# Genetics of leaf rust resistance in the spring wheats 'Ivan' and 'Knudson' spring wheat

**J.A. Kolmer and L.M. Oelke**

**Abstract:** Leaf rust caused by *Puccinia triticina* is a common and widespread disease of wheat in North America and worldwide. Durable genetic resistance to leaf rust in wheat has been difficult to achieve, since the virulence of the leaf rust pathogen to specific leaf rust resistance genes in wheat is highly variable. 'Ivan' and 'Knudson', hard red spring wheat cultivars recently released by AgriPro®, are highly resistant to leaf rust. The objective of this study was to determine the identity of leaf rust resistance genes present in both cultivars. 'Ivan' and 'Knudson' were crossed with 'Thatcher', a cultivar susceptible to leaf rust; the $F_1$ plants were backcrossed to 'Thatcher'; and the $BCF_1$ plants (approximately 80 from each cross) were selfed to develop $BCF_2$ families. The $BCF_2$ families were tested as seedlings with different *P. triticina* isolates to determine the number and identity of leaf rust resistance genes that segregated in the $BCF_2$ families. Both the $BCF_3$ lines from selected resistant $BCF_2$ plants and near-isogenic lines of 'Thatcher' were tested with different *P. triticina* isolates to confirm the identity of the resistance genes. Selected $BCF_2$ families were also tested as adult plants in the greenhouse and field to identify genes for adult plant leaf rust resistance. 'Ivan' was determined to have genes *Lr16* and *Lr24* and 'Knudson' to have *Lr3*, *Lr10*, *Lr13*, *Lr16*, *Lr23*, and *Lr34*. 'Ivan' has been highly resistant because of the rarity of leaf rust isolates with virulence to *Lr16* and *Lr24,* while the combination of *Lr16*, *Lr23*, and *Lr34* accounts for the resistance in 'Knudson'.

*Key words:* durable resistance, inheritance of resistance, *Puccinia recondita* f. sp. *tritici*, *Puccina triticina,* specific resistance.

**Résumé :** La rouille des feuilles, causée par le *Puccinia triticina*, est une maladie du blé fréquente et répandue en Amérique du Nord et partout dans le monde. La résistance génétique durable à la rouille des feuilles chez le blé est un but difficile à atteindre puisque la virulence de l'agent pathogène de la rouille des feuilles envers des gènes spécifiques de résistance à la maladie varie beaucoup. 'Ivan' et 'Knudson', commercialisés depuis peu par AgriPro®, sont des cultivars de blé de force roux de printemps très résistants à la rouille des feuilles. Le but de la présente étude était d'identifier les gènes de résistance à la rouille des feuilles présents dans les deux cultivars. 'Ivan' et 'Knudson' ont été croisés avec le cultivar 'Thatcher' sensible à la rouille des feuilles; les plantes de la $F_1$ ont été rétrocroisées avec 'Thatcher'; et les plantes de la $BCF_1$ (environ 80 pour chaque croisement) ont été autofécondées pour développer les familles $BCF_2$. Les familles $BCF_2$ ont été testées au stade plantule avec divers isolats du *P. triticina* afin de déterminer le nombre et l'identité des gènes de résistance à la rouille des feuilles qui ségrèguent dans les familles $BCF_2$. Les lignées $BCF_3$ issues de plantes résistantes $BCF_2$ sélectionnées et les lignées quasi-isogéniques de 'Thatcher' ont été testées avec divers isolats du *P. triticina* afin de confirmer l'identité des gènes de résistance. Des familles $BCF_2$ sélectionnées ont aussi été testées au stade adulte dans des serres et au champ afin d'identifier les gènes de résistance des plantes adultes à la rouille des feuilles. Les gènes *Lr16* et *Lr24* ont été identifiés chez 'Ivan' et *Lr3*, *Lr10*, *Lr13*, *Lr16*, *Lr23* et *Lr34* l'ont été chez 'Knudson'. 'Ivan' a été très résistant à cause de la rareté des isolats de rouille des feuilles avec de la virulence envers *Lr16* et *Lr24*, alors que la combinaison des gènes *Lr16*, *Lr2* et *Lr34* est responsable de la résistance de 'Knudson'.

*Mots clés :* résistance durable, transmission de la résistance, *Puccinia recondita* f. sp. *tritici*, *Puccina triticina,* résistance spécifique.

## Introduction

Leaf rust, caused by *Puccinia triticina* Eriks., is a common and widespread disease of bread wheat (*Triticum aestivum* L.) in North America (Chester 1946) and worldwide (Roelfs et al. 1992). Leaf rust is first observed on winter wheat crops in the southern United States in March and April, and urediniospores of the fungus are blown north-

Accepted 1 May 2006.

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ward to the spring wheat region of Minnesota, North Dakota, and South Dakota, where infections are usually first observed in mid-June. Leaf rust can be particularly severe in years when the rust infections are well established when the wheat crop is at the tillering stage, before heading, and when the maximum daytime temperature exceeds 25 °C. Losses due to leaf rust can vary from less than 1% to over 25%, depending on the stage of crop development when the initial infection occurs, subsequent temperature and moisture conditions, and the resistance or susceptibility of the wheat cultivar (Chester 1946). In fungicide-sprayed plots at Morris, Minnesota, in 2004, commonly grown spring wheat cultivars suffered up to 15% yield loss due to leaf rust (J.A. Kolmer, unpublished data).

Genetic resistance is the preferred method to control leaf rust in wheat. To date, over 55 leaf rust resistance genes have been mapped to chromosome locations and assigned gene symbols (McIntosh et al. 2005). However, many of these genes no longer condition effective resistance, since virulent isolates of *P. triticina* develop very rapidly in response to the release of wheat cultivars with race-specific resistance genes (Kolmer 1999). Leaf rust is highly variable for virulence, as 40–50 virulence phenotypes of *P. triticina* are detected annually in the United States (Kolmer et al. 2005). Certain genes have conditioned effective resistance over a long period of time in wheat cultivars that have been extensively grown. 'Chris', a hard red spring wheat cultivar released in 1965, was developed by backcrossing leaf rust resistance gene *Lr34* from the Brazilian 'Frontana' into selections of 'Thatcher' (Tc) wheat that had been improved for stem rust resistance. *Lr34* has provided a moderate level of resistance in US spring wheat cultivars for over 40 years (Samborski 1985). The highly resistant spring wheat cultivars often have combinations of *Lr34* with other leaf rust resistance genes (Dyck 1993; Dyck et al. 1966; Oelke and Kolmer 2005; Samborski and Dyck 1982). Identification of *Lr* gene combinations in resistant wheat cultivars can aid the development of wheat germplasm with highly effective resistance.

'Ivan' and 'Knudson', released in 2000 and 2002, respectively, are hard red spring wheat cultivars developed by AgriPro® for the spring wheat region in the United States. In a recent study of hard red spring wheats from the United States, 'Ivan' and 'Knudson' were found to be highly resistant to leaf rust (Oelke and Kolmer 2004). In 2004, 'Knudson' was the second most commonly grown wheat in Minnesota. The pedigree of 'Ivan' is MN74103 / Success / 3 / W87-0069 // Bergen. The pedigree of 'Knudson' is Karl / Krona / 3 / Bergen // Erik / MN 73167. The objective of this study was to determine the genetic basis of resistance to leaf rust in'Ivan' and 'Knudson'.

## Materials and methods

Seeds of 'Ivan', 'Knudson', and the leaf rust susceptible Tc wheat were planted in 15 cm diameter pots filled with steamed topsoil and grown in a growth cabinet equipped with a mixture of fluorescent and incandescent bulbs on a 16 h light : 8 h dark photoperiod. At the second-leaf stage, the plants were treated with Nutricote® Type 100 fertilizer (13-13-13 N-P-K) (Plantco Inc., Brampton, Ont.). At heading, the Tc plants were emasculated, and pollen-shedding anthers from 'Ivan' and 'Knudson' were used to pollinate the Tc female parent. The $F_1$ plants from Tc × 'Ivan' and Tc × 'Knudson' were backcrossed as the male parent to Tc. Approximately 80 $BCF_1$ seeds were obtained from each of the two crosses. The $BCF_1$ plants were grown in a greenhouse and selfed to obtain $BCF_2$ families.

The isolates of *P. triticina* used in this study were collected from wheat in the United States and Canada. The isolates were selected for their low infection type (IT) to specific *Lr* genes in spring wheat. The four-letter avirulence/virulence designations for each isolate are based on the 12 differential lines used by Long and Kolmer (1989) and high and low IT to differential lines with genes *LrB*, *Lr10*, *Lr14a*, and *Lr18*. Urediniospores of individual isolates were stored at –80 °C prior to use and were heat-shocked at 45 °C for 5 min prior to inoculation. Approximately 200 µg of urediniospores was mixed with 350 µL of Soltrol™ 170 oil (Phillips Petroleum, North Borger, Tex.) in a size 00 gelatin capsule (Gallipot Inc., St. Paul, Minn.). Plants were inoculated by spraying the spore–oil mixture with an atomizer attached to a positive air pressure line. Seedlings of 'Ivan', 'Knudson', and seven lines of Tc wheat near isogenic for leaf rust resistance genes were inoculated with eight isolates of *P. triticina* (Table 1). Additional isolates were used in subsequent tests.

Fifteen seeds from each $BCF_2$ family were planted in one 3.5 $cm^2$ plastic pot filled with vermiculite (Sunshine Strong-Lite Medium Vermiculite Premium Grade, JR Johnson Horticultural Supplies, St. Paul, Minn.) and placed in a plastic tray, with six pots per tray. The Tc lines were planted in clumps of four to eight seeds at the corners of 3.5 $cm^2$ plastic pots, with six pots per tray. All seedlings were treated at emergence with soluble 20-20-20 N-P-K fertilizer (Spectrum Group, St. Louis, Mo.), watered daily, and grown for 8 d at approximately 20 ± 3 °C and with 16 h supplemental light in a greenhouse. The seedlings were inoculated 8 d after planting at full emergence of primary leaves. The pots were left on a bench for 1 h after inoculation to allow oil to evaporate from leaf surfaces and then placed in a mist chamber at 18 °C for 24 h. The seedlings were then removed from the mist chamber and returned to the greenhouse bench.

The seedlings were evaluated for IT 10–12 d after inoculation in greenhouse tests. ITs were classified according to the 0–4 scale used by Long and Kolmer (1989): 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 = intermediate-sized uredinia lacking chlorosis; 4 = large uredinia lacking chlorosis. Designations "+" and "–" indicate uredinia that were larger and smaller than normal, respectively. ITs from 0 to $2^+$ were considered low, and ITs from 3 to 4 were considered high. Intermediate ITs (IT 23) had a mixture of small and intermediate-sized uredinia. In the $BCF_2$ populations, the ratio of families that segregated for plants with low ITs 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 $\chi^2$ test (Steel

**Table 1.** Seedling infection types, adult-plant infection types, and field rust severity and response of 'Ivan', 'Knudson', and 'Thatcher' wheat lines near isogenic for leaf rust resistance to isolates of *Puccinia triticina*.

| Wheat line/ cultivar | Isolate | | | | | | | | Field rust severity and response* |
|---|---|---|---|---|---|---|---|---|---|
| | BBBD 1-1 | MFBJ 94-2 | THBJ 588 | TDBJ82 | MJBJ406 | SBDG1-2 | MHDS237 | TLGF218 | |
| 'Ivan' | ; | ; | ; | $22^+$ | 23 | 0; | 0; | 0; | 5 R |
| 'Knudson' | 0; | ;1 | 2 | $22^+$ | $22^+$ | 0; | $;2^-$ | ;1 | 5 R |
| 'Thatcher' | $33^+$ | $33^+$ | $33^+$ | $3^+$ | $3^+$ | $3^+$ | $33^+$ | 3 | 50–80 S |
| RL 6003 *Lr3* | ; | $33^+$ | $33^+$ | $33^+$ | $3^+$ | ; | 3 | 3 | 70–90 S |
| RL 6005 *Lr16* | $22^-$ | $;22^+$ | $2^+3$ | $22^+$ | $2^+3$ | $22^-$ | $2^+3$ | 2 | 30–50 MR MS |
| RL 6064 *Lr24* | ; | $33^+$ | ; | $33^+$ | $3^+$ | 0; | ;1 | ; | 5–20 MR MS |
| RL 6004 *Lr10* | $;22^-$ | $33^+$ | $33^+$ | $33^+$ | $3^+$ | $3^+$ | 3 | ;2 | 70–90 S |
| RL 6012 *Lr23* | 23 | 23 | $2^+3^+$ | $2^+3^+$ | $22^+$ | ; | $;22^-$ | $2^+3$ | 5–20 R MR |
| RL 4031 *Lr13* | $3^+/;1^{-\dagger}$ | $3^+$ | $3^+$ | $3^+$ | $3^+$ | 3+ | 3+ | 3+ | 40–50 S |
| RL 6058 *Lr34* | $33^+/23^\dagger$ | 3 | 3 | $33^+$ | $3^+$ | $33^+$ | $3^+$ | 3 | 10–40 MR MS |

**Note:** Infection types (0–4 scale; Long and Kolmer 1989) are as follows: 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 = intermediate-sized uredinia without chlorosis; 4 = large uredinia lacking chlorosis. Designations "+" and "–" indicate uredinia that are larger and smaller than normal, respectively.

*A mixture of common isolates of *P. triticina* was used to inoculate field plots: *R* denotes resistant with very small uredinia surrounded by necrosis; MR denotes moderately resistant with intermediate-sized uredinia surrounded by necrosis; MS denotes moderately susceptible with intermediate-sized to large uredinia surrounded by chlorosis; and S denotes large uredinia lacking chlorosis or necrosis. Severity ratings are estimated according to the Cobb scale (Peterson et al. 1948).

†Adult-plant infection type.

et al. 1997). The $\chi^2$ test for independence in a contingency table was used to determine if segregations of $BCF_2$ families for resistance to different *P. triticina* isolates were significantly associated. If the segregation of $BCF_2$ families to one isolate was highly associated with the segregation of the same families to other isolates, resistance in the $BCF_2$ families to these isolates was then conditioned by the same gene(s). Selected resistant $BCF_2$ plants were transplanted into 15 cm diameter pots and grown out to obtain the $BCF_3$ seed. The $BCF_3$ lines and selected near-isogenic lines of Tc wheat 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 in the seedling tests 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, we ensured that 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 diameter 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 with 16 h of supplemental light per day. Three weeks after planting, plants were trimmed to three tillers each. When the plants had fully headed, the flag leaves were inoculated with isolate BBBD urediniospores suspended in Soltrol® 170 oil. The Tc lines with *Lr13* (RL 4031), *Lr34* (RL 6058), and Tc were also planted and inoculated with isolate BBBD as adult plants for comparison of ITs. The pots were left on a bench for 1 h after inoculation to allow oil to evaporate and 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 1 h and then placed on a greenhouse bench. Flag leaves of the $BCF_2$ plants and Tc lines were scored for IT 14 d after inoculation on the 0–4 scale previously described. Resistant plants were selected and progeny tested as $BCF_3$ lines with different isolates to confirm the identity of the postulated genes.

The $BCF_2$ families that were homozygous susceptible to isolate BBBD in seedling tests were also evaluated in a field rust nursery at St. Paul, Minnesota, in 2004 for segregation of genes for adult plant leaf rust resistance. Approximately 40–50 seeds of each family and the near-isogenic Tc lines were planted in a 2 m row, spaced 30 cm apart. Rows of a mixture of 'Max', 'Little Club', 'Thatcher', and 'Morocco', four leaf rust susceptible wheat cultivars, were planted at an angle perpendicular to the entries. The spreader rows were inoculated with a mixture of common leaf rust isolates from the Great Plains region in 2003 (Kolmer et al. 2005) when the spreader rows had fully tillered and heads had fully emerged. Urediniospores suspended in Soltrol® 170 oil were sprayed on the spreader rows using a backpack mist blower with a modified spray nozzle. Leaf rust infections that occurred naturally were also observed in rust nursery plots in 2004.

The severity ratings (percent infection of individual plants) for the adult plants in the field rust nursery test were based on the modified Cobb scale (Peterson et al. 1948). Between 5 and 10 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 intermediate-sized uredinia surrounded by necrosis; MS = moderately susceptible with intermediate-sized to large uredinia surrounded by chlorosis; S = large uredinia lacking chlorosis or necrosis. The $BCF_2$ families and Tc lines were rated for leaf rust when Tc had leaf rust severity of at least 70%.

## Results

'Ivan' had low ITs of 0; to ; (fleck) to all isolates that were tested (Table 1) except TDBJ and MJBJ, both of which

**Table 2.** Segregation for leaf rust resistance in greenhouse tests of seedlings of $BCF_2$ families of 'Thatcher' × 'Ivan'.

| Leaf rust isolate | *Lr* gene(s) detected | No. of families* | | Expected ratio | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|
| | | Seg. | Susc. | | | |
| BBBD 1-1 | *Lr16*, *Lr24* | 54 | 25 | 3:1 | 1.52 | 0.22 |
| THBJ 588 | *Lr24* | 34 | 45 | 1:1 | 1.26 | 0.26 |
| MHDS 237 | *Lr24* | 18 | 19 | 1:1 | 0.00 | 1.00 |
| MFBJ 94-1 | *Lr16* | 20 | 58 | 1:3 | 0.00 | 1.00 |
| SBDG 1-2 | *Lr16*, *Lr24* | 46 | 29 | 5:3 | 0.01 | 0.92 |

*"Seg." denotes families segregating for resistant and susceptible plants; "Susc." denotes homozygous susceptible families.

**Table 3.** Segregation for leaf rust resistance in greenhouse tests of seedlings of $BCF_2$ families of 'Thatcher' × 'Knudson'.

| Leaf rust isolate | *Lr* gene(s) detected | No. of families* | | Expected ratio | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|
| | | Seg. | Susc. | | | |
| BBBD 1-1 | *Lr3*, *Lr10*, *Lr16* | 67 | 11 | 7:1 | 0.07 | 0.79 |
| TDBJ 82 | *Lr16* | 30 | 48 | 1:1 | 3.70 | 0.05 |
| TLGF 218 | *Lr10*, *Lr16* | 52 | 16 | 3:1 | 0.01 | 0.92 |
| MHDS 237 | *Lr23* | 14 | 64 | 1:3 | 1.71 | 0.19 |

*"Seg." denotes families segregating for resistant and susceptible plants; "Susc." denotes homozygous susceptible families.

were virulent to Tc*Lr24*. 'Knudson' had low ITs of 0; to ;1 to all isolates except THBJ, TDBJ, MJBJ, and MHDS. These isolates were all virulent to Tc*Lr16*, except for TDBJ. In inoculated field plots, both 'Ivan' and 'Knudson' had leaf rust ratings of 5 R.

### $BCF_2$ families of Tc × 'Ivan'

In seedling tests to isolate BBBD, the $BCF_2$ families of Tc × 'Ivan' segregated in a 3:1 ratio for families that were segregating for resistant and susceptible plants to families that were homozygous susceptible (Table 2). This indicated that two genes controlled seedling resistance in 'Ivan' to isolate BBBD. To isolates THBJ and MHDS, both of which produced very low ITs on Tc*Lr24* and higher ITs on Tc*Lr16*, the $BCF_2$ families segregated in a 1:1 ratio, which indicated that a single gene in 'Ivan' controlled seedling resistance to these two isolates. When tested with isolate MFBJ, which produced a high IT on Tc*Lr24* and a lower IT on Tc*Lr16,* the $BCF_2$ families segregated in a 1:3 ratio for segregating and homozygous susceptible families, respectively. This indicated that a single gene conditioned resistance to MFBJ and that a second gene suppressed the expression of the resistance gene. To isolate SBDG, which produced low ITs on both *Lr16* and *Lr24,* the $BCF_2$ families segregated in a 5:3 ratio, indicating that resistance was conditioned by two genes and that a third gene suppressed the expression of one of the resistance genes. The segregation of the $BCF_2$ families to BBBD was highly associated with the segregation of the same families to isolates MFBJ, THBJ, SBDG, and MHDS ($P < 0.01$), which indicated that the same gene(s) conditioned resistance to these isolates. Likewise, the segregation of the families to isolate SBDG was highly associated with the segregation of the families to isolates BBBD, MFBJ, THBJ, and MHDS ($P < 0.01$). The segregation of the $BCF_2$ families to isolates THBJ and MFBJ was independent ($P = 0.81$), as was the segregation to isolates MFBJ and MHDS ($P = 0.74$).

### $BCF_2$ families of Tc × 'Knudson'

The $BCF_2$ families of Tc × 'Knudson' segregated in a 7:1 ratio for segregating homozygous to susceptible families when tested with isolate BBBD (Table 3), which indicated that three genes in 'Knudson' conditioned resistance to this isolate. To isolate TDBJ, which produced a low IT on Tc*Lr16*, the $BCF_2$ families segregated in a 1:1 ratio, which indicated that a single gene in 'Knudson' conditioned resistance to isolate TDBJ. To isolate TLGF, which produced low ITs on both Tc*Lr10* and Tc*Lr16*, the $BCF_2$ families segregated in a 3:1 ratio, indicating that two genes in 'Knudson' conditioned resistance to this isolate. To isolate MHDS, which produced a low IT on seedling plants with *Lr23* (Table 1), the $BCF_2$ families segregated in a 1:3 ratio. This indicated that a single gene conditioned resistance to MHDS and that a second independent gene suppressed the expression of the resistance gene. The segregation of the $BCF_2$ families to isolate BBBD was highly associated ($P < 0.01$) with the segregation of the families to isolates TDBJ and TLGF, which indicated that the same gene(s) in 'Knudson' conditioned resistance to these isolates. Segregations of the $BCF_2$ families to isolates TDBJ and TLGF were also highly associated ($P < 0.01$), which indicated that the same gene(s) conditioned resistance to both isolates. Segregations of the families to isolates MHDS and BBBD, MHDS and TDBJ, and MHDS and TLGF were all independent ($P > 0.05$), which indicated that different gene(s) in 'Knudson' conditioned resistance to these isolates.

### $BCF_3$ lines

The selected $BCF_3$ lines derived from Tc × 'Ivan' and Tc × 'Knudson' were tested as seedlings with the leaf rust isolates listed in Tables 4 and 5, respectively. Lines 8-2 and 51-1 derived from Tc × 'Ivan' were postulated to have *Lr16*, since both lines had IT 23 or 3 to isolates MJBJ and THBJ, and IT from $11^+$ to 2 to isolates MFBJ and MCDS (Table 4). Lines 30-1 and 38-1 derived from Tc × 'Ivan' were postulated

**Table 4.** Seedling infection types of $BCF_3$ lines of 'Thatcher' × 'Ivan' to isolates of *Puccinia triticina*.

| Wheat line | Isolate | | | | *Lr* gene(s) detected |
|---|---|---|---|---|---|
| | MFBJ 94-2 | MJBJ 406 | THBJ 588 | MCDS 520 | |
| 'Thatcher' | 3 | 3 | 3 | 3 | |
| RL 6005 *Lr16* | ;$22^-$ | $2^+3^+$ | $22^+$ | 12 | |
| RL 6064 *Lr24* | 3 | 3 | ;$1^-$ | 0; | |
| Tc*2 / 'Ivan' 8-2 | 2 | 3 | 3 | 2 | *Lr16* |
| Tc*2 / 'Ivan' 51-1 | ;$22^-$ | 23 | 23 | $11^+$ | *Lr16* |
| Tc*2 / 'Ivan' 30-1 | $3^+$ | $3^+$ | ;$1^-$ | 0; | *Lr24* |
| Tc*2 / 'Ivan' 38-1 | $3^+$ | $3^+$ | ;$1^-$ | 0; | *Lr24* |
| Tc*2 'Ivan' 11-1 | ;2 | 3 | ;$2^-$ | 0; | *Lr16*, *Lr24* |

**Note:** For infection types see Table 1.

**Table 5.** Seedling infection types of $BCF_3$ lines of 'Thatcher' × 'Knudson' to isolates of *Puccinia triticina.*

| Wheat line | Isolate | | | | | | | *Lr* gene(s) detected |
|---|---|---|---|---|---|---|---|---|
| | MJBJ 406 | SBDG 1-2 | MHDS 237 | BBBD 1-1 | KFBJ 94-1 | NBGS 629 | TLGF 218 | |
| 'Thatcher' | 3 | $33^+$ | $3^+$ | $33^+$ | $33^+$ | $3^+$ | 33+ | |
| RL 6003 *Lr3* | 3 | ; | $3^+$ | ; | 3 | ; | 3+ | |
| RL 6004 *Lr10* | 3 | $3^+$ | $3^+$ | ; | $33^+$ | $3^+$ | ; | |
| RL 6005 *Lr16* | 3 | ;$1^-$ | $2^+3$ | ;$1^-$ | ;$1^-$ | ;$1^-$ | ;1 | |
| RL 6012 *Lr23* | $2^+3$ | ; | ; | ;$22^+$ | $2^+3$ | $2^+3$ | $2^+3$ | |
| Tc*2 / 'Knudson' 20-1 | $2^+3^+$ | ; | $3^+$ | ; | $2^+3$ | ; | $3^+$ | *Lr3* |
| Tc*2 / 'Knudson' 27-1 | $33^+$ | ; | $3^+$ | ; | $2^+3$ | ; | 3 | *Lr3* |
| Tc*2 / 'Knudson' 6-1 | $3^+$ | ; | $3^+$ | ; | 3 | ; | ; | *Lr3*, *Lr10* |
| Tc*2 / 'Knudson' 27-2 | $33^+$ | ; | $3^+$ | ; | $33^+$ | ; | ; | *Lr3*, *Lr10* |
| Tc*2 / 'Knudson' 52-1 | $2^+3^+$ | ; | 3 | ;$1^-$ | ;$1^-$ | ;$1^-$ | ;$1^-$ | *Lr16* |
| Tc*2 / 'Knudson' 66-1 | $2^+3$ | ; | $2^+3$ | ; | ;$1^-$ | ; | ;$1^-$ | *Lr16* |
| Tc*2 / 'Knudson' 53-1 | $23^+$ | ; | ; | ; | ;$1^+$ | ; | ;1 | *Lr16*, *Lr23* |
| Tc*2 / 'Knudson' 56-1 | $22^+$ | ; | ;$1^-$ | ; | ;$1^-$ | ;$1^-$ | ;$1^-$ | *Lr16*, *Lr23* |

**Note:** For infection types see Table 1.

to have *Lr24*, since both had IT $3^+$ to isolates MFBJ and MJBJ and low IT of ;$1^-$ and 0; to isolates THBJ and MCDS. Line 11-1 derived from Tc × 'Ivan' had low ITs to isolates MFBJ and MCDS and high IT to isolate MJBJ and IT ;$2^-$ to THBJ, which indicated that this line had both genes *Lr16* and *Lr24*.

Lines 20-1 and 27-1 derived from Tc × 'Knudson' were postulated to have *Lr3*, since both had IT from $2^+3$ to $3^+$ to isolates MJBJ, MHDS, KFBJ, and TLGF and low IT of ; (fleck) to isolates BBBD, SBDG, and NBGS, which are avirulent to *Lr3* (Table 5). Lines 6-1 and 27-2 derived from Tc × 'Knudson' were postulated to have *Lr3* and *Lr10*, since both lines had low IT to isolates BBBD, TLGF, NBGS, and SBDG and high IT to the other isolates tested. Lines 52-1 and 66-1 derived from Tc × 'Knudson' were postulated to have *Lr16*, since both lines had IT from $2^+$ to 3 to isolates MJBJ and MHDS and low IT from ; to ;$1^-$ to the other isolates. Lines 53-1 and 56-1 derived from Tc × 'Knudson' were postulated to have *Lr16* and *Lr23*, since both had IT $22^+$ to isolate MJBJ and low IT from ; to ;$1^+$ to all other isolates.

## Tests of adult plants

Twenty-two $BCF_2$ families from Tc × 'Ivan' that were homozygous susceptible to isolate BBBD in the seedling tests were evaluated for adult-plant resistance in greenhouse tests with isolate BBBD. Of the 235 $BCF_2$ adult plants that were tested, none had ITs similar to those of the Tc lines with *Lr13* (IT ;$1^-$) or *Lr34* (IT 23, with few uredinia). All the $BCF_2$ plants had IT from 3 to $3^+$, similar to those of the susceptible Tc controls. Twenty-five $BCF_2$ families that were homozygous susceptible to isolate BBBD were evaluated for adult-plant resistance in field plots at St. Paul in 2004. All of the 25 families were homozygous susceptible, which indicated that 'Ivan' did not have any effective genes for adult-plant resistance. In a greenhouse test, adult $F_2$ plants of Tc*Lr34* × 'Ivan' were tested for segregation of resistance with isolate MJBJ, which had IT ;$22^-$ to adult plants of 'Ivan', IT $2^-3^-$ on Tc*Lr34*, and IT $3^+4$ to Tc. The $F_2$ plants segregated 143 resistant (IT from ; to 23) to 15 susceptible (IT $3^+4$), which fit a 15:1 ratio ($\chi^2 = 2.82$, $P = 0.09$). The $F_2$ segregation data indicated that *Lr34* was not present in 'Ivan', and that another gene, most likely *Lr16*, conditioned resistance in adult plants of 'Ivan' to isolate MJBJ. Seedling ITs to different *P. triticina* isolates of $F_3$ lines derived from Tc*Lr34* × 'Ivan' indicated that *Lr16* was the second gene segregating in the $F_2$ population (data not shown). Previous data have also shown that lines with *Lr16* can have high IT in seedlings and lower IT at the adult-plant stage to the same isolate (Kolmer 1992). The $F_2$ plants from Tc × 'Ivan' with IT; most likely had *Lr16* and *Lr34*. *Lr34* has previously been shown to interact with *Lr16* in adult plants, for a lower IT (German and Kolmer 1992).

Eight $BCF_2$ families from Tc × 'Knudson' that were homozygous susceptible to isolate BBBD in the seedling tests were evaluated for adult-plant resistance in greenhouse tests

**Table 6.** Adult-plant infection types $BCF_3$ lines of 'Thatcher' × 'Knudson' to specific isolates of *Puccinia triticina*.

| Wheat line | Isolate | | | *Lr* gene(s) detected |
|---|---|---|---|---|
| | TCTD 190 | MCRK 267 | MBBJ 72 | |
| 'Thatcher' | $3^+$ | $3^+$ | 3+ | |
| RL4031 *Lr13* | ; | $3^+$ | ;1 | |
| RL6058 *Lr34* | 2 f* | 23 f | 2 f | |
| Tc*2 / 'Knudson' 4-2 | 0; | $3^+$ | 0; | *Lr13* |
| Tc*2 / 'Knudson' 63-2 | 23 f | 23 f | 23 f | *Lr34* |
| Tc*2 / 'Knudson' 67-1 | $0;2^-$ | 23 f | $;1^-$ | *Lr13*, *Lr34* |

**Note:** For infection types see Table 1.
*Fewer pustules than 'Thatcher'.

with isolate BBBD. Of the 78 $BCF_2$ adult plants that were tested, 40 had ITs from ;12 to 23 with few pustules, and 38 had IT $3^+4$. The 40:38 segregation fit a 39:25 ratio ($\chi^2$ = 3.05, $P > 0.05$), which is expected for the segregation of two genes in a random $BCF_2$ population. A 39:25 ratio is expected for segregation of two genes in a random $BCF_2$ population, since 25% of the $BCF_1$ plants would produce $BCF_2$ progeny in a 15 resistant : 1 susceptible (15R:1S) ratio, 50% of the $BCF_1$ plants would produce $BCF_2$ progeny in a 3R:1S ratio, and 25% of the $BCF_1$ plants would produce $BCF_2$ progeny that would all be susceptible. This model also assumes that plants heterozygous for resistance gene(s) can be distinguished from plants that are homozygous susceptible. Adult plants of Tc*Lr13* had IT ;12, those of Tc*Lr34* had IT 23 with few pustules, and those of Tc had IT $3^+4$. Resistant $BCF_2$ plants were selected and progeny tested as $BCF_3$ adult plants in a greenhouse test with the isolates listed in Table 6. The $BCF_3$ line 4-2 derived from Tc × 'Knudson' had high IT to isolate MCRK and low ITs to isolates TCTD and MBBJ, which indicated that this line had *Lr13*. Line 63-2 derived from Tc × 'Knudson' was postulated to have *Lr34*, since this line had IT 23 with few uredinia to all three isolates tested. Line 67-1 was postulated to have *Lr13* and *Lr34*, since it had low IT to isolates TCTD and MBBJ and IT 23 to isolate MCRK. Nine $BCF_2$ families derived from Tc × 'Knudson' that were homozygous susceptible to isolate BBBD in the seedling tests were tested for adult-plant resistance in the inoculated field plots in 2004. Seven of the families segregated for resistance with resistant ratings of 10–50 MRMS, and two families were homozygous susceptible with ratings of 50–90 S. This fit a 3:1 ratio ($\chi^2$ = 0.04, $P$ = 0.84), which indicted that two genes segregated for resistance in these families. Since the Tc line with *Lr13* did not condition effective resistance in the field plots (Table 1), the two genes that expressed resistance in these selected $BCF_2$ families derived from Tc × 'Knudson' were most likely *Lr23* and *Lr34*. The Tc line with *Lr23* had a rating of 5–20 R MR and the Tc line with *Lr34* had a rating of 10–40 MR MS in field plots in 2004.

## Discussion

'Ivan' was determined to have leaf rust resistance genes *Lr16* and *Lr24*. 'Knudson' was determined to have *Lr3*, *Lr10*, *Lr13*, *Lr16*, *Lr23*, and *Lr34*. Although both cultivars have been highly resistant to leaf rust since their release in 2000 and 2002, respectively, they were found to have very different leaf rust genotypes, with only *Lr16* in common.

The high level of resistance in 'Ivan' is due to the rarity of leaf rust phenotypes with virulence to both *Lr16* and *Lr24*. In 2003 only two isolates (0.03% of the total population) with virulence to both *Lr16* and *Lr24* were detected in the United States (Kolmer et al. 2005). Virulence to *Lr24* was present throughout the United States in 11% of isolates in 2003, and virulence to *Lr16* was present in 17% of all isolates. The resistance in 'Ivan' will inevitably decrease if isolates with virulence to both *Lr16* and *Lr24* increase, especially since 'Ivan' did not have the adult-plant resistance gene *Lr34*. The hard red winter wheat 'Arapahoe', released in 1989, was postulated to have *Lr16* and *Lr24* (McVey and Long 1993), and the winter wheat 'Millennium' (J.A. Kolmer, unpublished data) may also have these two genes. However, we are not aware of any other US wheat cultivars with this combination of resistance genes. Although *Lr24* is present in soft red and hard red winter wheat cultivars in the United States (Kolmer 2003; McVey and Long 1993), 'Ivan' is the only spring wheat cultivar known to have *Lr24* in the United States. 'Ivan' may be useful as a parent, since *Lr24* is the same gene as *Sr24* (McIntosh et al. 2005), which gives resistance to the stem rust race TTKS from East Africa, and which is virulent to many other US spring wheat cultivars (Jin and Singh 2006). However since 'Ivan' did not have *Lr34*, progeny lines derived from 'Ivan' may not have any effective genes for adult-plant leaf rust resistance.

A gene that suppressed the expression of *Lr16* to isolates MFBJ and SBDG appeared to be segregating in the $BCF_2$ families derived from 'Ivan'. The origin of the suppressor gene is not obvious. If this gene had been present in 'Ivan', in combination with *Lr16* and *Lr24*, we would not have observed the low ITs in seedling tests of 'Ivan' to all isolates that have low ITs to Tc*Lr16* and Tc*Lr24*. The suppressor gene could also be present in Tc; however, this is unlikely, since suppression of *Lr16* resistance in lines derived from Tc has not been reported previously (Dyck 1989; Dyck et al. 1966; Oelke and Kolmer 2005). The action of the suppressor gene appeared to be isolate-specific, as *Lr16* was fully expressed in the segregating $BCF_2$ families to isolate BBBD.

The high level of resistance to leaf rust present in 'Knudson' is due to the combination of *Lr16*, *Lr23*, and *Lr34*. The Tc lines with these genes all had effective resistance in the field plots in St. Paul in 2004. *Lr3* and *Lr10*, also present in

'Knudson', would not condition any effective resistance, since nearly every leaf rust isolate in the United States is virulent to both of these genes (Kolmer et al. 2005). The adult-plant gene *Lr13*, present in many spring wheat cultivars, no longer conditions effective resistance in the northern Great Plains region. The adult-plant gene *Lr34* has conditioned effective resistance in US spring wheat cultivars since the release of 'Chris' in 1965, as isolates that are completely virulent to this gene have never been found (Kolmer et al. 2005). *Lr23* is difficult to detect in seedling tests, since it is highly temperature-sensitive (Dyck and Johnson 1983). At normal greenhouse temperatures of 18–22 °C, the Tc line with *Lr23* can produce ITs varying from 2 to $3^+$ with the same leaf rust isolate, depending on the ambient temperature and lighting conditions. Most of these isolates produce lower ITs from ; to ;1 on Tc*Lr23* in growth-cabinet tests at 25 °C. A few isolates, such as SBDG and MHDS, consistently produce lower ITs from ; to $;2^-$ on Tc*Lr23* in greenhouse tests. *Lr23* can also be difficult to detect in segregating populations derived from Tc, since Tc has a gene that suppresses the expression of *Lr23* (Dyck 1982). The segregation of the $BCF_2$ families derived from 'Knudson' to isolate SBDG indicated that a second gene suppressed the expression of *Lr23*.

Although isolates such as THBJ, MHDS, and MJBJ are present in the United States, *Lr16* still conditions effective resistance, especially in combination with other genes such as *Lr34*. The spring wheat 'Norm' was determined to have genes *Lr1*, *Lr10*, *Lr23*, *Lr16*, *Lr13*, and *Lr34* and has been highly resistant since release in 1992 (Oelke and Kolmer 2005). Wheat cultivars with the combination of *Lr16*, *Lr23*, and *Lr34* have displayed high levels of durable leaf rust resistance in the US spring wheat region.

## References


**Chester, K.S.** 1946. The nature and prevention of the cereal rusts as exemplified in the leaf rust of wheat. Chronica Botanica. Waltham, Mass.

**Dyck, P.L.** 1982. Genetic inhibition of expression of resistance gene *Lr23* in wheat to *Puccinia recondita*. Can. J. Plant Sci. 62: 219–220.

**Dyck, P.L.** 1989. The inheritance of leaf rust resistance in wheat cultivars Kenyon and Buck Manantial. Can. J. Plant Sci. 69: 1113–1117.

**Dyck, P.L.** 1993. The inheritance of leaf rust resistance in the wheat cultivar Pasqua. Can. J. Plant Sci. 73: 903–906.

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

**Dyck, P.L., Samborski, D.J., and Anderson, R.G.** 1966. Inheritance of adult-plant leaf rust resistance derived from the common wheat varieties Exchange and Frontana. Can. J. Genet. Cytol. 8: 665–671.

**German, S.E., and Kolmer, J.A.** 1992. Effect of gene *Lr34* in the enhancement of resistance to leaf rust of wheat. Theor. Appl. Genet. 84: 97–105.

**Jin, Y., and Singh, R.P.** 2006. Resistance in U.S. wheat to recent East African isolates of *Puccinia graminis* f. sp. *tritici* with virulence to resistance gene *Sr31*. Plant Dis. 90: 476–480.

**Kolmer, J.A. 1992.** Enhanced leaf rust resistance in wheat conditioned by resistance gene pairs with *Lr13*. Euphytica, 61: 123–130.

**Kolmer, J.A.** 1999. Virulence dynamics, phenotypic diversity, and virulence complexity in two populations of *Puccinia triticina* in Canada from 1987–1997. Can. J. Bot. 77: 333–338.

**Kolmer, J.A.** 2003. Postulation of leaf rust resistance genes in selected soft red winter wheats. Crop Sci. 43: 1266–1274.

**Kolmer, J.A., Long, D.L., and Hughes, M.E.** 2005. Physiological specialization of *Puccinia triticina* on wheat in the United States in 2003. Plant Dis. 89: 1201–1206.

**Long, D.L., and Kolmer, J.A.** 1989. A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopathology, 79: 525–529.

**McIntosh, R.A., Yamazaki, Y., Devos, K.M., Dubcovsky, J., Rogers, J., and Appels, R.** 2005. Catalogue of gene symbols for wheat. KOMUGI Integrated Wheat Science Database. Available online at http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbo/ClassList.jsp [accessed 13 June 2006].

**McVey, D.V., and Long, D.L.** 1993. Genes for leaf rust resistance in hard red winter wheat cultivars and parental lines. Crop Sci. 33: 1373–1381.

**Oelke, L.M., and Kolmer, J.A.** 2004. Characterization of leaf rust resistance in hard red spring wheat cultivars. Plant Dis. 88: 1127–1133.

**Oelke, L.M., and Kolmer, J.A.** 2005. Genetics of leaf rust resistance in spring wheat cultivars Norm and Alsen. Phytopathology, 95: 773–778.

**Peterson, R.F., Campbell, A.B., and Hannah, A.E.** 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can. J. Res. Sect. C, 26: 496–500.

**Roelfs, A.P., Singh, R.P., and Saari, E.E.** 1992. Rust diseases of wheat: concepts and methods of disease management. CIMMYT [International Maize and Wheat Improvement Center], Mexico, D.F., Mexico.

**Samborski, D.J.** 1985. Wheat leaf rust. *In* The cereal rusts. Vol. 2. *Edited by* A.P. Roelfs and W.R. Bushnell. Academic Press, Orlando, Fla. pp. 39–59.

**Samborski, D.J., and Dyck, P.L.** 1982. Enhancement of resistance to *Puccinia recondita* by interactions of resistance genes in wheat. Can. J. Plant Pathol. 4: 152–156.

**Steel, R.G.D., Torrie, J.H., and Dickey, D.A.** 1997. Principles and procedures of statistics: a biometrical approach. 3rd ed. McGraw–Hill, Boston, Mass.