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Genetics of stem rust resistance in the spring wheat cultivar Thatcher and the enhancement of stem rust resistance by *Lr34*

Stem rust resistance in TcLr34

Carmen D. Gavin Vanegas · David F. Garvin · James A. Kolmer

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Abstract Three recombinant inbred line populations from the crosses RL6071/Thatcher, RL6071/RL6058 (Thatcher Lr34), and Thatcher/RL6058, were used to study the genetics of stem rust resistance in Thatcher and TcLr34. Segregation of stem rust response in each population was used to determine the number of genes conferring resistance, as well as the effect of the leaf rust resistance gene *Lr34* on stem rust resistance. The relationship between resistance in seedling and adult plants was also examined, and an attempt was made to identify microsatellite markers linked to genes that were effective in adult plants. In field plot tests at least three additive resistance genes segregated in the RL6071/RL6058 population, whereas two resistance genes segregated in the RL6071/Thatcher population. The presence of the gene *Lr34* permitted the expression of additional stem rust resistance in Thatcher-derived lines both at the seedling and adult plant stages. Seedling resistance to races TPMK and RKQQ was significantly associated with resistance in adult plants, whereas seedling resistance to races QCCD and QCCB may have made a minor contribution. The seedling resistance genes *Sr16* and *Sr12* may have contributed to resistance in adult plants. A molecular marker linked to resistance in adult plants was identified on chromosome 2BL.

Keywords Durable resistance · *Puccinia graminis* f. sp. *tritici* · Specific resistance

Abbreviation
IT Infection type

Introduction

Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, has historically caused devastating yield losses on spring wheat in the United States (Kolmer 2001). Epidemics from 1910–1954 led wheat breeders to incorporate more stem rust resistance into North American cultivars. One important source of stem rust resistance has been the cultivar Thatcher (Kolmer et al. 1991).

Thatcher wheat (Marquis/Iumillo durum//Marquis/Kanred) was one of the first wheat cultivars specifically bred for stem rust resistance (Hayes et al. 1936). Thatcher derived stem rust resistance from both the tetraploid (AABB) Iumillo durum parent and Kanred winter wheat (AABBDD). Thatcher was not immune to stem rust infection; however, it was much more

C. D. G. Vanegas
Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA

D. F. Garvin
USDA-ARS Plant Science Research Unit, University of Minnesota, St. Paul, MN 55108, USA

J. A. Kolmer (✉)
USDA-ARS Cereal Disease Laboratory, University of Minnesota, St. Paul, MN 55108, USA
e-mail: jkolmer@umn.edu

resistant than the susceptible cultivars Reward and Marquis that preceded it. As a result, and combined with its hard red spring wheat quality, Thatcher was widely used as a resistant parent in subsequent breeding programs in the spring wheat regions of the US and Canada (Kolmer et al. 1991).

Thatcher has four race-specific seedling stem rust resistance genes that have been mapped to chromosome arms: *Sr5* (chromosome arm 6DS), *Sr9g* (2BL), *Sr12* (3BS), and *Sr16* (2BL). An additional seedling resistance gene temporarily designated *SrTc* has not been mapped, and other seedling resistances have not been fully described (Knott 2001; Nazareno and Roelfs 1981). The *Sr* genes confer resistance to some races of stem rust, but generally do not confer effective resistance to the stem rust races currently found in the US (Kolmer et al. 1991; Nazareno and Roelfs 1981).

Inheritance of seedling and adult plant stem rust resistance in Thatcher has been shown to be complex (Knott 1999, 2001). In addition to the seedling stem rust resistance genes, Thatcher also possesses two or more adult plant stem rust resistance genes (Nazareno and Roelfs 1981). These adult resistance genes confer a generalized resistance that has proven durable since the cultivar was released (Kolmer et al. 1991; Nazareno and Roelfs 1981). There is some evidence that chromosomes 6A and 2B are associated with adult plant resistance (Brennan 1975). Tetraploid Iumillo durum is the source of the adult plant stem rust resistance in Thatcher (Hayes et al. 1936). Therefore, the adult plant resistance genes must be located on the A or B genomes.

Leaf rust resistance gene *Lr34* has conditioned nonspecific, durable resistance to leaf rust for over 40 years in wheats developed in the US and other countries (Kolmer 1996). This gene is also associated with stripe rust resistance, barley yellow dwarf resistance, and powdery mildew resistance (Spielmeyer et al. 2005). Dyck (1987) mapped *Lr34* to chromosome 7D and found that Thatcher backcross lines with *Lr34* had superior stem rust resistance compared to the recurrent parent Thatcher. Gene *Lr34* may counter the effect of a stem rust resistance suppressor gene that is present on chromosome 7D in many common hexaploid wheat cultivars (Kerber and Aung 1999).

The objective of the present study was to further characterize the seedling and adult plant stem rust resistances in Thatcher by examining the segregation of response in seedling and adult plants in three recombinant inbred line populations derived from Thatcher. The effects of *Lr34* on stem rust responses in seedling and adult plants, as well as the association between resistance in seedling and adult plants, were examined. We hypothesized that segregants with *Lr34* among recombinant inbred lines with the genetic background of Thatcher would show enhanced stem rust resistance, making it easier to detect and map individual resistance genes. Microsatellite (SSR) markers were employed to search for chromosomal regions associated with adult plant resistance in one of the populations.

Materials and methods

Parental lines and recombinant inbred line populations

Three parents were used for the development of three recombinant inbred populations. The stem rust resistant line RL6058 (Tc*6/PI58548) (Dyck 1987) is a backcross-derived near-isogenic line of cultivar Thatcher with resistance gene *Lr34*. RL6058 was crossed with the stem rust and leaf rust susceptible line RL6071 (Prelude/8*Marquis). Line RL6058 was also crossed with Thatcher, and Thatcher was crossed with RL6071. Progeny from the three crosses were advanced through single seed descent to produce approximately 100 $F_{5:6}$ recombinant inbred lines per cross. The $F_{5:6}$ RL6071/RL6058 lines were used for DNA extractions, field testing and seedling tests. In 2005, seed from field plots of the RL6071/RL6058 population was harvested ($F_{5:7}$ seed) and also used for seedling tests. Since results of the seedling tests with the $F_{5:6}$ lines and $F_{5:7}$ lines were consistent, data from only the $F_{5:7}$ lines are presented. The original $F_{5:6}$ lines from the Thatcher/RL6058 and RL6071/Thatcher populations were used for seedling and field tests.

Additional wheat genotypes were evaluated in seedling tests with isolates of stem rust races TPMK and RKQQ (Roelfs and Martens 1988). Lines tested included the parents of Thatcher: Iumillo durum, Kanred, and Marquis; a series of Thatcher backcross lines with *Lr34*: RL6050 (Tc*6/Terenzio), RL6070 (Tc*5/PI321999), RL6077 (Tc*6/PI250413), and RL6059 (Tc*6/PI58548); and two *Lr34* donors, namely Terenzio and Chinese Spring.

For seedling tests, a differential set of wheat lines that included both the 16 wheat genotypes used for *Pgt* race nomenclature in North America (Roelfs and Martens 1988) and single gene wheat lines with the stem rust resistance genes present in Thatcher was inoculated at the same time as each recombinant line set. The lines with the seedling *Sr* genes in Thatcher included: ISr16-Ra (CI 14173) with *Sr16* in Chinese Spring background; *Sr9g* in a Chinese Spring background; BtSr12Tc with *Sr12* in a background derived from Baart and Thatcher, and *Sr12* in a W2691 background. The lines RL6058, Thatcher, RL6071were also tested with the same stem rust isolates.

Field tests

Stem rust and leaf rust response data for the three $F_{5:6}$ recombinant inbred populations and parental lines were collected in 1999 from plots at North Dakota State University, Fargo, North Dakota. Planting density was 50 seeds per 2 m row. Spreader rows of the stem rust susceptible wheats Baart and Little Club and Wolfe barley were planted among the entries. The three parental lines (RL6058, Thatcher, and RL6071) were also planted twice among entries of each population. Planting took place in late April. The spreader rows were needle-inoculated with a mixture of isolates of stem rust races (TMLK, TPMK, RTQQ, QFCQ, and QTHJ) in mid-June. Inoculations were not necessary for leaf rust (*Puccinia triticina*), because sufficient inoculum blew into the plots from surrounding areas. The predominant leaf rust races common in the Upper Midwest in 1999 were THBJ, MBDS, MGDS, MDRJ, and MCDS (Long et al. 2002). The three recombinant inbred populations were rated for percentage stem rust and leaf rust severities at the end of July, using the modified Cobb scale (Peterson et al. 1948). Each recombinant inbred line was also rated as resistant, moderately resistant, moderately susceptible, or susceptible, based on the amount of chlorosis and necrosis associated with the pustules. Resistant lines had small uredinia with necrosis, moderately resistant lines had a mixture of small and large uredinia with necrosis, moderately susceptible lines had moderate to large uredinia with chlorosis, and completely susceptible lines had large uredinia without necrosis or chlorosis (Liu and Kolmer 1998).

Seedling tests

The three recombinant inbred populations were tested for segregation of seedling stem rust response. Four to six seeds of each line were planted in the four corners of 3.5 cm pots containing vermiculite. Trays contained six pots each, with 24 entries per tray. Seedlings were fertilized upon emergence with 20:20:20 NPK fertilizer. Seedling inoculations were carried out in the greenhouse approximately 7–9 days after planting (when most plants had reached the two leaf stage). Isolates of five races of stem rust were used for seedling tests: isolate 2–84 of race TPMK, isolate 74 of race BCCB, isolate 351–89 of race RKQQ, isolate 75-32-1644-A of race QCCD, and isolate 70-13-458 A of race QCCB. These isolates were chosen based on low IT to line RL6058 and to the individual stem rust resistance genes *Sr5, Sr12*, and *Sr16* present in Thatcher.

Inoculum of individual stem rust races, increased previously on seedlings of the susceptible cultivar McNair 701, dried for 2–3 days in a desiccator at 4°C, and stored at −80°C, was subjected to a heat shock (7 min at 40 C in a heating block) prior to inoculation to increase spore germination rates. Approximately 4 μg of spores were placed in size 00 gelatin capsules in approximately 330 μl Soltrol 170 oil. Each tray was inoculated with one capsule of inoculum. After inoculation, the seedlings were placed in a dew chamber at approximately 20°C for 16 h including four hours of light at the end of incubation. The seedlings were then removed from the dew chamber, fertilized with approximately one-half teaspoon of standard 20:20:20 NPK fertilizer per tray, and placed on a bench in the greenhouse. Greenhouse temperatures ranged between 18 and 25°C, and natural light was supplemented by metal halide lighting. Seedlings were fertilized once again seven days after inoculation with the same quantity of fertilizer. The seedling infections were allowed to develop over 12–14 days.

Seedlings were scored for IT at the two-leaf stage using the scale developed by Stakman and Levine (1922). Plants with no visible signs of infection were rated as zero; plants with hypersensitive flecks were indicated with a semicolon (;); plants with small uredinia surrounded by necrotic rings were rated as 1; plants with small to medium size uredinia surrounded by chlorotic circles were rated as 2; plants with medium size uredinia with no surrounding necrosis or

chlorosis were rated as 3; plants with large diamond-shaped uredinia that coalesced and lacked chlorosis or necrosis were rated as 4. Pluses (+) and minuses (−) were used to indicate larger or smaller uredinia for each IT.

Data analysis

Seedling IT data were sorted to determine both the minimum and maximum possible number of segregating resistance genes in each population. To determine the minimum number of segregating genes, ITs to each race were divided into two groups: a resistant group with distinct low ITs and a susceptible group with higher ITs. The threshold between the high and low groups was based on the IT of the single gene lines with *Sr5, Sr12*, and *Sr16*, as well as the range and relative prevalence of ITs segregating in the population. The maximum number of segregating genes was determined by raising the threshold for the resistant group to just below either the IT of the susceptible parent or to the highest IT in the progeny set.

The association between the segregation of seedling stem rust response and field response was tested using both Student's *t*-test and χ^2 contingency table tests (Samuels 1999). The *t*-test is commonly used to check for associations between a binary data set and a metric data set, such as a set of marker scores and a set of field disease severity scores (Kearsey 1998). The *t*-tests were conducted only for seedling segregations of progenies that fit a 1:1 resistant: susceptible ratio. A data set consisting of binary seedling response data and corresponding metric field data was sorted into resistant and susceptible classes, and then *t*-tests were undertaken on field stem rust data to determine whether there was a significant difference in response in adult plants between classes. When a significant association was found, the proportion of resistance in adult plants explained by the seedling resistance genes was determined by simple linear regression (Samuels 1999).

Contingency table tests were carried out using field data in three classes for the RL6071/RL6058 population (resistant = 0–5MSS; intermediate resistant = 10MRMS−40MSS; and susceptible = 50–60MSS), two classes for the RL6071/Thatcher population (intermediate resistant = 10MRMS–50 MSS; susceptible = 60–70 MSS), and two classes for the Thatcher/RL6058 population (resistant = 0–5MSS; intermediate resistant = 10MRMS–30MSS). Seedling data were organized into the same resistant and susceptible classes used for the *t*-tests. However, since resistance to TPMK and RKQQ did not segregate 1:1 in the RL6071/Thatcher population, that population could not be divided into single resistant and susceptible classes of approximately the same size. In this cross there were three categories: low IT (0; to ;2), intermediate IT (22+ to 2+3), and high IT (33− to 4).

Marker testing

DNA was extracted from each of the 107 $F_{5:6}$ RL6071/RL6058 recombinant inbred lines and parental lines using a scaled-down version of the protocol described in Riede and Anderson (1996). DNA was stored at −20°C.

Marker analysis of $F_{5:6}$ progeny from the RL6071/RL6058 population and parents was carried out using microsatellite primers from the *Xbarc* series (Song et al. 2005). PCR amplification of the markers was carried out in a total volume of 10 µl with 0.25 units of Taq polymerase, 1× PCR buffer, 5 µl (15–30 ng) of DNA, 0.2 mM each dNTPs, and 1 µl of each 0.33 µM primer (0.0328 µM per primer final concentration). PCR cycling conditions were: 3 min. at 94°C (one cycle), 1 min. at 94°C, 1 min. at 50, 55, 60, or 65°C (depending on primer), 2 min. at 72°C (repeated for 35 total cycles), and one final cycle of 72°C for 10 min.

Screening for microsatellite polymorphism among the progeny lines was carried out on 6.5% denaturing polyacrylamide gels with silver staining using methods as indicated by Litt et al. (1993) and Bassam et al. (1991). A total of 450 *Xbarc* microsatellite markers (Song et al. 2005) were screened to identify polymorphisms between RL6071 and RL6058. A selective genotyping strategy was employed to search for markers associated with stem rust resistance. About 12 progeny from the group with highest field disease severity (stem rust rating 50–60 MS-S) and 12 from the group with lowest disease severity (stem rust rating between 0 and 10 R-MR) were used to screen for microsatellites potentially linked to field resistance, based on linkage disequilibrium in the two groups. Data for promising markers were then obtained for all

of the recombinant inbred lines. The program QTX (Meer, Manly, and Cudmore, Roswell Park Cancer Institute, Buffalo, New York, 2002) was used to produce a linkage map from the marker data.

Results

Seedling response

In seedling response tests, RL6071 was susceptible to all races tested, with ITs from 33^+ to 3^+ 4 (Table 1). Thatcher had low IT 0; to races BCCB and QCCD, IT 2 to race QCCB, IT 2^+ to race TPMK, and IT that varied from ;23 to 33^- to race RKQQ. RL6058 had low IT, ranging from 0; to $;2^-$ to all races tested. The differential line with *Sr5* had a low IT of 0; to race BCCB and intermediate to high IT from 2^+ 4 to 3^+ 4 to all other races. The differential line with *Sr9g* had ITs of 33^- to 4 to all races. The differential line with *Sr16* had a low ITs that ranged from 2 to 2^+ 3 to races QCCD, BCCB, QCCB, and TPMK, and an IT of 33^- to race RKQQ. The two differential lines with *Sr12*, had a low IT of 0; to race QCCD, ITs of 22^+ to 2^+ 3 to races QCCB and BCCB, and high IT of from 33^- to 3^+ 4 to races RKQQ and TPMK.

When tested with race QCCB the RL6071/RL6058 population segregated in a 3:1 ratio, indicating the segregation of two resistance genes, when the resistant class included ITs of 0; to 2^+(Table 2). When the resistant IT class was redefined from 0; to 3 the segregation data fit a three gene ratio. One of the genes conditioning resistance may be *Sr16*, which had a 2^+ IT to race QCCB, or *Sr12*, which had a 22^+ IT. To race QCCD, the population segregated for a single gene that conditioned ITs 0; to ;1. When the resistant class included ITs from 0; to 2^+, the population segregated for two resistance genes to QCCD (Table 2). The first gene that conditioned resistance was likely *Sr12*, which had a 0; IT to QCCD. The second gene may have been *Sr16*, which had an IT 2. To race BCCB, the population segregated for a single resistance gene that conditioned IT 0 to ;1. When the resistant class included ITs from 0; to 2^+, the segregation fit a three gene ratio (Table 2). The first resistance gene was likely *Sr5*, which had a IT 0; to race BCCB. The other resistance genes might have been *Sr12*, which had a IT 2^+ 3 to BCCB, and *Sr16*, which had a IT 23 to race BCCB. To race RKQQ, approximately one-half of the $F_{5:7}$ lines of RL6071/RL6058 had low ITs of 0; to 2^+ (Table 2). The gene(s) that conditioned resistance to RKQQ could not be any of the previously identified seedling stem rust resistance genes in Thatcher (*Sr5, Sr9g, Sr12*, and *Sr16)*, since RKQQ had high ITs to all of those genes. Approximately one-half of the RL6071/RL6058 population had low to intermediate ITs from 0; to 2^+ to race TPMK. The segregating resistance may have involved *Sr16*, which had a IT 2^+ 3 to race TPMK However, some of the recombinant inbreds had a lower IT of 0; to TPMK than could have been due to *Sr16* alone.

Table 1 Seedling infection types[a] of RL6071, RL6058, Thatcher, and differential wheat lines with single stem rust resistance genes

Wheat line	Stem rust race					Field response
	BCCB	QCCB	QCCD	RKQQ	TPMK	
RL6071	3^+ 4	3^+ 4	3^+ 4	33^+ 4	33^+ 4	50MSS
RL6058	$;1^-$	0;	0;	$;2^-$	0;	Trace—10MS
Thatcher	0;	2	0	$;23^-$–33^-	2^+	10—20MRMS
*I*Sr5 Ra CI 14159	0;	3^+ 4	3^+ 4	3	3^+ 4	–[b]
Sr9g CNS	3^+ 4	4	4	33^-	3^+	–
*I*Sr16 Ra CI 14173	23	2^+	2	33^-	2^+ 3	–
BtSr12/Tc	2^+ 3	22^+	0;	33^-	33^+	–
*Sr*12-W2691	2^+ 3	2^+	0	3	3^+ 4	–

[a] Stem rust infection types: 0; = faint hypersensitive flecks; 1 = small uredinia surrounded by necrosis; 2 = small uredinia surrounded by chlorosis; 3 = moderate size uredinia without necrosis or chlorosis; 4 = large uredinia without necrosis or chlorosis. "+" and "−" indicate larger and smaller uredinia for that infection type, respectively. A range of infection types is indicated by listing the infection types together

[b] Not tested for field response

Table 2 Segregation of seedling stem rust response in recombinant inbred lines of RL6071/RL6058, RL6071/Thatcher, and Thatcher/RL6058

Cross	Race	*Sr* genes detected	Resistant infection types[a]	Susceptible infection types	Number of resistant lines	Number of susceptible lines	Expected ratio	χ^2	*P*-value
RL6071/RL 6058	RKQQ	+[b]	0;–2^+	3–3^+	51	59	1:1	0.57	0.47
	TPMK	+	0;–2^+	3–4	47	62	1:1	2.06	0.15
	QCCB	+	0;–2^+	3–4	72	32	3:1	1.85	0.17
		+	0;–3	3^+–4	90	14	7:1	1.27	0.26
	QCCD	*Sr12*	0;–;1^+	2–4	45	58	1:1	1.64	0.20
		Sr12 +	0;–2^+	3–4	82	26	3:1	0.01	0.82
	BCCB	*Sr5*	0–;1	;2–3^+ 4	62	49	1:1	1.52	0.22
		Sr5 +	0;–2^+	3–4	98	15	7:1	0.06	0.80
RL6071/Thatcher	RKQQ	+	;2–3	3^+–4	37	89	1:3	1.28	0.26
	TPMK	+	2^+–3	3^+–4	16	108	1:7	0.14	0.71
	QCCB	+	0;–2^+ 3	3^+–4	91	33	3:1	0.17	0.68
	QCCD	*Sr12*	0;–;2	3–4	63	61	1:1	0.03	0.86
	BCCB	*Sr5*	0;–;1	;2^-–3^+ 4	69	56	1:1	1.35	0.25
		Sr5 +	0;–2^+	3–4	94	31	3:1	<0.01	0.96
Thatcher/RL6058	RKQQ	+	0;–;2	2^+–2^+ 3	52	58	1:1	0.33	0.57
	TPMK	+	0;–;23	3–3^+	54	51	1:1	0.09	0.77

[a] See footnote Table 1

[b] Additional resistance gene

The RL6071/Thatcher population segregated for two resistance genes that conditioned ITs of 0; to 2^+ 3 (Table 2) to race QCCB. To race QCCD, the population segregated for a single resistance gene, likely *Sr12*, that conditioned ITs 0; to ;2 (Table 2). To race BCCB, the population segregated for a single resistance gene, likely *Sr5*, that conditioned ITs of 0; to ;1 (Table 2). When the resistant class included ITs of 0; to 2^+ 3, the segregation fit a two gene ratio. To race RKQQ the RL6071/Thatcher population segregated for a minimum of two additive resistance genes that conditioned ITs from ;2 to 3 (Table 2). To race TPMK, the population segregated for three additive resistance genes that conditioned ITs from 2^+ to 3.

To race TPMK, approximately half of the Thatcher/RL6058 population segregated for low ITs 0; to ;23 (Table 2). To race RKQQ, approximately half of the Thatcher/RL6058 population segregated for low ITs 0; to ;2.

Seedlings of five Thatcher/*Lr34* backcross lines, parents of Thatcher, and additional wheat cultivars with *Lr34* were tested for IT with races TPMK and RKQQ (Table 3). Among the Thatcher backcross lines with *Lr34*, there was some variation in ITs. Line RL6058 had IT 0;2 (hypersensitive flecks with a few type 2 uredinia) to race TPMK, whereas Thatcher/*Lr34* backcross lines RL6050 and RL6070 had IT

Table 3 Seedling infection types[a] to stem rust races TPMK and RKQQ for parents of Thatcher, RL6071, and backcross lines of Thatcher with *Lr34*

	Stem rust race	
Wheat line	TPMK	RKQQ
Thatcher	2^+ 3	;23–33^-
Marquis	3^+ 4	33^+
Kanred	3^+ 4	3^+ 4
Iumillo durum	0;	0;
RL6071	4	3^+ 4
Terenzio	3^+ 4	3^+ 4
Chinese Spring	3^+ 4	3^+ 4
RL6050 (Tc*6/Terenzio)	0;22^+	22^+
RL6070 (Tc*6/PI321999)	0;22^+	0;23
RL6077 (Tc*6/PI250413)	0;22^-	0;23
RL6058 (Tc*6/PI58548)	0;2	0;1
RL6059 (Tc*6/PI58548)	0;2	0

[a] See footnote Table 1

0;22+. Line RL6058 had IT 0;1 to race RKQQ, whereas lines RL6070 and RL6077 had IT 0;2+ 3, and line RL6050 had IT 22+. Two parents of Thatcher, Marquis and Kanred, were susceptible to both races, with ITs from 33+ to 3+ 4. Iumillo durum, also a parent of Thatcher, was highly resistant to both races, with an IT 0;. Cultivars Terenzio and Chinese Spring, which have *Lr34*, were susceptible to both races with IT 3+ 4.

Field response

In the field evaluation, the susceptible parent RL6071 had a stem rust rating of 50MSS, the resistant parent RL6058 had a rating of Trace-10MS, and Thatcher had a rating of 10–20MRMS (Table 1). The RL6071/RL6058 population segregated for leaf rust resistance, 58 lines had leaf rust responses and severities of 10–20 MS and 51 lines had responses and severities of 90S (Table 4). This fit a 1:1 ratio for the segregation of *Lr34*. It also permitted the stem rust responses of lines with and without *Lr34* to be analysed separately. For stem rust response, the $F_{5:6}$ progeny lines with *Lr34* segregated in three classes of highly resistant equivalent to RL6058, intermediate resistant equivalent to Thatcher, and susceptible equivalent to RL6071. The observed segregation of these lines did not fit the expected 1:2:1 ratio for segregation of two independent genes, and also did not fit a 1:6:1 ratio expected for the segregation of three genes with two distinct classes of resistance. However the observed segregation did fit the expected 7:1 ratio for three resistance genes when the two resistant classes were pooled together. The $F_{5:6}$ progeny lines without *Lr34* segregated in a 3:1 ratio for two resistance genes separately conditioning disease severities of 10MRMS-40 MSS. None of the lines lacking *Lr34* had a level of resistance as high as RL6058. Two of the genes detected in the two subsets of the population were likely identical. Five $F_{5:6}$ progeny lines with *Lr34* were susceptible, indicating that *Lr34* alone did not condition stem rust resistance. Overall the total number of resistant, intermediate resistance, and susceptible lines in the RL6071/RL6058 population fit a 1:6:1 ratio, which also indicated that three additive genes from RL6058 conditioned resistance in adult plants (Table 4).

In the RL6071/Thatcher population segregation of intermediate resistant and susceptible individuals fit a 3:1 ratio, indicating the presence of two segregating resistance genes. In the Thatcher/RL6058 population, approximately one-half of the lines were very resistant to stem rust, with resistance equal to RL6058, and the other half of the lines had resistance equal to Thatcher. The F_6 lines in this population also segregated in a single gene ratio for leaf rust resistance, due to the segregation of *Lr34*. There was a complete correlation between high levels of stem rust resistance and the presence of *Lr34* as determined by leaf rust severity.

Table 4 Segregation of adult plant field stem rust response in recombinant inbred lines from the RL6071/RL6058, RL6071/Thatcher, and Thatcher/RL6058 crosses

Cross	Stem rust response and severity				Expected ratio	χ^2	*P*
RL6071/RL6058	Resistant 0-5MSS	Intermediate 10MRMS-40 MSS	Susceptible 50-60 MSS	*Lr34* phenotype			
	14	39	5	+	1:2:1	9.69	0.008
					1:6:1	7.44	0.024
					7:1 (53:5)	0.79	0.372
	0	39	12	−	3:1	0.06	0.808
Total	14	78	17	Pooled	1:6:1	1.02	0.601
RL6071/Thatcher	Resistant 0-5MS	Intermediate 10MRMS-50 MSS	Susceptible 60-70 MSS				
	0	87	27	–	3:1	0.105	0.746
Thatcher/RL6058	Resistant 0-5MSS	Intermediate 10MRMS-30MSS	Susceptible 60-70 MSS				
	57	55	0	Seg	1:1	0.036	0.850

[a] + = *Lr34* present; − = *lr34* absent

Association between field and seedling resistance

Associations between seedling and field resistance were evaluated using *t*-tests and χ^2 contingency tests (Table 5). In the RL6071/RL6058 population, associations between seedling resistance to races RKQQ and TPMK and field resistance were found to be significant ($P < 0.01$) by both *t*-tests and χ^2 tests. Seedling resistance to race RKQQ was associated with 25% of the phenotypic variation in field resistance. Similarly, resistance to race TPMK accounted for 21.7% of the phenotypic variation. The associations between seedling resistance to races QCCB and QCCD and field resistance were determined to be significant ($P < 0.05$) by *t*-tests, but not by the χ^2 tests. Seedling resistance to races QCCB and QCCD accounted for 6.7 and 6.9% of the variation in field resistance, respectively. The presence of *Lr34* accounted for 39.5% of the variation in field resistance to stem rust. In the RL6071/Thatcher population, seedling resistance to race RKQQ was associated with field resistance according to the χ^2 test (Table 5), however resistance to TPMK was not associated with field resistance. Seedling resistance to race QCCD was significantly associated with field resistance as determined by both the *t*-test and χ^2 analysis, and accounted for 5% of the variation in field resistance. Resistance to races QCCB and BCCB was not significantly associated with field stem rust resistance. In the Thatcher/RL6058 population, seedling resistance to race TPMK was not associated with field resistance (Table 5). Seedling resistance to race RKQQ in this cross was associated with field resistance as determined by the *t*-test, and accounted for 5.7% of the variation in field resistance, but was not significantly associated with field resistance in the χ^2 analysis (Table 5). The presence of *Lr34* was highly associated ($P < 0.001$) with field resistance.

Table 5 Associations determined by Student's *t*-test and χ^2 analysis between seedling response to specific races, *Lr34*, and microsatellite marker *Xbarc128* with adult plant field stem rust response, evaluated in recombinant inbred lines from three wheat crosses

Cross	Race	*t*-test		χ^2 test	
		P	R^2	χ^2	*P*
RL6071/RL6058	RKQQ	<0.001	0.250	19.7	<0.01
	TPMK	<0.001	0.217	17.2	<0.01
	QCCB	0.016	0.067	7.54	0.183
	QCCD	0.012	0.069	9.24	0.100
	BCCB	0.139	NA[a]	3.17	0.674
	Lr34	<0.001	0.395	13.6	0.018
	Xbarc 128	0.026	0.049	10.2	0.069
RL6071/Thatcher	RKQQ	–[b]	–	37.3	<0.01
	TPMK	–[b]	–	5.31	0.379
	QCCB	0.077	NA	1.16	0.763
	QCCD	0.017	0.052	8.59	0.035
	BCCB	0.112	NA	3.22	0.359
Thatcher/RL6058	RKQQ	0.015	0.057	6.15	0.104
	TPMK	0.917	NA	0.00	1.000
	Lr34	<0.001	0.909	96.7	0.000

[a] Not applicable since probability of *t*-test was greater than 0.05

[b] *t*-tests could not be carried out for seedling resistance to RKQQ and TPMK in the RL6071/Thatcher population because the number of resistant individuals was too small

Molecular markers

A region on chromosome 2BL was found to be associated with field stem rust resistance using the selective genotyping method. A linkage map of four *Xbarc* microsatellite markers spanning 25.7 cM was generated from data obtained from 98 recombinant inbred lines (data not shown). The marker most closely associated with field resistance was identified by the *t*-test to be *Xbarc128*. Linear regression revealed that *Xbarc128* was associated with 4.9% of the variation in field resistance (Table 5). The χ^2 test did not indicate a significant association between the marker and field resistance, and there were no significant associations between *Xbarc128*, seedling resistance, and *Lr34* according to either *t*-tests or χ^2 analysis.

Discussion

In this study, the segregation of *Lr34* in the recombinant inbred lines was associated with enhanced stem rust resistance in some genotypes of adult plants and seedlings. The presence of *Lr34* allowed additional stem rust resistance genes(s)to be expressed in the segregating populations. Seedling resistance to race RKQQ was strongly associated with adult plant field resistance in both the RL6071/RL6058 population and the Thatcher/RL6058 population. Resistance to race RKQQ was also associated with resistance in adult plants in the Thatcher/RL6071 population.

Resistance to TPMK was associated with field resistance in the RL6071/RL6058 population. One of the genes determining seedling resistance to race RKQQ may be the same as one of the genes that segregated in the adult plant tests in both the RL6071/RL6058 and the RL6071/Thatcher populations. In seedling evaluations of the Thatcher/RL6058 population, where only *Lr34* was segregating and all other resistance genes were fixed, the proportion of lines with low ITs to both races was greater than in the other populations. This suggests that some of the genes determining resistance to TPMK and RKQQ were expressed more strongly in the presence of *Lr34* at the seedling stage. Since race RKQQ is virulent to genes *Sr5*, *Sr9g, Sr12, and Sr16*, the resistance to this race that is associated with resistance in the field must be due to the enhanced effects of the adult plant resistance genes in Thatcher.

Other studies of stem rust resistance in Thatcher have also found associations between seedling and field stem rust resistance, as well as enhanced stem rust resistance associated with *Lr34*. The results of the present study indicate that seedling genes *Sr12* and *Sr16* may have contributed to field resistance. Nazareno and Roelfs (1981) studied segregating F_6 lines of Baart/Thatcher, and found no relationship between seedling resistance genes *Sr9g, Sr5*, and *Sr16* and field resistance. They did, however, observe that lines with both *Sr12* and *SrTc* had better field resistance than lines lacking both genes, although they concluded that the resistance must have come from a linked gene and not from either seedling gene per se, since there were susceptible lines that had both *Sr12* and *SrTc*.

Knott (2001) found that seedling resistance was associated with field resistance in recombinant inbred lines of LMPG-6/Thatcher. The seedling *Sr* genes which might have contributed to field resistance were not identified, except that *Sr5* did not contribute to field resistance. That study faced limitations similar to those in our study, including the inability to precisely determine gene number and identity in each line. Knott (1999) concluded that resistance in adult plants derived from Thatcher is genetically complex, heterogeneous in different accessions of Thatcher, and difficult to transmit to progeny in breeding programs because of the number of genes involved. The present study confirmed the genetic complexity of resistance expressed in adult plants, while at the same time providing a basis for future researchers to map loci associated with adult plant resistance.

This study demonstrated a strong association between *Lr34* and stem rust resistance at both the seedling and adult stages, as well as an association between seedling resistance to races TPMK and RKQQ and field resistance. Dyck (1993) studied stem rust resistance in the cultivar Roblin, which has a Thatcher type background and *Lr34*. In F_6 lines of RL6071/Roblin, seedling resistance to races RTH and TMR was associated with field resistance. Dyck (1987) also found an association between the presence of *Lr34* (as determined by leaf rust phenotype and leaf tip necrosis) and both seedling and field stem rust resistance.

Liu and Kolmer (1998) examined stem rust resistance in F_6 lines from the cross RL6071/Pasqua. Pasqua has a partial Thatcher background and *Lr34*. They found a strong association between the presence of *Lr34* and stem rust resistance in the field. However, in contrast to the present study, no association was observed between the presence of *Lr34* and seedling resistance. Pasqua may not have the full complement of stem rust resistance genes present in Thatcher.

Many other cultivars such as Chinese Spring and Terenzio, which possess *Lr34*, are highly susceptible to stem rust. Therefore, *Lr34* alone does not condition stem rust resistance. The mechanism by which *Lr34* mediates the enhancement of stem rust resistance in Thatcher is not well understood. *Lr34* appears to inhibit a suppressor of stem rust resistance on chromosome 7D (Kerber and Aung 1999). This suppressor is commonly found in hexaploid wheat and is present in the donor of the D genome, *Triticum tauschii* (Kerber 1983). The stem rust suppressor gene would not have segregated in the populations in this study since Thatcher, RL6058, and RL6071 have the suppressor (Kerber 1983). When the suppressor locus is mutated or eliminated as in nullisomic 7D lines, superior stem rust resistance is observed (Kerber 1983). *Lr34* has the same effect on the expression of resistance as elimination or mutation of the suppressor locus (Dyck 1987). *Lr34* is not an allele of the suppressor (Kerber and Aung 1999); it appears to indirectly mediate the collective expression of resistance genes by inactivating the suppressor (Kerber and Aung 1999).

The hypothesis that *Lr34* acts as an anti-suppressor of resistance is consistent with the results of the

present study. The detection of additional field resistance and seedling resistance to races QCCD, QCCB, BCCB, TPMK, and RKQQ in the RL6071/RL6058 population compared to the RL6071/Thatcher population is likely due to the anti-suppressor effect of *Lr34*. The segregation of resistance in the Thatcher/RL6058 population can also be attributed to the anti-suppressing effect of *Lr34*. Tetraploid Iumillo durum, a source of stem rust resistance in Thatcher, was highly resistant in seedling tests to races TPMK and RKQQ. The transfer of resistance from tetraploid to hexaploid wheat would have been limited by the 7D suppressor (Kerber 1983), and the presence of the suppressor in Thatcher may explain why it is less resistant than Iumillo. RL6058 is much more resistant than Thatcher and has a level of field resistance similar to Iumillo, presumably because *Lr34* inactivates the resistance suppressor and enhances expression of the stem rust resistance genes (Kerber and Aung 1999). The very high association between the field stem rust resistance and *Lr34* indicates that epistatic interactions are important in the analysis of these populations. This contribution to resistance cannot be due to *Lr34* alone, but most likely, could be due to unlinked stem rust resistance gene(s) dependent on the anti-suppressor effect of *Lr34* for expression.

There is a possibility that the difference in resistance between Thatcher and RL6058 is due to an additional introgressed resistance gene on chromosome 7DS, tightly linked to *Lr34*. The likelihood that a resistance gene was introgressed at other locations in the genome is low since *Lr34* was crossed six times into Thatcher. The variation in resistance among the five Thatcher backcross lines, also documented by Dyck (1987), could also be explained by an additional gene being introgressed into RL6058. However, it is more likely that not all backcross lines recovered the full complement of resistance genes present in Thatcher and RL6058. Also, all five Thatcher *Lr34* backcross lines showed greater resistance than the recurrent parent, and it is unlikely that all of them would contain the same introgression because the donors of *Lr34* were different. The low IT of Iumillo durum (0;) in the seedling tests provides support for the hypothesis that the stem rust resistance genes involved in adult resistance were derived from Thatcher rather than introgressed from the donor of *Lr34*.

Microsatellite marker *Xbarc128* on chromosome 2BL was associated with resistance in adult plants. The resistance associated with this marker was not enhanced in the presence of *Lr34*. This indicated that other chromosome regions are associated with the enhanced stem rust resistance conditioned by *Lr34* in a Thatcher background. There was no association between *Xbarc128* and seedling resistance, so it is unlikely that it is tightly linked to *Sr16*, which is also located on chromosome 2BL. Since flanking markers were not found, it is possible that the resistance locus is not tightly linked to *Xbarc128*. If linkage is not tight, the relative contribution to field stem rust resistance of this chromosomal region would be underestimated. Using chromosome substitution lines of Thatcher, Brennan (1975) identified regions on chromosomes 6A and 2B where adult resistance genes were located. The identification of additional molecular markers polymorphic between RL6071 and RL6058 and associated with field resistance will allow further characterization of other chromosome regions in Thatcher wheat associated with field stem rust resistance.

In 1999 an isolate of stem rust with virulence to *Sr31, Sr38*, and to many CIMMYT and US wheat cultivars was found in Uganda (Wanyera et al. 2006). By 2007, this isolate designated Ug-99, had spread to Kenya, Sudan, and Yemen. Selections of Thatcher varied for seedling IT ; to 3 to stem rust isolate Ug 99 (Jin and Singh 2006). The cultivar Chris which was derived from Thatcher and has *Lr34* (Kolmer, unpublished data) had seedling IT of ;2 to Ug 99. In field tests in Kenya, RL6058 and Chris showed good resistance to Ug 99 (Jin, personnel communication). Since many of the resistance genes found in wheats from CIMMYT and the US are ineffective to this isolate, germplasm with Thatcher stem rust resistance and *Lr34* will be an important source of resistance to Ug 99.

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