Genetics of Leaf Rust Resistance in Spring Wheat Cultivars Alsen and Norm

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ABSTRACT

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Alsen is a recently released spring wheat cultivar that has been widely grown in the United States because it has resistance to Fusarium head blight and leaf rust caused by *Puccinia triticina*. Norm is a high yielding wheat cultivar that has been very resistant to leaf rust since it was released. Alsen and Norm were genetically examined to determine the number and identity of the leaf rust resistance genes present in both wheats. The two cultivars were crossed with leaf rust susceptible cv. Thatcher and F_1 plants were backcrossed to Thatcher. Eighty one and seventy three BCF_1 of Thatcher × Alsen and Thatcher × Norm respectively, were selfed to obtain BCF_2 families. The BCF_2 families were tested as seedlings with different isolates of *P. triticina* that differed for virulence to specific leaf rust resistance genes. The BCF_2 families that lacked seedling resistance were also tested as adult plants in greenhouse tests and in a field rust nursery plot. Segregation of BCF_2 families indicated that Alsen had seedling genes *Lr2a*, *Lr10*, and *Lr23* and adult plant genes *Lr13* and *Lr34*. Norm was determined to have seedling genes *Lr1*, *Lr10*, *Lr16*, and *Lr23* and adult plant genes *Lr13* and *Lr34*. The characterization of *Lr23* in the segregating populations was complicated by the presence of a suppressor gene in Thatcher and the high temperature sensitivity of resistance expression for this gene. The effective leaf rust resistance in Alsen is due to the interaction of *Lr13* and *Lr23*, with *Lr34*; and the effective leaf rust resistance in Norm is due to the interaction of *Lr13*, *Lr16*, and *Lr23*, with *Lr34*.

Additional keywords: durable resistance, *P. recondita* f. sp. *tritici*, specific resistance.

Leaf rust of wheat, caused by *Puccinia triticina* Eriks., is the most common and widespread disease of wheat world-wide. In the spring wheat region of Minnesota and the Dakotas, yield losses to leaf rust range from 5 to 15% (18) or even greater if the initial infections occur before the wheat has headed.

Spring wheats with leaf rust resistance genes have been released in North America since 1937, when the cv. Renown with *Lr14a* was released in Canada. Stable leaf rust resistance in wheat has been difficult to achieve because the *P. triticina* population in the United States and Canada is highly diverse for virulence. In 2001 over 40 *P. triticina* virulence phenotypes or races were described in the United States (8). Races of *P. triticina* with virulence to the corresponding leaf rust resistance gene almost inevitably increase to a point in the population to render the resistance gene ineffective. Genes that are effective in the seedling plant stage have been the most vulnerable to selection and increase of virulent races. In contrast, wheat cultivars with the adult plant resistance gene *Lr34* in combination with other leaf rust resistance genes have maintained effective resistance for a longer period of time compared with cultivars that lack *Lr34* (4,17,21). The U.S. spring wheat, Era, with the seedling resistance gene *Lr10*, and the adult plant genes *Lr13* and *Lr34* (6) has been rated as highly resistant to moderately resistant (14) since release in the early 1970s. The Canadian wheats AC Domain (10), CDC Teal (9), Roblin (2), and Glenlea (5) all have *Lr34* in combination with other leaf rust resistance genes and have maintained at least a moderate level of leaf rust resistance. Identification of gene combinations that have conditioned durable resistance can aid in the development of germplasm with high levels of leaf rust resistance.

Since the mid-1990s, Fusarium head blight caused by *Fusarium graminearum* Schwabe has become a major disease in the spring wheat region of the United States (13). This has led to increased emphasis on developing spring wheat germplasm with head blight resistance derived from Chinese wheats, notably Sumai 3 (23). The wheat cv. Alsen (pedigree = ND 674//ND 2710/ND 688), released by the North Dakota Agricultural Experiment Station in 2000, was developed by incorporating the head blight resistance derived from Sumai 3 into an adapted background that also had good stem and leaf rust resistance, yield, and quality characteristics. In recent years Alsen has been widely grown in Minnesota and North Dakota. The cv. Norm (pedigree = MN 73167/MN 81070) was released by the Minnesota Agricultural Experiment Station and the U.S. Department of Agriculture-Agricultural Research Service in 1992. Although Norm is highly susceptible to head blight and is no longer commonly grown, it is a high yielding cultivar that has maintained very high levels of leaf rust resistance since it was released. In a recent study, Alsen and Norm were among the most leaf rust resistant cultivars in a group of hard red spring wheats that have been grown in Minnesota and the Dakotas (14). Both Alsen and Norm have been used extensively as parents in spring wheat breeding programs in Minnesota and the Dakotas. The objective of this study was to determine the identity of the leaf rust resistance genes present in wheat cvs. Alsen and Norm.

MATERIALS AND METHODS

The isolates of *P. triticina* used in this study (Table 1) were collected from wheat in the United States and Canada. The isolates were selected for their low infection types to specific *Lr*

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genes in spring wheat. Isolate BBBD is avirulent to all the lines with seedling resistance genes in Table 1, except for the line with *Lr14a*. The total number of seedling genes that were segregating in the populations was estimated with isolate BBBD, in combination with segregation of resistance to other leaf rust isolates.

Seed of Alsen, Norm, and the leaf rust susceptible wheat Thatcher were planted in 15-cm-diameter pots that were filled with steamed topsoil and grown in a growth cabinet with a mixture of fluorescent and incandescent bulbs with a 16-h day length. The plants were treated with Nutricote 13-13-13 micronutrients, Type 100 (Plantco Inc., Brampton, ON). At heading, Thatcher plants were emasculated, and pollen bearing anthers from Alsen and Norm were used to pollinate the Thatcher female parent. The F_1 seed from Thatcher × Alsen and Thatcher × Norm was planted and backcrossed as the male parent to Thatcher. Approximately 80 BCF_1 seed were obtained from each of the two crosses. The BCF_1 plants were grown in a greenhouse and selfed to obtain BCF_2 families.

Fifteen seeds from each BCF_2 family were planted in one 3.5 cm^2 plastic pot in vermiculite (Sunshine Strong-Lite Medium Vermiculite Premium Grade, JR Johnson Horticultural Supplies, St. Paul, MN) and placed in a plastic tray, with six pots per tray. Differentials of Thatcher near-isogenic lines with single genes (Thatcher; RL 6003 *Lr1;* RL 6016 *Lr2a*; RL 6002 *Lr3*; RL 6005 *Lr16*; RL 6004 *Lr10*; RL 6013 *Lr14a*; and RL 6012 *Lr23*) were also planted for comparison of infection types. The Thatcher lines were planted in clumps of four to eight plants at the corners of 3.5 cm^2 plastic pots, with six pots per tray. All seedling plants were treated with soluble 20-20-20 N-P-K fertilizer (Spectrum Group, St. Louis, MO) at emergence, watered daily, and grown for 8 days at approximately 20 ± 3°C with 16 h supplemental light in a greenhouse. The seedlings were inoculated 8 days after planting at full emergence of primary leaves with a mixture of rust spores and Soltrol 170 oil. The leaves were allowed to dry for 1 h after inoculation, and then the seedlings were placed in a mist chamber at 18°C for 24 h. The seedlings were removed from the mist chamber and returned to the greenhouse bench.

The seedlings were evaluated for infection types at 12 days after inoculation in greenhouse tests, and 10 days after inoculation in tests done at 25°C in a growth cabinet. The infection types were classified on a 0 to 4 scale used by Long and Kolmer (11): 0 = immunity, no hypersensitive flecks or uredinia; 0; = faint hypersensitive flecks; ; = distinct hypersensitive flecks; 1 = small uredinia surrounded by distinct necrosis; 2 = small uredinia surrounded by distinct chlorosis; 3 = moderate size uredinia without chlorosis; and 4 = very large uredinia lacking chlorosis. Designations of "+" or "–" indicate larger than normal uredinia and smaller uredinia, respectively. Infection types from 0 to 2^+ were considered low infection types, and infection types 3^+ to 4 were considered high. Intermediate infection types occurred with a 23 (mixture of 2 to 3 type uredinia) infection type. In the BCF_2 families, the ratio of families that segregated for plants with low infection types to families that were homozygous for susceptible plants was used to determine the number of seedling resistance genes that were effective to each *P. triticina* isolate. Goodness of fit of observed ratios to expected ratios was determined using a χ^2 test (22). Selected resistant BCF_2 plants were transplanted into 15-cm-diameter pots and grown out to obtain BCF_3 seed. The BCF_3 lines were tested with different *P. triticina* isolates to confirm the identity of leaf rust resistance genes that segregated in the BCF_2 populations.

The BCF_2 families that were homozygous susceptible to isolate BBBD were also evaluated for resistance as adult plants to the same isolate in greenhouse tests. By using families that were homozygous susceptible to this isolate in seedling tests, any resistance in adult plants would be conditioned by adult plant resistance genes. From each BCF_2 family that was tested, four seeds per 15 cm pot were planted, with two pots for each family. The plants were treated with Nutricote fertilizer and grown in the greenhouse at 20 ± 3°C and 16 h of supplemental light per day. At 3 weeks after planting, the plants were trimmed to three tillers each. The adult plant flag leaves were inoculated with a suspension of isolate BBBD urediniospores in Soltrol 170 oil when the plants had fully headed. The Thatcher lines with *Lr13* (RL 4031), *Lr34* (RL 6058), and Thatcher were also planted and inoculated with isolate BBBD as adult plants for comparison of infection types. The plants were allowed to dry for 60 min after inoculation and were then placed in a mist chamber at approximately 18°C and 100% relative humidity. After 24 h of incubation, the plants were removed from the mist chamber and allowed to dry for 60 min and then placed on a greenhouse bench. The BCF_2 plants and Thatcher lines were scored for infection type at 14 days after inoculation. Resistant plants were selected and progeny were tested as BCF_3 plants with isolate BBBD.

TABLE 1. Seedling infection types[a], adult plant infection types, and field rust severity and response of the wheat cvs. Alsen and Norm, and 10 lines of Thatcher wheat near-isogenic for leaf rust resistance genes to specific isolates of *Puccinia triticina*

	Isolates										
Cultivar/ wheat line	BBBD 1-1	MCDS 520-1	TLGF 218-1	SBDG 1-2	KFBJ 64-1	MJBJ 406-1	MBRJ 16-2	PBLR 432-1	THBJ 588-1	THBJ 588-1[b]	Field rust severity[c]
Alsen	;	0;	;1	;1$^-$	3	;	;	0;	;23	;	5 MR
Norm	;	;1	0;	0	;1$^-$	;1	;1	;2	22$^-$	0;	5 R
Thatcher	33$^+$/3$^+$[d]	33$^+$	33$^+$	33$^+$	33$^+$	3$^+$	33$^+$	3$^+$	3$^+$	3	70–80 S
Tc*Lr1* RL 6003	0;	3$^+$	3$^+$	33$^+$	;1	3$^+$	3	3$^+$	33$^+$	3	70–80 S
Tc*Lr2a* RL 6016	0;	;	33$^+$	3	3	0;	;	;2$^-$	33$^+$	3	20 S
Tc*Lr3* RL 6002	;	3	3$^+$	0;	33$^+$	3$^+$	3$^+$	3$^+$	33$^+$	3	70–80 S
Tc*Lr16* RL 6005	22$^-$	22$^-$	1	;1	22$^-$	2$^+$3	22$^-$	22$^+$	3	2	60 MS
Tc*Lr10* RL 6004	;22$^-$	3$^+$	0;	3	33$^+$	3$^+$	3	3$^+$	33$^+$	3	70–80 S
Tc*Lr14a* RL 6013	33$^+$	3$^+$	33$^+$	;12	3$^+$	3$^+$	3	;12	3	3	70–80 S
Tc*Lr23* RL 6012	2-3$^+$	;1-2	2-3$^+$	;	3$^+$	22$^+$	2-3$^+$	3$^+$	2-3$^+$	;1$^-$	20 MR MS
Tc*Lr13* RL 4031	3$^+$/;1$^-$[d]	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	50–80 MRMS
Tc*Lr34* RL 6058	33$^+$/23[d]	3	3	33$^+$	3$^+$	33$^+$	33$^+$	3$^+$	3	3	40–60 MR MS

[a] Infection types as described in Long and Kolmer (11): 0 = immunity, no hypersensitive flecks or uredinia; 0; = faint hypersensitive flecks; ; = distinct hypersensitive flecks; 1 = small uredinia surrounded by distinct necrosis; 2 = small uredinia surrounded by distinct chlorosis; 3 = moderate size uredinia without chlorosis; and 4 = very large uredinia lacking chlorosis. Designations of "+" or "–" indicate larger than normal uredinia and smaller uredinia, respectively. Infection types from 0 to 2^+ were considered low infection types, and infection types 3 to 4 were considered high.

[b] Seedling plants were inoculated with isolate THBJ and placed in a growth cabinet at 25°C.

[c] A mixture of common isolates of *P. triticina* was used to inoculate field plots. R = resistant with very small uredinia surrounded by necrosis; MR = moderately resistant with moderate size uredinia surrounded by necrosis; MS = moderately susceptible with moderate to large uredinia surrounded by chlorosis; and S = large uredinia lacking chlorosis or necrosis.

[d] Adult plant infection type.

The BCF_2 families that were homozygous susceptible to isolate BBBD in seedling tests were also evaluated in field rust plots for segregation of adult plant leaf rust resistance genes. Approximately 40 to 50 seed of each family and the near-isogenic Thatcher lines previously described were planted in a 2 m row, spaced 30 cm apart. Rows of the leaf rust susceptible wheat cv. Max were planted at a perpendicular angle to the entries. The spreader rows were inoculated with isolates THBJ 588, MCDS 520, and MBRJ 16-2B when the spreader rows had fully tillered and the heads had fully emerged. Isolates with race designation of THBJ and MCDS are common in the current leaf rust population, and MBRJ was common in the 1990s. The mixture of leaf rust isolates was sprayed on the spreader rows with Soltrol 170 oil with a backpack mist blower with a modified spray nozzle.

The severity ratings for the adult plants in the field rust nursery test were based on the modified Cobb scale (15). Five to ten flag leaves of each wheat line were evaluated for rust severity and resistance response. The rust severity and response ratings were an average of the estimates. The host infection response was rated as follows: R = resistant with very small uredinia surrounded by necrosis; MR = moderately resistant with moderate size uredinia surrounded by necrosis; MS = moderately susceptible with moderate to large uredinia surrounded by chlorosis; and S = large uredinia lacking chlorosis or necrosis. The BCF_2 families and Thatcher lines were rated for leaf rust when the susceptible line Thatcher had leaf rust severity of at least 70%.

RESULTS

Alsen. Seedling plants of Alsen had low infection types of 0; to ;1 to isolates BBBD, MCDS, TLGF, SBDG, MJBJ, MBRJ, and PBLR (Table 1). Isolate THBJ had an intermediate infection type of ;23, and isolate KFBJ had a high infection type of 3 to Alsen. Since THBJ and KFBJ are virulent to *Lr2a*, and BBBD, MCDS, MJBJ, MBRJ, and PBLR are avirulent to *Lr2a*, it was postulated that Alsen has *Lr2a*. Since isolate TLGF is avirulent to *Lr10* and virulent to *Lr2a*, it was postulated that Alsen also has *Lr10*. Isolate SBDG is virulent to *Lr2a* and *Lr10*; therefore an additional gene(s) in Alsen was postulated to condition resistance to this isolate.

The BCF_2 families of Thatcher (Tc) × Alsen segregated in a 7:1 ratio for segregating to homozygous susceptible families when tested with isolate BBBD (Table 2), which indicated that at least three genes in Alsen conditioned resistance to this isolate. To isolate MCDS, the Tc × Alsen BCF_2 families segregated in a 3:1 ratio, with resistant plants having infection types of 0; to ;1. The segregation data indicated that at least two genes in Alsen conditioned resistance to MCDS. Isolate MCDS had low infection types to *Lr2a*, *Lr16*, and *Lr23* (Table 1). To isolate TLGF, the BCF_2 families of Tc × Alsen segregated to fit both a 3:1 and a 7:1 ratio, with resistant plants in the segregating families having infection types of 0; to 22^+. The segregation data indicated that at least two genes in Alsen conditioned resistance to TLGF. Isolate TLGF had low infection types to *Lr16* and *Lr10*, and intermediate infection type to *Lr23*. To isolate SBDG, the BCF_2 families of Tc × Alsen segregated to fit a 1:1 ratio, which indicated that a single gene in Alsen conditioned resistance to SBDG. The resistant plants had infection types of ; (fleck) to 22^+ in the segregating families. Isolate SBDG had low infection types to *Lr3*, *Lr16*, *Lr14a*, and *Lr23*. To isolate MBRJ, the BCF_2 families of Tc × Alsen segregated to fit a 1:1 ratio, with segregating families having resistant plants with ; (fleck) infection types. The segregation data indicated that a single gene in Alsen conditioned resistance to MBRJ. Isolate MBRJ had low infection type to genes *Lr2a* and *Lr16*, and had an intermediate infection type of 2^+3 to *Lr23*. To isolate KFBJ, the BCF_2 families of Tc × Alsen were all homozygous susceptible, indicating that Alsen did not have any seedling resistance genes that conditioned resistance to KFBJ. Isolate KFBJ is avirulent to genes *Lr1* and *Lr16*; therefore, these genes were determined not to be present in Alsen.

The BCF_2 families of Tc × Alsen that segregated for resistance to isolate BBBD were highly associated ($P < 0.01$) with the families that segregated for resistance to isolates MCDS, MBRJ, and TLGF in contingency table tests of independence. This indicated that the genes that conditioned resistance to BBBD also conditioned resistance to MCDS, MBRJ, and TLGF. The BCF_2 families of Tc × Alsen that segregated for resistance to isolate MBRJ were highly associated with the families that segregated for resistance to MCDS ($P < 0.01$), which indicated that the same gene in Alsen conditioned resistance to both isolates. Isolates MBRJ and MCDS both have low infection type to gene *Lr2a*, which was determined to be in Alsen. The BCF_2 families of Tc × Alsen that segregated for resistance to isolate TLGF were independent ($P = 0.46$) of the families that segregated for resistance to isolate MBRJ, which indicated that different genes in Alsen conditioned resistance to the two isolates. Isolate TLGF has a 0; infection type to *Lr10*, and MBRJ is virulent to *Lr10*. Gene *Lr10* was determined to be in Alsen.

Based on the segregation data with isolates SBDG, TLGF, MBRJ, and MCDS, it was not possible to determine which of genes *Lr3*, *Lr14a*, or *Lr23* conditioned the resistance of Alsen to SBDG. The BCF_2 families of Tc × Alsen were tested for segregation with isolate PBLR, which had low infection types to genes *Lr2a* and *Lr14a*, and high infection types to *Lr3* and *Lr23*. If *Lr14a* were present in Alsen, then a two gene segregation would be expected with PBLR. The 1:1 segregation ratio (Table 2) indicated that a single gene in Alsen conditioned resistance to isolate PBLR. The families that segregated for resistance to PBLR had infection types of ;2, which was very similar to the infection type of *Lr2a* to this isolate. The segregation data with PBLR eliminated the possible presence of *Lr14a*. To confirm that *Lr23* was present, the BCF_2 families of Tc × Alsen were tested with isolate THBJ at 25°C. The Thatcher line with *Lr23* is temperature sensitive for expression of resistance. In varying greenhouse temperatures of 18 to 25°C, Tc*Lr23* expresses infection types ranging from 2 to 3^+ to many isolates, including THBJ (Table 1). At 25°C in a growth cabinet, Tc*Lr23* has low infection type of $;1^-$ to isolate THBJ. The BCF_2 families of Tc × Alsen segregated in a 1:3 ratio to isolate THBJ in growth cabinet test at 25°C. Since THBJ had high infection types to genes *Lr3* and *Lr14a* in both greenhouse and growth cabinet tests, the resistance in Alsen to THBJ is most likely due to the presence of *Lr23*. The 1:3 ratio of segregating to homozygous susceptible families indicated that a second gene suppressed the expression of *Lr23* in the segregating populations. The seedling resistance genes in Alsen were determined to be *Lr2a*, *Lr10*, and *Lr23*.

TABLE 2. Segregation for leaf rust resistance in greenhouse tests of seedling plants of BCF_2 families of Thatcher × Alsen

Leaf rust isolate	Gene(s) detected	Number of families[a]		Expected ratio	χ^2	*P* value
		Seg.	Susc.			
BBBD	*Lr2a*, *Lr10*, (*Lr23*)[b]	68	13	7:1	0.27	0.64
MCDS	*Lr2a*, (*Lr23*)	59	21	3:1	0.01	0.92
TLGF	*Lr10*, (*Lr23*)	64	15	3:1	1.21	0.27
				7:1	2.48	0.16
SBDG	*Lr23*	36	40	1:1	0.12	0.73
MBRJ	*Lr2a*	43	32	1:1	1.34	0.25
KFBJ	...	0	63	0:1	0.00	1.00
PBLR	*Lr2a*	17	19	1:1	0.02	0.89
THBJ[c]	*Lr23*	9	33	1:3	0.13	0.72

[a] Seg. = segregating for resistance and susceptible plants; Susc. = homozygous susceptible.

[b] Expression of the low infection type of *Lr23* to these isolates is variable due to temperature sensitivity and a suppressor gene in Thatcher.

[c] Seedling plants were inoculated and then placed in a growth chamber at 25°C.

The identities of the seedling genes in Alsen based on the segregation of the BCF_2 families were supported by testing derived BCF_3 lines (Table 3). Single gene lines with *Lr2a* and *Lr23* were derived from the Tc^2 × Alsen F_2 families as identified by infection type to six *P. triticina* isolates. A line with *Lr10* and *Lr23* was also derived from this cross.

Individual plants from 13 BCF_2 families of Tc × Alsen that were homozygous susceptible to isolate BBBD in the seedling tests were tested for resistance to the same isolate at the adult plant stage. The BCF_2 plants segregated 58:35 for resistant/susceptible plants, which fit a 39:25 ratio, which is the expected ratio for two independent genes segregating in a random BCF_2 population (Table 4). The resistant BCF_2 adult plants had infection types varying from ;1^+, ;2^-, and 23. Adult plants of the Thatcher lines with *Lr13* and *Lr34* had infection types of ;1^- and 23, respectively, to isolate BBBD (Table 1). Based on the segregation data and infection types of the individual BCF_2 plants, it is most likely that *Lr13* and *Lr34* are present in Alsen. Lines derived from the resistant BCF_2 adult plants were progeny tested as BCF_3 lines (Table 3). Single gene lines with *Lr13* and *Lr34* were identified based on infection type to isolate BBBD in adult plants. The 13 BCF_2 families of Tc × Alsen that lacked seedling resistance genes were also tested in a field rust nursery to a mixture of common *P. triticina* isolates. Nine of the families segregated for resistance, with leaf rust severity and response varying between 10 MR to 50 MR MS and four of the BCF_2 families were homozygous susceptible. The 9:4 segregation fit a 3:1 ratio ($\chi^2 = 0.03$) that indicated that two genes, likely *Lr13* and *Lr34*, conditioned adult plant resistance. The Thatcher lines with *Lr13* and *Lr34* had leaf rust severity and response of 50 to 80 MR MS and 40 to 60 MR MS, respectively (Table 1).

Norm. Seedling plants of Norm had low infection types of 0 to ;2 to isolates BBBD, MCDS, TLGF, SBDG, KFBJ, MJBJ, MBRJ, and PBLR in greenhouse tests (Table 1). Isolate THBJ had a 22^- infection type to Norm in greenhouse tests. Based on these data, it was postulated that Norm had *Lr16*, since THBJ had a high infection of 3 to *Lr16.*

To isolate BBBD, the BCF_2 families of Tc × Norm segregated to fit a 7:1 ratio of families that segregated for resistance to families that were homozygous susceptible, which indicated that at least three genes in Norm conditioned resistance to this isolate (Table 5). The resistant plants in the segregating families had infection types that varied from ; (fleck) to 2. To isolate TLGF, the BCF_2 families of Tc × Norm segregated to fit a 3:1 ratio, which indicated that at least two genes in Norm conditioned resistance to this isolate. Isolate TLGF had low infection types to *Lr10* and *Lr16* and infection types of 2 to 3^+ to *Lr23* (Table 1). The resistant plants in the segregating families had infection types of 0; to 22^+. Genes *Lr10* and *Lr16* most likely segregated for resistance to TLGF in the BCF_2 families. The BCF_2 families of Tc × Norm segregated to isolate KFBJ to fit a 3:1 ratio (Table 5), which indicted that *Lr16* and a second gene in Norm conditioned resistance to this isolate. The resistant plants in the segregating families had infection types of 0; to 22^-. Isolate KFBJ had low infection types to *Lr1* and *Lr16* (Table 1). Genes *Lr1* and *Lr16* mostly likely segregated for resistance to KFBJ in the BCF_2

TABLE 3. Seedling and adult plant infection types[a] in greenhouse tests of Tc × Alsen BCF_3 lines and Tc × Norm BCF_3 lines to specific isolates of *Puccinia triticina*

BCF_3 line	TLGF[b]	MCDS[b]	THBJ[b]	SBDG[b]	MJBJ[b]	KFBJ[b]	BBBD[c]	*Lr* gene postulation
Tc^2 × Alsen								
22-1	3	;	3	3	;	3	...	*Lr2a*
23-1	;	3	3	;2^-	23	3	...	*Lr10*, *Lr23*
49-1	;2	;2^-	3	;2^-	22^+	3	...	*Lr23*
50-2	...	...	...	...	...	...	;1^+	*Lr13*
75-2	...	...	...	...	...	...	23	*Lr34*
Tc^2 × Norm								
32-1	;	3^+	...	3	3^+	;	...	*Lr1*, *Lr10*
17-1	;	3^+	...	3	3^+	3^+	...	*Lr10*
25-1	2^+	22^+	...	22^+	3^+	22^-	...	*Lr16*
56-2	3	;-;2	3	;2^-	23	3	...	*Lr23*
55-4	...	...	...	...	...	...	;1^+	*Lr13*
48-4	...	...	...	...	...	...	;2^-	*Lr34*

[a] Infection types as described in Long and Kolmer (11): 0 = immunity, no hypersensitive flecks or uredinia; 0; = faint hypersensitive flecks; ; = distinct hypersensitive flecks; 1 = small uredinia surrounded by distinct necrosis; 2 = small uredinia surrounded by distinct chlorosis; 3 = moderate size uredinia without chlorosis; and 4 = very large uredinia lacking chlorosis. Designations of "+" or "–" indicate larger than normal uredinia and smaller uredinia, respectively. Infection types from 0 to 2^+ were considered low infection types, and infection types 3 to 4 were considered high.
[b] Seedling infection types.
[c] Adult plant infection types.

TABLE 4. Segregation of individual Thatcher × Alsen BCF_2 adult plants and Thatcher × Norm BCF_2 adult plants for infection type to leaf rust isolate BBBD in greenhouse tests

	Infection types[a]								
	R[b]				S[c]				
Cross	0;	;1	;22^-f[d]	23f	3^+ 4	Expected ratio R:S	χ^2	*P* value	Genes detected
Thatcher × Alsen	...	4[e]	21	33	35	39:25	0.03	0.86	*Lr13*, *Lr34*
Thatcher × Norm	10	9	12	30	52	39:25	2.02	0.25	*Lr13*, *Lr34*

[a] Infection types as described in Long and Kolmer (11): 0 = immunity, no hypersensitive flecks or uredinia; 0; = faint hypersensitive flecks; ; = distinct hypersensitive flecks; 1 = small uredinia surrounded by distinct necrosis; 2 = small uredinia surrounded by distinct chlorosis; 3 = moderate size uredinia without chlorosis; and 4 = very large uredinia lacking chlorosis. Designations of "+" or "–" indicate larger than normal uredinia and smaller uredinia, respectively. Infection types from 0 to 2^+ were considered low infection types, and infection types 3 to 4 were considered high.
[b] Resistant infection types.
[c] Susceptible infection types.
[d] Few uredinia compared with Thatcher.
[e] Four BCF_2 plants of Thatcher × Alsen had infection ;1^- to isolate BBBD.

families. To isolate SBDG, the BCF_2 families of Tc × Norm segregated to fit a 3:1 ratio, which indicated that *Lr16* and a second gene were segregating. The resistant plants in the segregating BCF_2 families had infection types of 0; to 22^+. Isolate SBDG had low infection types to genes *Lr3*, *Lr16*, *Lr14a*, and *Lr23* (Table 1). The BCF_2 families of Tc × Norm segregated to fit a 1:3 ratio when tested with isolate THBJ (Table 5). This indicated that two genes, a seedling resistance gene and an independent gene that suppressed the expression of the resistance gene, which was postulated to be *Lr23*, were present in the segregating population. The resistant plants in the segregating populations had infection types of ;2 to ;23. Isolate THBJ had infection types varying from 2 to 3^+ to Tc*Lr23* in greenhouse tests, and had high infection types to the other genes listed in Table 1. To confirm the presence of *Lr23* in Norm, the BCF_2 families were tested with isolate PBLR, which has low infection types to *Lr16* and *Lr14a*, and high infection types to *Lr3* and *Lr23* (Table 1). The BCF_2 families segregated for a single gene, *Lr16*, when tested with PBLR (Table 5), which eliminated the presence of *Lr14a*. The BCF_2 families segregated in a 3:1 ratio (Table 5) to isolate THBJ in a growth cabinet at 25°C, which indicated that two genes conditioned resistance to this isolate at high temperatures. Gene *Lr16* had a 2 infection type to THBJ at 25°C and *Lr23* had a $;1^-$ infection type (Table 1). The seedling resistance genes in Norm were determined to be *Lr1*, *Lr10*, *Lr16*, and *Lr23*. In the BCF_3 derived lines, single gene lines with *Lr10*, *Lr16*, and *Lr23* were identified (Table 3), which confirmed the identity of these genes in the segregating BCF_2 populations. A line with *Lr1* and *Lr10* was also identified.

Individual adult plants from 11 BCF_2 families of Tc × Norm that were homozygous susceptible to isolate BBBD in the seedling tests were tested for segregation of leaf rust resistance. The BCF_2 plants segregated 61:52 for resistant/susceptible plants, which fit a 39:25 ratio, which is the expected ratio for two independent genes segregating in a random BCF_2 population. The resistant BCF_2 adult plants had infection types varying from 0; to 23 (Table 4). Adult plants of the Thatcher lines with *Lr13* and *Lr34* had infection types of $;1^-$ and 23, respectively, to isolate BBBD (Table 1). Based on the segregation data and infection types of the individual BCF_2 plants, *Lr13* and *Lr34* are most likely present in Norm. The BCF_2 adult plants with 0; infection types likely had both *Lr13* and *Lr34*. Lines derived from resistant BCF_2 plants were progeny tested as BCF_3 lines (Table 3). Single gene lines with *Lr13* and *Lr34* were identified based on infection type to isolate BBBD to adult plants. The 11 BCF_2 families of Tc × Norm that lacked seedling resistance genes were also tested in a field rust nursery with a mixture of common *P. triticina* isolates. Seven of the families segregated for resistance, with leaf rust severity and response varying between 5 R to 40 MR MS, and four of the BCF_2 families were homozygous susceptible. The 7:4 segregation fit a 3:1 ratio ($\chi^2 = 0.27$), indicating that two genes, likely *Lr13* and *Lr34*, conditioned adult plant resistance. The Thatcher lines with *Lr13* and *Lr34* had leaf rust severity and response of 50 to 80 MR MS and 40 to 60 MR MS, respectively (Table 1).

DISCUSSION

Wheat cv. Alsen was determined to have genes *Lr2a*, *Lr10*, *Lr13*, *Lr23*, and *Lr34*. Wheat cv. Norm was determined to have genes *Lr1*, *Lr10*, *Lr13*, *Lr16*, *Lr23*, and *Lr34*. Effective leaf rust resistance in both cultivars is conditioned by the combination of *Lr13*, *Lr23*, and *Lr34*. Gene *Lr16*, present in Norm, also contributes to effective leaf rust resistance. In field plots, the Thatcher lines with *Lr13*, *Lr16*, *Lr23*, and *Lr34* have some effective resistance compared with Thatcher. In recent years, virulence to *Lr16* has increased in the north central region of the United States (8), yet single gene lines with *Lr16* still have some effective resistance in field plots. Virulence to *Lr13* has also increased in recent years (8), thus greatly reducing the effectiveness of this gene. Isolates with complete virulence to adult plants with *Lr34* have not been described (8). By itself, *Lr34* does not condition a high level of effective resistance, yet in combination with *Lr13*, *Lr16*, and *Lr23*, wheat lines with *Lr34* will have better leaf rust resistance compared with lines that have fewer of these genes. Genes *Lr1* and *Lr10* are completely ineffective because nearly every *P. triticina* isolate in the north central region of the United States (8) has virulence to these two genes. Virulence to *Lr2a* is also very common.

Gene *Lr23* was determined to be present in both Alsen and Norm. The resistance of *Lr23* is best expressed at temperatures above 25°C (3). With isolates BBBD, TLGF, and THBJ, infection types to Tc*Lr23* can range from ;2 to 3, depending on temperatures during the greenhouse tests. The Tc*Lr23* line had lower and more stable infection types of ; (fleck) to isolate SBDG and $;1\text{-}2^-$ to isolate MCDS. Expression of *Lr23* in the segregating populations was further complicated by the presence of a suppressor gene in Thatcher. Previous studies have also indicated that a gene in Thatcher suppressed the expression of *Lr23* (1,12). A gene in Thatcher that inhibited the expression of *Lr23* to Canadian isolates of *P. triticina* acted as a partial inhibitor to Australian isolates (12). Dyck (1) found that the suppressor gene was also present in a backcross line of Marquis wheat, a progenitor of Thatcher. McIntosh and Dyck (12) showed that in some crosses *Lr23* was recessive, while in other crosses it was partially dominant. The dominance effect of *Lr23* increased at higher temperatures. The Thatcher line with *Lr23* was developed by selecting a final backcross line that lacked the suppressor gene (1). Expression of *Lr23* is highly temperature and isolate dependent, which makes it more difficult to reliably detect in segregating Thatcher backcross populations.

Virulence to *Lr23* in the *P. triticina* population in the United States is not assayed regularly due to the variable infection types that a single isolate can produce on the Thatcher line with *Lr23*. Wheat cultivars with *Lr23* would have some leaf rust resistance, because the Thatcher line with *Lr23* had effective resistance in the field rust nursery. The cv. Lee, the original source of *Lr23* in the Thatcher line, had rust severity and response of 20 to 30 MR MS in 2003 and 5 MR MS in 2004. Wheat genotypes with combinations of *Lr23* and *Lr34* would be expected to have better resistance relative to the single gene lines, based on the enhancement effect observed of *Lr34* in general with other effective leaf rust resistance genes (7,19).

Alsen (ND 674//ND 2710/ND 688) (ND 674 = Grandin*2/Glupro) (ND 2710 = Sumai 3/Wheaton/Grandin) most likely derived *Lr2a*, *Lr10*, *Lr13*, and *Lr34* from Grandin (9). Wheaton (Crim/*2 Era//Buitre/Gallo) may have been the source of *Lr23* in Alsen since Crim was postulated to have *Lr23* (16). Norm (MN 73167/MN 81070) (MN 73167 = Crim/*2 Era//MN 6925s) (MN 81070 = Era/Kitt/3/Fletcher Ciano 67//ND 264/4/II-60-46/5/

TABLE 5. Segregation for leaf rust resistance in greenhouse tests of seedling plants of BCF_2 families of Thatcher × Norm

Leaf rust isolate	Gene(s) detected	Number of families[a]		Expected ratio	χ^2	*P* value
		Seg.	Susc.			
BBBD	*Lr1*, *Lr10*, *Lr16* (*Lr23*)[b]	62	11	7:1	0.24	0.62
SBDG	*Lr16*, *Lr23*	58	14	3:1	0.91	0.34
KFBJ	*Lr1*, *Lr16*	47	17	3:1	0.03	0.86
TLGF	*Lr10*, *Lr16*, (*Lr23*)	57	16	3:1	0.23	0.63
PBLR	*Lr16*	19	23	1:1	0.22	0.64
THBJ	(*Lr23*)	14	59	1:3	1.03	0.31
THBJ[c]	*Lr16*, *Lr23*	25	10	3:1	0.08	0.78

[a] Seg. = segregating for resistance and susceptible plants; Susc. = homozygous susceptible.
[b] Expression of the low infection type of *Lr23* to these isolates is variable due to temperature sensitivity and a suppressor gene in Thatcher.
[c] Seedling plants were inoculated and then placed in a growth chamber at 25°C.

Waldron/Era/6/Fletcher Bajo-66//Era/3/*2 Era) may have derived *Lr10*, *Lr13*, and *Lr34* from Era (6). Kitt or Crim may have been the source of *Lr23* (16). The sources of *Lr1* and *Lr16* in Norm were not apparent from the pedigree; however, both genes are common in U.S. hard red spring wheats (14).

Spring wheat cultivars grown in Minnesota and the Dakotas can have a fairly complex inheritance of leaf rust resistance based on the genetics of Alsen and Norm. The Sumai 3 material and other lines from China that have head blight resistance have poor leaf rust resistance (J. A. Kolmer, *unpublished data*). Introgression of head blight resistant germplasm has diluted the leaf rust resistance in the spring wheat breeding programs in the United States and made recovery of highly resistant germplasm more difficult. Another complicating factor is the continued selection for virulence in the *P. triticina* populations. Many *Lr* genes such as *Lr1*, *Lr3*, *Lr10*, and *Lr14a*, no longer provide effective resistance even though they may be common in wheat germplasm. Development of wheat germplasm with good leaf rust resistance will depend on the ability to select genotypes that have combinations of effective seedling genes such as *Lr16* and *Lr23* in combination with the adult plant resistance gene *Lr34*, which has conditioned durable nonspecific resistance. Genes *Lr16*, *Lr13*, *Lr23*, and *Lr34* are likely present in a number of U.S. spring wheats (14). This will increase the selection for virulence in *P. triticina* populations to these resistance genes. Additional sources of unique nonspecific adult plant leaf rust resistance (20) may complement and enhance the leaf rust resistance present in U.S. spring wheats.

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