difference in infection types of Tc*Lr34* and the homozygous F_4 lines in the adult stage was small and difficult to evaluate to many of the isolates. Pustule size and percentage of infection were variable between and within tests with different isolates. The expression of resistance of *Lr34* appears to involve quantitative aspects such as receptivity and spore production (Drijepondt and Pretorius 1989). A quantitative comparison of the components of resistance conditioned by *Lr34* singly and combinations with other resistance genes in the adult plant stage would allow a more precise characterization of the interactions between resistance genes to specific isolates.

In the rust nursery, the F_4 lines that expressed enhanced resistance compared to the parental 'Thatcher' lines were the lines whose seedling resistance gene expressed some degree of effective resistance when present singly. Virulence to these genes was at a very low level or not present at all in the rust nursery inoculum (Table 1). F_4 lines that expressed enhanced resistance in the field did not necessarily express enhanced resistance to most of the avirulent isolates in the seedling tests. An example of this was the Tc*Lr34,16* line, which had enhanced resistance

relative to either Tc*Lr34* or Tc*Lr16* in the rust nursery, but expressed enhanced resistance to only 5 of 26 *Lr16* avirulent isolates in the seedling test. It is possible that interactions for enhanced resistance are seen more easily in field conditions than in the seedling stage since *Lr34* is most effective in the adult plant stage. Knott and Weller (1988) detected more interactions between stem rust resistance genes in field tests than in seedling tests. F_4 lines that did not express enhanced resistance in the field were those whose seedling genes did not condition effective resistance when present singly. The F_4 line Tc*Lr34,B* did not express enhanced resistance to isolates avirulent to *LrB* in seedling tests, and it was not more resistant than Tc*Lr34* in the rust nursery even though virulence to *LrB* was at a very low level in the rust population.

Lr34 enhanced the resistance of effective resistance genes. Other studies examining resistance gene combinations also showed that enhanced resistance is mostly expressed to isolates avirulent to at least one of the genes. Samborski and Dyck (1982) determined that in general, isolates avirulent to both of the combined genes had lower infection types than expected, and several combinations of genes also expressed enhanced resistance to isolates avirulent to one of the genes and virulent to the other. Knott and Weller (1988) detected enhanced resistance in seedling tests only to isolates avirulent to the stem rust resistance genes.

However, one exception to this general conclusion was found: the combination *Lr34,18* had enhanced resistance to certain *Lr18* virulent isolates in seedling and adult tests. In the seedling tests, enhanced resistance in Tc*Lr34,18* to certain *Lr18* virulent isolates was found in two of the four tests. In a third test warmer temperatures may have caused the higher infection type observed on Tc*Lr18* (Dyck and Johnson 1983). Four isolates that were avirulent to *Lr18*, and expressed enhanced resistance to Tc*Lr34,18* in cooler conditions, were virulent to *Lr18* and Tc*Lr34,18* at warmer temperatures. Apparently the expression of enhanced resistance in the Tc*Lr34,18* F_4 lines can be affected by temperature. In adult plant tests *Lr34* interacted markedly with *Lr18* for enhanced resistance to isolates virulent to *Lr18*.

Another gene with some degree of temperature sensitivity was *Lr17*, which in the greenhouse adult plant test expressed a low infection type to certain isolates that had been classified as virulent to this gene in the seedling tests. Since the adult plant test was in July, the higher temperatures could have changed the infection type, as *Lr17* has been reported to be more resistant at higher temperatures than at lower temperatures (Dyck and Johnson 1983).

In the adult plant test, some F_4 line-isolate combinations of virulent and avirulent isolates had higher infection types on the homozygous F_4 lines than on Tc*Lr34*, although the differences were small, except in the Tc*Lr34,17*-215 combination (0;13 and ;23+ on Tc*Lr34* and Tc*Lr34,17*, respectively). Apparently certain combinations of genes express less resistance to specific leaf rust isolates than the most resistant single gene. This effect was also reported by Knott and Weller (1988). In field tests the ineffective stem rust resistance gene *Sr11* increased susceptibility in combinations with other resistance genes to stem rust race 15B-1. *Sr7a* appeared to increase susceptibility to race 56 in the combination *Sr7a,8a,9b*.

Reduced chlorosis associated with the low infection types in the F_4 lines when compared with the seedling gene was variably expressed among resistance gene combinations. This effect has not been reported previously in leaf rust resistance gene combinations with *Lr34*. The expression of reduced chlorosis in certain F_4 line-isolate combinations may be related to the expression of *Lr34*. The reduced pustule size typical of *Lr34* is accompanied by little or no chlorosis or necrosis in adult plants (Drijepondt and Pretorius 1989) or in seedlings (Dyck 1977).

Tc*Lr34,13* was more resistant than Tc*Lr34* or Tc*Lr13* for both years in the rust nursery. The combination *Lr34,13* has been identified as a durable source of leaf rust resistance (Roelfs 1988 b). The percentage of isolates virulent to the adult plant gene *Lr13* is not regularly determined, but some tests have indicated that isolates virulent to *Lr13* are present in the North American wheat leaf rust population (Kolmer unpublished data). However, virulence to *Lr13* must be at low or moderate level, because this gene conditions an effective level of resistance in the rust nursery and when present singly in commercial cultivars (Kolmer et al. 1991)

The *Lr34,13* combination continues to provide more resistance than either *Lr34* or *Lr13* singly. Schafer et al (1963) obtained lines with enhanced seedling resistance by intercrossing the cvs 'Aniversario', 'La Prevision 25', 'Frontana', and 'Exchange'. As found in later studies, 'Frontana' has *Lr34,13,T3* (Dyck and Samborski 1982; Dyck et al. 1966), and 'Exchange' has *Lr10,Lr16* (Anderson 1961), *Lr12* (Dyck et al. 1966), and probably *Lr34* (Samborski and Dyck 1982). 'Aniversario' (*Lr3ka*) and 'La Prevision 25', two Argentinean cultivars, are derivatives of 'Americano 44d' (Perez and Roelfs 1989), which probably has *Lr34,13* or *Lr34,12* (Roelfs 1988 b). The higher level of resistance in the lines obtained by Schafer et al. (1963) was most likely due to *Lr34* and/or *Lr13* interacting with other resistance genes since Kolmer (unpublished data) demonstrated that *Lr13* also interacts for superior resistance with seedling genes that condition effective resistance.

In F_2 families of Tc*Lr34* × 'Columbus' three resistance genes were segregating as 'Columbus' has *L13* and *Lr16*. *Lr34* must be present in the F_4 lines because they were more resistant than 'Columbus' in the rust nursery. It was not possible to directly determined the presence of

Lr13, since the field responses of the Tc*Lr34* × 'Columbus' and Tc*Lr34,16* F_4 lines were very similar. The seedling tests detected the presence of *Lr16*; the F_4 lines were resistant to all *Lr16* avirulent isolates. Infection types from the F_4 seedling tests indicated that *Lr13* was probably present in the Tc*Lr34* × 'Columbus' F_4 lines; these lines had lower infection types than Tc*Lr34,16* to most isolates. The isolates that were virulent to Tc*Lr16* and Tc*Lr34,16* F_4 lines were avirulent to 'Columbus' and Tc*Lr34* × 'Columbus' F_4 lines. If 'Columbus' does not have another resistance gene of modifier, the only possible resistance gene combination that confers seedling resistance to *Lr16* virulent isolates is *Lr13,16*, since *Lr13* and *Lr16* interact for resistance in seedling and adult plants to isolates virulent to *Lr16* (Samborski and Dyck 1982). Therefore it appears that the F_4 lines are homozygous for all three genes. Since the genetic background can modify the expression of resistance genes, the presence of *Lr13* in the F_4 lines from Tc*Lr34* × 'Columbus' should be confirmed through genetic analysis.

Lr34 appears to express resistance in a manner similar to the effect of other resistance genes that confer slow rusting or partial resistance in several cereal rust diseases (Wilcoxon 1981). The phenotype of reduced pustule size expressed by *Lr34* may be one of the factors considered to be a slow rusting characteristic (Dyck 1977; Broers 1989). Drijepondt and Pretorius (1989) found that *Lr34* affected infection frequency, latent period, and pustule size. Another similarity between partial resistance and the effect of *Lr34* is that both are best expressed in the adult plant stage (Dyck and Samborski 1982; Jacobs and Buurlage, 1990). The enhanced resistance *Lr34* expressed in combinations of resistance genes may be regarded as additive (Dyck and Keber 1985), which is similar to the action of partial resistance genes (Jacobs and Broers, 1989).

It is notable that *Lr34* and *Lr13* have provided long-lasting resistance in combination with each other and other resistance genes. Interaction between genes for enhanced resistance may be involved in durable resistance. However not all interacting pairs of genes confer durable resistance. Resistance in the Australian wheat cv 'Gatcher', which has the complementary genes *Lr27* and *Lr31* (Singh and McIntosh 1984), did not prove to be durable. The identity and effectiveness of the resistance genes involved in interacting combinations is most likely important in determining the durability of the resistance. In addition to enhanced resistance in certain combinations and durability of leaf rust resistance, *Lr34* can contribute to improved resistance to stem rust, since Tc*Lr34* has considerably more stem rust resistance than 'Thatcher' (Dyck 1987).

Acknowledgements. We thank the Canadian International Development Agency for financial support of S.E.G., and P.L. Dyck for useful advice and discussion.

References

Anderson RG (1961) The inheritance of leaf rust resistance in seven varieties of common wheat. Can J Plant Sci 41:342–359

Broers LHM (1989) Influence of development stage and host genotype on three components of partial resistance to leaf rust in spring wheat. Euphytica 44:187–195

Drijepondt SC, Pretorius ZA (1989) Greenhouse evaluation of adult plant resistance conferred by the gene *Lr34* to leaf rust of wheat. Plant Dis 73:669–671

Dyck PL (1977) Genetics of leaf rust reaction in three introductions of common wheat. Can J Genet Cytol 19:711–716

Dyck PL (1987) The association of a gene for leaf rust resistance with chromosome 7D suppressor of stem rust resistance in common wheat. Genome 29:467–469

Dyck PL (1991) Genetics of adult-plant resistance in 'Chinese Spring' and 'Sturdy' cultivars. Crop Sci 31:309–311

Dyck PL, Johnson R (1983) Temperature sensitivity of genes for resistance in wheat to *Puccinia recondita*. Can J Plant Pathol 5:229–234

Dyck PL, Kerber ER (1985) Resistance of the race-specific type. In: Roelfs AP, Bushnell WR (eds) The cereal rusts, vol II. Diseases, distribution, epidemiology and control. Academic Press, Orlando, pp 469–500

Dyck, PL, Samborski DJ (1982) The inheritance of resistance to *Puccinia recondita* in a group of common wheat cultivars. Can J Genet Cytol 24:273–283

Dyck PL, Samborski DJ, Anderson RG (1966) Inheritance of adult-plant leaf rust resistance derived from the common wheat varieties 'Exchange' and 'Frontana'. Can J Genet Cytol 8:665–671

Dyck PL, Samborski DJ, Martens JW (1985) Inheritance to leaf rust and stem rust in the wheat cultivar 'Glenlea'. Can J Plant Pathol 7:351–354

Ezzahiri B, Roelfs AP (1989) Inheritance and expression of adult plant resistance to leaf rust in 'Era' wheat. Plant Dis 7:549–551

Jacobs T, Broers LHM (1989) The inheritance of host plant effect on latency period of wheat leaf rust in spring wheat. II: Estimation of gene action and number of effective factors in F_1, F_2 and backcross generations. Euphytica 44:197–206

Jacobs T, Buurlage MB (1990) Growth of wheat leaf rust colonies in susceptible and partially resistant spring wheats. Euphytica 45:71–80

Knott DR, Weller J (1988) Genetic interactions among four genes for resistance to stem rust in bread wheat. Genome 30:182–185

Kolmer JA (1989) Physiologic specialization of *Puccinia recondita* f. sp. *tritici* in Canada in 1988. Can J Plant Pathol 11:431–434

Kolmer JA (1990) Physiologic specialization of *Puccinia recondita* f. sp. *tritici* in Canada in 1989. Can J Plant Pathol. 12:428–430

Kolmer JA, Dyck PL, Roelfs AP (1991) An appraisal of stem and leaf rust resistance in North American hard red spring wheats and the probability of multiple mutations to virulence in populations of cereal rust fungi. Phytopathology 81:237–239

Long DL, Kolmer JA (1989) A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopathology 79:525–529

McIntosh RA (1988) A catalogue of gene symbols for wheat. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genetics Symp. UK Agric and Food Res Council, Institute of Plant Science Research, Cambridge, UK, pp 1225–1323

Perez BA, Roelfs AP (1987) Differences in host response of near isogenic wheat lines to leaf rust. Phytopathology 77:1763

Perez BA, Roelfs AP (1989) Resistance to wheat leaf rust of land cultivars and their derivatives. Phytopathology 79:1183

Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can J Res 26 [sec C]:496–500

Roelfs AP (1988 a) Genetic control of phenotypes in wheat stem rust. Annu Rev Phytopathol 26:351–367

Roelfs AP (1988 b) Resistance to leaf and stem rust in wheat. In: Simmonds NW, Rajaram S (eds) Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico DF, pp 10–22

Samborski DJ (1985) Wheat leaf rust. In: Roelfs AP, Bushnell WR (eds) The cereal rusts, vol. II. Diseases, distribution epidemiology, and control. Academic Press, Orlando, pp 39–59

Samborski DJ, Dyck PL (1982) Enhancement of resistance to *Puccinia recondita* by interactions of resistance genes in wheat. Can J Plant Pathol 4:152–156

Sawhney RN, Nayar SK, Sharma JB, Bedi R (1989) Mechanism of durable resistance: a new approach. Theor Appl Genet 78:229–232

Schafer JF, Caldwell RM, Patterson FL, Compton LE (1963) Wheat leaf rust resistance combinations. Phytopathology 53:569–573

Shang HS, Dyck PL, Samborski DJ (1986) Inheritance of resistance to *Puccinia recondita* in a group of resistant accessions of wheat. Can J Plant Pathol 8:123–131

Singh RP, McIntosh RA (1984) Complementary genes for reaction to *Puccinia recondita tritici* in *Triticum aestivum*. I. Genetic and linkage studies. Can J Genet Cytol 26:723–735

Stakman EC, Stewart DM, Loegering WQ (1962) Identification of physiologic races of *Puccinia graminis* var. *tritici*. US Dept Agric ARS-E 6/7

Wilcoxon RD (1981) Genetics of slow rusting in cereals. Phytopathology 71:989–993