Genes for Adult-Plant Resistance to Leaf Rust in Soft Red Winter Wheat

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ABSTRACT

Wamishe, Y. A., and Milus, E. A. 2004. Genes for adult-plant resistance to leaf rust in soft red winter wheat. Plant Dis. 88:1107-1114.

Host plant resistance in wheat (*Triticum aestivum*) has been the principal means of managing leaf rust caused by *Puccinia triticina*. The need for durable resistance has changed the focus from the use of seedling resistance to adult-plant resistance. The objectives of this study were to determine the genetic basis for adult-plant resistance and to determine the most effective method to identify adult-plant resistance genes *Lr12*, *13*, and *34* among 116 contemporary soft red winter wheat cultivars and breeding lines. Adult-plant resistance was detected by inoculating flag leaves with a race that was virulent on seedlings. Approximately 90% of the lines expressed resistance under controlled conditions. It was postulated that the adult-plant resistance in 67 lines was due in part to either *Lr12*, *13*, or *34*; the adult-plant resistance detected in 17 lines was attributed to *Lr12* based on a distinctive low infection type very similar to that on the isolate TeLr12; the adult-plant resistance in 27 lines was attributed to *Lr34*, as all of these lines expressed a "leaf tip necrosis" in the field (a phenotype controlled by a gene known to be tightly linked with *Lr34*); and the adult-plant resistance in 23 lines was attributed to *Lr13* based on a high infection type at 18.1°C and low infection type at 25.5°C with one or more pathogen isolates that were virulent on *Lr13* at 18.1°C and avirulent on *Lr13* at 25.5°C. The adult-plant resistance detected in the remaining 40% of the lines was due to one or more unidentified genes for adult-plant resistance. In a 4-year field study at several locations, nearly 29% of the lines were resistant at all locations, no line was susceptible at all locations, and only 30% of the lines were susceptible at one or more locations. Given that many of the lines in this study were resistant to all known races of *P. triticina* before being released as cultivars, the high frequency of adult-plant resistance in this study demonstrates that adult-plant resistance can be incorporated even in the presence of highly effective seedling resistance.

Soft red winter wheat (*Triticum aestivum* L.) is grown in the eastern United States where the environment is often favorable for the development of leaf rust caused by *Puccinia triticina* Eriks. (Syn.: *Puccinia recondita* Rob. ex. Desm. f. sp. *tritici*). Use of resistant cultivars has been the principal and most economical means of managing leaf rust. Wheat lines susceptible to a given race at the seedling stage but resistant to that race at the adult stage are considered to have adult-plant resistance (1,2). Some genes for adult-plant resistance have been associated with slow rusting (33) and partial resistance (29) and may have the potential to provide durable leaf rust resistance (8,26).

Genes *Lr12*, *13*, and *34* are the most likely designated genes providing adult-plant resistance to leaf rust in North America (26). *Lr12* has been used widely in North America and is expressed on leaves of adult plants as infection types ;1 or 2 with prominent necrosis (21). Virulence to *Lr12* has been common in North America

(17,20,33), and therefore, *Lr12* alone is not likely to provide adequate resistance.

Gene *Lr13* was initially described as being expressed only in the adult stage (11); however, Dyck and Johnson (7) detected *Lr13* at the seedling stage and observed increased resistance with increasing temperature. Pretorius et al. (25) detected *Lr13* in seedlings at 25.5°C but not at 18.1°C when inoculated with specific isolates. Low infection type for *Lr13* was reported as ;1 through X to 3 on seedlings depending on factors such as pathogen race, environmental conditions, and background genotype (21). Virulence to *Lr13* was common after wheat cultivars with *Lr13* were grown in North America (16), and *Lr13* alone is not likely to provide an adequate level of resistance (16,26). *Lr13* is tightly linked with *Ne2m*, a gene for progressive hybrid necrosis (31). Most wheat lines with *Ne2m* have *Lr13*, and all lines with *Lr13* appear to have *Ne2m* (21). The presence of *Lr13* in wheat lines was postulated by crossing lines with the Australian spring wheat cultivar Spica that was known to have *Ne1*, the complementary gene for progressive hybrid necrosis (31,37). If F₁ plants from a cross between a test line and Spica showed progressive hybrid necrosis, then the test line was determined to have *Lr13*.

Gene *Lr34* has been shown to condition slow-rusting resistance (33,36), partial

resistance (29), and durable resistance (15) to leaf rust. Gene *Lr34* was only partially effective by itself, and moderate to high levels of leaf rust infection can occur on cultivars with *Lr34* alone (16,19,37). Detecting *Lr34* at the seedling stage has been difficult because of a high degree of variability in expression (9). Expression of *Lr34* increased as plant maturity progressed (33) and as temperature and light intensity decreased (5,10,21,33). Dyck (5) suggested testing for *Lr34* at 10°C. The gene for leaf tip necrosis (*Ltn*) is tightly linked with *Lr34*, and the presence of *Lr34* was almost certain in lines that showed leaf tip necrosis (31). Leaf tip necrosis has been used as a marker to detect *Lr34* in the adult stage (6,33). However, detecting leaf tip necrosis can be obscured by genetic backgrounds (32) and variable influences of environments (6). Therefore, wheat lines that do not show leaf tip necrosis in some environments may still carry *Lr34* (32). A combination of leaf tip necrosis, temperature-sensitive seedling reaction, and slow rusting in the field were collectively a good indication for the presence of *Lr34* (31).

Highly effective adult-plant resistance to leaf rust was ascribed to an "*Lr34* complex" that was considered to be the product of additive interaction among *Lr34* and two or three undesigned genes for slow rusting (10,30,34,35,37). The interactive effect of *Lr34* with adult-plant and/or seedling resistance genes to enhance leaf rust resistance has been widely documented (14,16,19,34). The combination of *Lr34* with other genes has been hypothesized as the basis for durable resistance in some cultivars (6,26).

In addition to the three designated adult-plant resistance genes described above, there are numerous reports of unnamed genes that confer adult-plant resistance to leaf rust. The high level of adult-plant resistance in cultivar Era was reported to involve the interaction of *Lr13* with *Lr34* and another unidentified adult-plant resistance gene (13,24). Two or three additive genes in addition to *Lr34* were reported to be in Frontana (33,34). Three accessions of Spelt wheat indicated the presence of uncharacterized adult-plant resistance genes (12). Two or more unidentified genes for slow rusting were proposed in Ciano 79 and Papago 86 (34). Several unidentified genes contributed to prolong the latent period in the line BH1146 (3).

There is little information on adult-plant resistance for leaf rust in contemporary soft red winter wheat cultivars and breed-

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Accepted for publication 26 May 2004.

ing lines. The objectives of this research were to determine the genetic basis for adult-plant resistance among 116 contemporary cultivars and breeding lines and to determine the most effective method to identify genes *Lr12*, *13*, and *34*.

MATERIALS AND METHODS

Wheat lines. The 116 cultivars and breeding lines from the 1998–99 Arkansas Wheat Cultivar Performance Test and the Uniform Eastern and Southern Soft Red Winter Wheat Nurseries were selected as representative of contemporary soft red winter wheat lines. These lines have been characterized for race-specific seedling resistance in a previous study (39).

Field studies. To determine field reactions to leaf rust and expression of leaf tip necrosis, the 116 lines were planted at multiple locations in Arkansas each year from 1999 to 2002. In 1999 to 2001, the lines were planted at Rohwer (Southeast Branch Station), Kibler (Vegetable Substation), and Keiser (Northeast Research and Extension Center). In 2002, they were planted at Kibler and Marianna (Cotton Branch Station). Depending on the year and location, individual plot size varied from a single row 1 m long to nine rows 1.8 m long, and the number of replications varied from two to four.

To enhance the development of leaf rust epidemics, all field experiments at Kibler were irrigated, and experiments at Kibler in 2001 and 2002 were artificially inoculated. In 2001, experiments were inoculated separately with races MCRN (a race selected to overcome *Lr26*) and TNRL (a common race in Arkansas during this time). In 2002, the experiment was inoculated with race TNRL. All other experiments relied on natural inoculum and were not irrigated. Race designations were as described previously (39).

To determine field reaction to leaf rust, rust intensity (percentage of foliage with symptoms) and host response (infection type) (28,38) were estimated visually during soft dough stage. Rust intensity percentages (and their ranges) that were recorded were 0, 2 (trace to 4), 7 (5 to 10), 15 (11 to 20), 30 (21 to 40), 50 (40 to 60), 70 (61 to 80), 85 (81 to 90), 93 (91 to 96), and 98 (>96). Rust intensity and host response were combined into a coefficient of infection (CI) by multiplying the rust intensity by a constant assigned to various categories of host response (28,38). The categories of host response (and associated constants) were infection type 0 to ; (constant = 0), infection type 1 (constant = 0.2), infection type 2 (constant = 0.4), infection type 3 (constant = 0.8), and infection type 4 (constant = 1). Coefficients of infection were averaged across replications for each line in each experiment.

For experiments in which leaf tip necrosis was observed, presence (+) or absence (–) of leaf tip necrosis on flag leaves of the

lines was recorded at the same time as rust intensity and host response. In order to postulate the presence of gene *Lr34*, frequency of positive observations was calculated across the 12 replications in which leaf tip necrosis was observed. Lines that expressed leaf tip necrosis in six or more of the replications were considered positive, and lines that expressed leaf tip necrosis in four or fewer replications were considered negative.

Growth chamber studies on adult plants. To determine which lines had adult-plant resistance and to postulate named adult-plant resistance genes by matching infection types, adult plants of 110 lines that were susceptible at the seedling stage were evaluated for infection type under controlled conditions. Five lines were not tested because seedlings were resistant to all races used, and seedlings of TW94315 died during vernalization. Sixty-two lines that were hypothesized to have adult-plant resistance based on moderate resistance to moderate susceptibility in the field or postulated to have unknown genes in the seedling tests (39) were evaluated during the winter of 2000–2001. The remaining 48 lines were evaluated during the winter of 2001–2002. In both groups, seedlings were vernalized for 4 weeks in petri dishes at 4 to 6°C and then transplanted to 7.5-cm square pots filled with potting mix (peat moss, vermiculite, perlite, Roxanna silt loam, and sand in a 6:4:2:3:3 ratio by volume, respectively). Plants were vernalized in growth chambers for an additional 2 weeks at 6°C followed by 1 week at 12/8°C day/night. After vernalization, plants were transplanted to 15-cm-diameter pots and grown at 20/15°C day/night in a greenhouse with supplemental light on a 14-h photoperiod. Plants were fertilized with Peters 20-20-20 NPK fertilizer and treated with insecticides for thrips and aphids as needed.

At Zadoks (40) growth stage 50 to 59 (beginning to completion of ear emergence), the adaxial surface of at least three flag leaves per pot were inoculated by spraying with fresh urediospores suspended in Soltrol 170 mineral oil (Chevron-Phillips Chemical Co., Houston, TX). Urediospores were produced on seedlings of susceptible cultivars as described previously (39). Each line was inoculated with only one race of *P. tritici* (MCRN, TLRT, TFRP, TDRN, or PNMT) depending on which race was most virulent on seedlings of the line. Adult plants of Thatcher isolines with genes *Lr12*, *13*, *34*, and *13*, *34* (*TcLr12*, *TcLr13*, *TcLr34*, and *TcLr13*, *34*, respectively), Sturdy (*Lr12*, *34*), Stephens (*Lr13*, *34*), Auburn (*Lr12*+), and Collin (*Lr34*+), and two susceptible cultivars (Thatcher and Morocco) were included as controls. After 18 to 24 h in a dew chamber at 20°C, plants were incubated in a growth chamber at 22/18°C day/night with a 12-h photoperiod. This

environment was selected as the most appropriate environment for the expression of *Lr12*, *13*, and *34*. Infection types were recorded 16 to 18 days after inoculation when the susceptible controls showed a high infection type. Each line was tested three times.

Detection of *Lr34* under controlled conditions. Because *Lr34* had been detected in seedlings at 10°C and low light intensity (9) and at 15°C (7), the 62 lines that were most likely to have adult-plant resistance were tested at seedling stage under different temperatures and light intensities. Cultivars Morocco, *TcLr12*, *TcLr13*, *TcLr34*, *TcLr13*, *34*, Caldwell (*Lr12*, *13*+), Auburn, Knox (*Lr12*, *34*), and Sturdy were included as controls. Races MCRN, TLRT, and TFRP were used to inoculate seedlings of all lines as described previously. After the dew period, seedlings were incubated in growth chambers at 10°C (12-h photoperiod at 58,125 lux), 15/10°C day/night (12-h photoperiod at 58,125 lux), or 22/18°C day/night (12-h photoperiod at 77,500 lux). Infection types were recorded 16 to 18 days after inoculation when the susceptible controls showed a high infection type. The experiment was done twice.

Because Singh and Gupta (33) reported that lines with *Lr34* had a lower infection type on primary leaves of 13-day-old seedlings than on primary leaves of 8-day-old seedlings, 29 wheat lines that were suspected to have *Lr34* were inoculated at 8 days and 13 days after planting. *TcLr12*, *TcLr13*, *TcLr34*, *TcLr13*, *34*, Knox, Sturdy, Caldwell, and Thatcher were included as controls. Each line was inoculated with one race (MCRN, TFRP, or TLRT) that was most virulent on seedlings of the line (39). Plants were incubated in a growth chamber at 18/15°C day/night and 12-h photoperiod at an intensity of 58,125 lux. Infection type was recorded on primary leaves 14 days after inoculation. The experiment was done twice.

Detection of *Lr13*. Because Pretorius et al. (25) were able to detect *Lr13* at the seedling stage, 10-day-old seedlings of the 116 lines were inoculated with the same two isolates of *P. tritici* (81-LCB-CH and 82-BHL-MD) and were incubated under similar conditions. Isolates were obtained from D. Long at the USDA-ARS Cereal Disease Laboratory in St. Paul, MN. Inoculation and incubation in a dew chamber were as described previously. After the dew period, plants were incubated in growth chambers programmed to maintain 18.1 or 25.5°C and light intensity of 77,500 lux. Infection types were recorded 14 days after inoculation as described previously. The experiment was done twice.

To detect the presence of *Lr13* by progressive hybrid necrosis (a trait controlled by a gene, *Ne2m*, that is tightly linked with *Lr13*), 45 wheat lines suspected to have

Lr13 were crossed to the Australian spring wheat cultivar, Spica, that was reported to have the complementary gene, *Ne1*, for progressive hybrid necrosis (31,37). Seeds of Spica were obtained from J. Kolmer (USDA-ARS, Cereal Disease Laboratory, St. Paul, MN). Spica was used as the female parent in all crosses. Thatcher was included as a negative control, and *TcLr13* and *TcLr13*, 34 were included as positive controls. The F₁ seeds were dried and treated intermittently with 1% hydrogen peroxide to enhance germination. Nonvernalized seedlings were transplanted to 15-cm-diameter pots filled with potting mix and grown in a greenhouse.

RESULTS

Field studies. During the 4-year study, leaf rust developed late in the season and not uniformly at all locations. However, disease pressure was sufficiently high in six of the 14 field experiments that were planted (Table 1). Nearly 29% of the lines were resistant (CI < 10) in all six experiments, no line was susceptible (CI > 40) in all six experiments, and only 30% of the lines were susceptible in one or more of the six experiments.

Six field experiments (12 replications) were suitable for evaluating the presence or absence of leaf tip necrosis (Table 1). Evaluations in the other experiments were confounded by stripe rust, drought stress, or leaf damage from windblown soil. Twenty-seven lines expressed leaf tip necrosis in six or more of the replications and were considered positive. Sabbe and M95*2883 expressed leaf tip necrosis in 11 of 12 replications and had the most consistent expression of this trait. Eighty-nine lines that expressed leaf tip necrosis in four or fewer of the replications were considered negative, and of these lines, 33 did not express leaf tip necrosis in any of the replications.

Growth chamber studies on adult plants. Of the 110 lines evaluated for adult-plant resistance in the growth chamber, 103 expressed some level of adult-plant resistance (infection types less than 3) to a race that was virulent on seedlings (Table 1). Only seven lines did not express adult-plant resistance (infection type 3 or greater). Seedlings of TW94315 died during vernalization, and five lines were not tested because seedlings were resistant to all races used. The susceptible controls, Thatcher, Morocco, and Ernie, displayed a high infection type with all races (Table 2). The races tested produced a distinctive low infection type with prominent necrosis on *TcLr12*. Seventeen lines had a low infection type with prominent necrosis similar to that on *TcLr12* and were postulated to have *Lr12*. All races produced a range of low infection type on *TcLr13* and *TcLr34* in which the ; infection type did not predominate. However, all races produced a range of low infection type on *TcLr13*, 34,

Auburn, and Stephens in which the ; infection type did predominate. All races produced a ; infection type on Sturdy that matched the infection type of 40 lines. Excluding the 17 lines postulated to have *Lr12*, nearly 65% of the lines had a low infection type in which the ; infection type predominated, indicating that these lines likely had more than one gene for adult-plant resistance. For lines postulated to have one known adult-plant resistance gene, an additional unidentified (+) gene was postulated if the infection type from the growth chamber study and/or the field leaf rust coefficient of infection was lower than expected for the known gene. No leaf tip necrosis was observed on any of the lines.

Detection of *Lr34* under controlled conditions. Seedling tests at 10°C or 15/10°C did not provide useful information on the presence or absence of *Lr34* because there was no clear difference between the positive and the negative controls (data not shown). Tests at 10°C resulted in a ; infection type on nearly all lines. More than 93% of the lines tested at 15/10°C showed lower infection types when compared with infection types obtained from tests at 22/18°C. The infection type on the susceptible controls (Thatcher and Morocco) and the negative controls (*TcLr12* and *TcLr13*) also showed a similar trend.

Differences between infection types produced on primary leaves of 8-day-old and 13-day-old seedlings of lines with *Lr34* were too small and inconsistent to reliably postulate the presence of *Lr34* (data not shown). Most of the tested lines had slightly lower infection types on primary leaves of 13-day-old seedlings than on 8-day-old seedlings.

Detection of *Lr13*. Seedling tests conducted at 25.5 and 18.1°C using appropriate pathogen isolates (virulent on *Lr13* at 18.1°C but avirulent on *Lr13* at 25.5°C) were useful for postulating *Lr13*. Twenty-three lines that showed high infection types at 18.1°C and low infection types at 25.5°C with one or both of the pathogen isolates were considered positive for *Lr13* (Table 3). Twenty-one lines that showed high infection type at both 18.1 and 25.5°C with one or both pathogen isolates were considered negative for *Lr13*. The presence or absence of *Lr13* could not be determined in 72 lines that were resistant to both isolates at both temperatures (data not presented).

None of the F₁ plants obtained by crossing 45 lines with Spica showed progressive hybrid necrosis. The F₁ plants obtained by crossing *TcLr13* and *TcLr13*, 34 with Spica also did not show the symptom for hybrid necrosis, indicating that the Spica seeds were not the correct genotype. Therefore, no information was obtained for *Lr13* from tests for progressive hybrid necrosis.

DISCUSSION

Results of this study indicated that more than 90% of the wheat lines that were susceptible at the seedling stage had some level of adult-plant resistance. The high frequency of adult-plant resistance in soft red winter wheat lines suggests that methods used by wheat breeders to select lines have indirectly selected for adult-plant resistance to leaf rust. In several lines, such as NK Coker 9184, NK Coker 9663, and Jaypee, adult-plant resistance was incorporated even in the presence of effective seedling resistance during the years that selections were being made. Generally it has been assumed that selecting adult-plant resistance in the presence of effective seedling resistance would be difficult or impossible (22,23). Breeders of soft red winter wheat have relied on field selections more than on seedling tests for selecting resistance to leaf rust, and perhaps adult-plant resistance genes have become linked to beneficial traits that are selected in field.

Genes *Lr12*, *13*, and *34* were identified in approximately 15, 20, and 23% of the lines, respectively, and these genes are important components of adult-plant resistance to leaf rust in contemporary soft red winter wheat cultivars and breeding lines. These genes have been reported in sources of leaf rust resistance used in soft red winter wheat breeding programs (15,21,26) and in early cultivars of soft red winter wheat (27). Virulence for *Lr12* and *13* has been reported in North America (17,20,33), making these genes less effective in the field. All lines postulated to have *Lr13* alone and two lines postulated to have *Lr12* alone were susceptible in the field at some locations, supporting previous reports (16,18,20,33) that these genes do not provide adequate adult-plant resistance unless they are combined with other *Lr* genes.

Gene *Lr34* alone did not provide adequate resistance in most lines. High susceptibility was observed with cultivars Sabbe (CI = 98), Terra E218 (CI = 77.5), and Stine 488 (CI = 70) to race TNRL in the field. The frequency of leaf tip necrosis was among the highest in Sabbe and Terra E218, and therefore, the presence of *Lr34* is very likely. McIntosh et al. (21) and Kolmer (16) also have reported high levels of leaf rust on lines with *Lr34* alone. The level of resistance conferred by *Lr34* appears to depend on the interaction with other *Lr* genes as reported by Drijepontd et al. (4) and Singh (30). Race TNRL was virulent to all seedling resistance genes in Sabbe, and there were no seedling resistance genes in Terra E218 and Stine 488 (39).

Several lines likely have some combination of two or more genes; however, only eight lines were postulated to have a two-way combination among *Lr12*, *13*, and *34*. Over 40% of the lines were determined to have unidentified (+) adult-plant resistance

Table 1. Average coefficient of infection for leaf rust across years, locations, and races, frequency of leaf tip necrosis (LTN), infection type on flag leaves in growth chamber tests using the listed races of *Puccinia triticina*, and adult-plant resistance (APR) genes postulated for 116 contemporary soft red winter wheat lines

Line	Coefficient of infection ^a							Growth chamber		
	1999			2001		2002		Race	Infection type ^c	APR gene
				Kibler	MCRN					
	Keiser	Rohwer	Kibler	TNRL	MCRN	Marianna	LTN ^b			
Agripro Foster	27	4	69	3	85	0	0	TFRP	;122+	+
Agripro Hickory	15	2	37	74	0	0	1	MCRN	;1N	12
Agripro Mallard	7	1	3	3	20	1	2	TFRP	;12N	12+
Agripro Marion	0	0	0	16	2	0	9	NT	NT ^d	34
Agripro Mason	0	0	1	6	1	0	0	TLRT	3	None
Agripro Patton	6	0	4	3	6	0	0	TFRP	;12N	12+
Agripro Shelby	21	1	18	0	2	2	10	TFRP	33–;2	34
Agripro Shiloh	3	0	0	30	0	45	0	TLRT	3	None
AGS 2000	12	6	12	2	18	0	2	TFRP	;12N	12+
AP-D94-5282	0	1	0	0	0	0	4	NT	NT	ND ^d
AP-D95*8811-1	0	0	0	3	0	2	3	TLRT	;	+
AP-D95*8811-2	0	0	0	... ^d	0	0	0	TLRT	1;22+	+
AP-D95-7763	0	0	0	0	0	0	3	PNMT	;	+
Armor 3235	23	40	...	28	20	0	9	TFRP	;	13,34
AR584A-3-1	3	3	0	28	0	6	9	TFRP	;	34+
BL931167	0	0	0	12	0	1	1	TLRT	;12–N	12+
BL940026	0	0	1	26	0	4	4	TFRP	;	+
Buckshots’ DS9193	0	0	0	35	0	14	0	TLRT	;122+N	12
Caldwell	0	6	3	0	3	6	7	TFRP	;0	13,34+
Cardinal	7	12	32	55	37	1	4	TFRP	;22+1	13+
Delta Grow 3300	1	0	1	14	0	0	2	MCRN	;1N	12,13
Delta King 1551W	30	56	60	20	0	2	1	MCRN	;122+	13+
Delta King 9027	6	1	2	1	0	1	8	TFRP	2+;33–	34
Delta King 9051	1	2	7	0	0	2	2	TFRP	;	+
Delta King 9121	0	0	3	0	0	0	0	TFRP	;	+
Dixie 2000	0	0	0	0	0	0	0	TLRT	;1N	12+
Dixie 911	4	1	0	23	2	6	8	TFRP	;2+N	12,34
EK 103	30	4	36	6	0	0	0	TFRP	;3–	+
EK 114	9	6	26	3	6	4	2	TFRP	;3–3	+
EK 156	1	4	13	4	0	2	3	TFRL	;22+N	12+
F322W	9	1	37	3	4	0	1	TFRP	;22+N	12+
FFR 522W	0	1	0	0	0	0	1	TLRT	;	+
FFR 558W	9	4	89	55	71	0	0	TLRT	21;	+
Florida 302	30	50	55	12	70	3	0	MCRN	32+	None
Florida 8868	0	0	3	47	0	1	1	TLRT	;3	+
G41332	6	6	0	35	0	1	4	TFRP	;0	+
G41333	9	9	12	38	0	0	3	TFRL	;	13+
G60232	1	1	0	1	0	1	4	MCRN	;1	+
GA881404E56	9	2	60	1	0	0	3	TLRT	;	+
GA88622E51	0	0	0	0	0	1	8	TFRP	;	34+
GA90524E35	0	...	1	6	0	2	6	TLRT	;1	34
HT98-10033	4	11	33	26	3	0	2	MCRL	;22+	13+
HT98-10291	1	3	40	18	0	0	1	MCRN	;	+
IL91-15911	19	4	2.9	6	0	0	3	TFRP	;2+	+
IL94-2894	2	4	12	1	0	0	2	TFRP	;	13+
IL94-6727	1	7	45	1	3	0	0	TFRP	;	13+
Jackson	21	2	31	16	1	3	7	MCRL	;122+	34
Jaypee	9	4	6	25	0	0	10	TFRP	0	34+
KY86C-127-3	8	9	26	2	35	1	0	TFRL	;22+	+
LA 87167D8-10-2	0	0	0	23	0	1	1	TLRT	;3–2	+
LA8513B1-7-B-1-4-2	0	0	14	4	0	32	0	MCRN	12;N	12+
LA90144B16-3-2	0	0	0	0	0	0	0	TLRT	;12–N	12+
LA90412F14-1-4	0	0	0	0	0	0	0	TLRT	;12–N	12+
LA9070G45-3-3-1	3	0	0	0	6	0	1	TLRT	;1N	12+
M95*2883	9	15	23	50	38	2	11	TFRP	;0	13,34
M95*3330	9	12	70	43	71	7	3	TFRP	;	+
M95*3349	3	11	78	28	78	9	0	MCRN	;21+2+	+
Madison	10	4	67	12	58	6	3	MCRN	3	None

(continued on next page)

^a Product of rust intensity and host response. Categories of host response (and associated constants) were infection type 0 to ; (0), infection type 1 (0.2), infection type 2 (0.4), infection type 3 (0.8), and infection type 4 (1).

^b Frequency of leaf tip necrosis among 12 replications in the field. Frequencies ≥ 6 were considered positive for *Lr34*, those ≤ 4 were considered negative.

^c 0 = no macroscopic symptoms, ; = hypersensitive necrotic or chlorotic flecks, 1 = small uredia surrounded by necrosis, 2 = medium uredia surrounded by necrosis or chlorosis, 3 = medium uredia without necrosis or chlorosis, 4 = large uredia without necrosis or chlorosis, + = uredia somewhat larger than normal, and - = uredia somewhat smaller than normal. A range of infection types is indicated by listing more than one type, with the predominant one listed first. N = prominent necrosis.

^d NT = not tested, ND = not determined, ... = missing data.

genes that were important components of adult-plant resistance to leaf rust in contemporary lines. These unidentified genes may be two-way or three-way combinations of *Lr12*, *13*, and *34* that could not be identified, other adult-plant genes, minor genes with additive effects, or modifying genes. Highly effective and durable resistance to leaf rust in bread wheat cultivars has been reported to result from the inter-

action of *Lr34* with other minor additive genes and was described as an “*Lr34* complex” (30,34,35,37). The high level of adult-plant resistance in cultivar Era was reported to result from the interaction between *Lr13* with *Lr34* and another unidentified adult-plant resistance gene (13,24).

Adult-plant resistance was easily detected by evaluating infection types on flag leaves inoculated with a race virulent to

seedlings. However, matching the infection type produced on a line with that on isolines with adult-plant resistance genes was only able to postulate the presence of *Lr12*. The distinctive low infection type with prominent necrosis produced on flag leaves of *TcLr12* and 17 lines postulated to have *Lr12* appeared to be a reliable and reproducible method for postulating *Lr12* and was in agreement with the infection

Table 1. (continued from previous page)

Line	Coefficient of infection ^a							Growth chamber		
	1999			2001		Marianna	LTN ^b	Race	Infection type ^c	APR gene
	Keiser	Rohwer	Kibler	Kibler						
				TNRL	MCRN					
MO94-317	7	11	82	77	55	9	4	TFRP	;	+
NC94-7197	0	0	0	3	0	0	0	NT	NT	ND
NC95-25305	4	15	0	3	0	12	4	MCRN	;	+
NC95-25707	0	0	9	6	0	4	1	TLRT	;1N	+
NK Coker 9025	8	2	0	44	0	0	6	TFRP	;	34+
NK Coker 9152	0	0	3	4	0	0	2	TDRL	;	+
NK Coker 9184	0	0	3	35	0	2	8	TFRP	;	34+
NK Coker 9543	3	3	25	0	0	0	1	MCRN	;3-2	+
NK Coker 9663	0	0	0	4	0	1	9	TLRT	;22+3-	34
NK Coker 9704	4	3	6	0	0	0	6	TFRP	;2N	12, 34
NK Coker 9835	2	0	0	0	12	26	0	TLRT	1;22+	+
OH 536	3	4	1	0	0	0	0	TFRP	;	+
OH 552	1	7	25	0	0	1	8	MCRL	;123-	13, 34
OH 599	29	30	55	25	85	0	0	TFRP	43	None
OK91P648-41	0	1	20	6	0	0	0	TFRP	;	13+
P86364RA1-4-2-7	4	6	6	35	7	2	1	TFRP	;	13+
P88204RB1-2-1-6	6	6	24	1	0	0	0	TFRP	0;	+
P92227C5-1-1	6	14	12	0	0	0	1	TFRP	0;	13+
Pioneer 2548	18	12	45	12	41	3	0	MCRN	;122+	13
Pioneer 2684	6	14	12	3	0	0	0	TFRP	;13-	+
Pioneer 26R46	12	23	35	3	0	0	0	MCRN	;	+
Pioneer 26R61	6	3	6	1	0	0	7	TFRP	;0	34+
Pioneer XW672	4	6	24	0	4	0	1	MCRN	;	13+
Pioneer 26R24	9	4	1	8	0	0	4	TFRP	;0	+
Pocahontas	15	4	18	6	47	1	2	TLRT	33+	None
Quantum 708	1	16	45	20	0	1	0	TFRP	3	None
Roane	14	0	40	25	45	1	8	TFRP	;2	34
Roberts	2	4	20	0	0	1	9	TFRP	;3-2	34+
Sabbe	14	4	50	98	0	7	11	TLRT	2;2+	34
SC9412192	27	40	45	43	70	0	3	MCRN	;	+
SC921285	0	16	30	44	0	1	3	MCRN	0;	+
SC921299	0	11	26	3	1	0	2	TFRP	0	+
SS36803	23	11	13	0	0	0	6	TFRP	;0	34+
SS550	15	3	3	3	85	0	1	TFRP	;	+
Stine 455	30	19	56	1	36	0	1	MCRN	;12-	13
Stine 488	14	4	70	0	41	1	6	MCRN	;22+	34
Stine 950455	70	47	72	0	35	4	3	MCRN	;12-	13
T104	33	23	25	70	78	0	0	MCRN	;12-2+	+
T105	2	33	26	20	32	0	1	MCRN	;122+	+
T106	7	18	27	1	9	0	3	MCRN	;12-N	12+
T109	7	9	8	0	3	0	0	TFRP	;	+
Terra E218	14	1	78	12	23	4	9	MCRN	;123-	34
Terra SR204	7	4	85	20	46	3	3	MCRN	;122+	13
Terra SR216	2	1	1	49	1	2	2	TFRP	;23-	+
Terral TV 8555	0	1	1	1	0	0	0	TFRP	;122+	+
Terral TV 8768	7	2	13	1	28	0	0	NT	NT	ND
Terral TV 8825	1	1	37	12	0	0	1	TFRP	;	+
Terral TVX8670	6	8	6	0	0	1	0	MCRN	;	+
TW94315	7	23	70	6	23	0	0	NT	NT	13
TX87-20	0	3	5	0	0	0	1	PNMT	;1	+
TX91-13	0	0	0	1	3	0	6	PNMT	;	34
USG 3408	16	6	23	20	1	0	6	TFRP	;	13,34
USG Exp 97-4	10	28	70	25	78	9	0	MCRN	;1	+
USG 3209	14	0	4	15	32	0	3	MCRN	;23-	+
VA96-54-326	5	4	4	3	38	0	2	TFRP	;3-	+
VA97W-375	0	0	1	0	0	1	7	NT	NT	34
Wakefield	21	4	63	0	44	2	2	MCRN	;122+	13
Wilfarm 488	23	4	78	20	78	3	4	MCRN	1;22+	13

type described in McIntosh et al. (21). Even though all the races used in the adult-plant experiments were avirulent on *TcLr13* and *TcLr34*, the low infection types were not distinctive enough to be useful for postulating the presence of *Lr13* or *34*.

Seedling tests conducted at 25.5 and 18.1°C using the two races virulent on *Lr13* at 18.1°C but avirulent on *Lr13* at 25.5°C appeared to be definitive for postulating *Lr13* as reported by Pretorius et al. (25). However, 62% of the lines were resistant to these races at both temperatures and could not be evaluated for *Lr13*. Additional races avirulent on *Lr13* and with combinations of virulence to overcome epistatic effects of the other *Lr* resistance genes are needed to identify *Lr13* in these lines. In retrospect, including *TcLr13* in the set of isolines used in the previous seedling study (39) may have identified additional races avirulent to *Lr13*, and some of these races may have been useful for postulating *Lr13* in additional lines.

Compared with the other methods we used to detect *Lr34*, the presence of leaf tip necrosis in the field appeared to be the best method for postulating *Lr34*. Leaf tip necrosis has been reported as a reliable marker to detect the presence of *Lr34* (30); however, its expression can be influenced by environment (6) and genetic background (32). Of the locations where leaf tip necrosis was evaluated, Kibler was the only irrigated location and had the most favorable environment for expression of leaf tip necrosis. Irrigation at Kibler likely alleviated drought stress and may have enhanced the expression of leaf tip necrosis. However, no line showed leaf tip necrosis 100% of the time, and some lines that were postulated not to have *Lr34* expressed leaf tip necrosis in a few replications. Six of 12 replications with leaf tip necrosis appeared to be an appropriate threshold for postulating *Lr34* because none of the lines expressed leaf tip necrosis in five replications, thus dividing the lines into two distinct groups. However, postulation of *Lr34* based on leaf tip necrosis under these conditions should not be considered definitive. Multiple recordings of leaf rust intensity in the field may have been useful for determining slow rusting types of resistance to support the presence or absence of *Lr34* that confers slow rusting. However, leaf rust developed late in the season and allowed a maximum of only two recordings. Seedling tests at low tem-

peratures and light intensity were not useful for identifying *Lr34*.

It may have been useful to include lines known to have *Lr34* as controls for evaluating leaf tip necrosis in the field. However, of the 137 lines listed as having *Lr34* in the USDA-ARS Cereal Disease Laboratory database (27), none are soft red winter lines adapted to Arkansas. The lack of soft red winter lines and the preponderance of CIMMYT lines listed as having *Lr34* may indicate that conditions are favorable for the expression of leaf tip necrosis where CIMMYT evaluates its lines and unfavorable where soft red winter lines are evaluated. Lines showed clear differences for leaf tip necrosis at CIMMYT's El Batán headquarters in Mexico, where nurseries were irrigated regularly and night temperatures after heading stage were much cooler than in Arkansas after heading stage (E. A. Milus, *personal observation*). The combination of optimal moisture for plant development and cool night temperatures may be required for consistent expression of leaf tip necrosis. Expression of resistance attributed to *Lr34* generally is considered to be greater at lower temperature ranges (21), but no reports describing the effect of temperature on leaf tip necrosis were found.

The most reliable method for identifying combinations of adult-plant resistance genes was to identify them individually using the methods described above. For example, Dixie 911 was postulated to have *Lr12* based on the distinctive infection type in the growth chamber test and *Lr34* based on leaf tip necrosis in the field. Likewise, M95*2883 was postulated to have *Lr13* based on the seedling test for *Lr13* and *Lr34* based on leaf tip necrosis. This method identified two-way combinations among *Lr12*, *13*, and *34* in only eight of 103 lines with adult-plant resistance.

Matching infection types with controls believed to have combinations of adult-plant resistance genes did not allow definitive identification of adult-plant resistance genes. One problem with the method was that, except for *TcLr13*, *34*, the controls did not have a truly known combination of adult-plant resistance genes. For example, Sturdy was determined to have *Lr12* and *34* by genetic analysis (6), but the infection type on Sturdy was consistently a hypersensitive fleck that was lower than for other lines postulated to have *Lr12* and *34*. Likewise, Auburn was reported to have

Lr12+ (D. Marshall, *personal communication*), but the infection type was similar to that on *TcLr13*, *34* (Table 2). A second problem with this method was that more than one combination of adult-plant resistance genes appeared to produce the same infection type. For example, of the lines observed to have a ;122+ infection type on flag leaves in the growth chamber test (Table 1), Pioneer 2548, Terra SR204, and Wakefield were postulated to have *Lr13* alone, Jackson and Terra E218 were postulated to have *Lr34* alone, and T105 was postulated as + and did not have *Lr34* or *13*. Furthermore, these lines had the same infection type as *TcLr13*, *34*. Therefore, matching infection types on the flag leaves was not useful for postulating genes *Lr13*, *34* or combinations of adult-plant genes.

Caldwell was postulated to have *Lr12*, *13+* (D. Marshall, *personal communication*); however, in this study it was postulated to have *Lr13*, *34+*. Caldwell had high infection type at 18.1°C and low infection type at 25.5°C with the pathogen isolates that were virulent on *Lr13* at 18.1°C and avirulent on *Lr13* at 25.5°C and therefore was considered positive for *Lr13*. The frequency of leaf tip necrosis on Caldwell indicated the presence of *Lr34*. Moreover, the infection types produced on USG 3408, Armor 3235, and M95*2883, which were postulated to have the combination of *Lr13* and *34*, were similar to that on Caldwell. Despite the absence of seedling *Lr* genes in Caldwell (39), the level of resistance in the field was higher compared with these lines. Therefore, it is likely that Caldwell had at least one adult-plant resistance gene in addition to *Lr13* and *34*. Although *Lr12* was not postulated in Caldwell based on infection type in growth chamber experiments in this study, *Lr12* may be present because infection type was not useful for identifying genes in combinations.

This study clearly demonstrated the high frequency of adult-plant resistance and its value for protecting soft red winter wheat from leaf rust. However, none of the published methods that we used to postulate genes for adult-plant resistance give definitive results, and consequently, the genes postulated in this study should be interpreted as tentative. This study underscores the difficulty of postulating genes for adult-plant resistance. Currently, genetic studies are the most reliable methods for

Table 2. Infection type^a produced on flag leaves of controls by the three races of *Puccinia triticina* used in the growth chamber study

Race	Thatcher	Morocco	Ernie	<i>TcLr12</i>	<i>TcLr13</i>	<i>TcLr34</i>	<i>TcLr13+34</i>	Auburn	Collin	Stephens	Sturdy
MCRN	33+	33+	33+	;12N	22+;3-	2;2+3-	;122+	;122+3	;1	;12-	;
TLRT	33+	4	3	;12-N	2;2+3-	1;22+	;122+	;122+	;1	;2+1	;
TFRP	33+	33+	3+4	;22N	22+;	2;3-	;122+	;122+3-	;12	;2	;

^a 0 = no macroscopic symptoms, ; = hypersensitive necrotic or chlorotic flecks, 1 = small uredia surrounded by necrosis, 2 = medium uredia surrounded by necrosis or chlorosis, 3 = medium uredia without necrosis or chlorosis, 4 = large uredia without necrosis or chlorosis, + = uredia somewhat larger than normal, and - = uredia somewhat smaller than normal. A range of infection types is indicated by listing more than one type, with the predominant one listed first. N = prominent necrosis.

determining the presence or absence of particular resistance genes, but genetic studies are laborious, expensive, and still require phenotypic evaluations. Molecular markers for adult-plant resistance genes may eventually facilitate postulation studies, but development of these markers

likely will require phenotypic characteristics similar to those used in this study.

ACKNOWLEDGMENTS

We thank David Long and Jim Kolmer, USDA-ARS, Cereal Disease Laboratory, for providing pathogen isolates, seeds of isolines, advice, and information, and Ravi Singh from CIMMYT for his

comments and suggestions. Technical support from the wheat pathology team of University of Arkansas and financial support from the Arkansas Wheat Promotion Board are gratefully appreciated.

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Table 3. Infection type^a produced by isolates of *Puccinia tritricina* on primary leaves of controls and lines of soft red winter wheat at two temperatures to detect *Lr13*

	<i>P. tritricina</i> isolate ^b				
	82-BHL-MD		81-LCB-CH		
Control/line	18.1°C	25.5°C	18.1°C	25.5°C	Lr13 ^c
Morocco (susceptible)	4	4	4	4	—
Ernie (susceptible)	3	4	4	4	—
Thatcher (susceptible)	3+	3+	3+	33+	—
<i>TcLr12</i>	4	4	2+	2+	—
<i>TcLr34</i>	3	3	3	3	—
<i>TcLr13</i>	3	2	3+	2	+
<i>TcLr13+34</i>	3	22+	2+3	2	+
Coastal (13+)	4	;	32+	;	+
Hadden (13+)	;12	;	33–1;	;	+
Armor 3235	2+	2+	4	2+	+
Caldwell	4	;12	4	;1	+
Cardinal	4	;2	;	;1	+
NK Coker 9543	;1	4	4	4	—
NK Coker 9704	3	3	;	;	—
Delta Grow 3300	4	;22+	4	22+	+
Delta King 1551W	;	;1	4	22+;	+
Delta King 9027	;2	22+	4	4	—
Delta King 9051	2+	4	2	4	—
Delta King 9121	;2+	4	;1	4	—
Dixie 911	;2	4	4	4	—
EK 103	;1	;1	3	3	—
EK 114	;	;1	4	4	—
EK 156	4	4	4	4	—
F322W	4	4	4	4	—
G41332	4	4	4	4	—
G41333	4	;2+	4	2;	+
HT98-10033	;	;	4	;22+	+
IL91-15911	4	4	4	4	—
IL94-2894	;	;	4	2	+
IL94-6727	4	2	4	2	+
M95*2883	4	2+	3	3	+
M95*3330	4	4	4	4	—
OH 552	;	;	4	;	+
OK91P648-41	4	;12	;12+	;1	+
P86364RA1-4-2-7	;	;	4	2	+
P92227C5-1-1	3	22+;	4	2	+
Pioneer 2548	;	;1	4	22+	+
Pioneer XW672	;	;	4	2	+
Roane	4	4	4	4	—
Stine 455	3	;	4	;2	+
Stine 488	4	4	4	3+	—
Stine 950455	;	;	4	;22+	+
T104	4	4	4	4	—
T105	4	4	4	3+	—
T106	4	4	4	4	—
Terra E218	4	4	4	3+	—
Terra SR204	4	4	4	2+	+
Terra SR216	4	4	4	4	—
TW94315	;1	;12	4	;2+	+
USG 3408	;	;	4	22+	+
VA96-54-326	4	4	4	4	—
Wakefield	4	;	4	;2	+
Wilfarm 488	3+	;22+	4	2+	+

^a 0 = no macroscopic symptoms, ; = hypersensitive necrotic or chlorotic flecks, 1 = small uredia surrounded by necrosis, 2 = medium uredia surrounded by necrosis or chlorosis, 3 = medium uredia without necrosis or chlorosis, 4 = large uredia without necrosis or chlorosis, + = uredia somewhat larger than normal, and — = uredia somewhat smaller than normal. A range of infection types is indicated by listing more than one type, with the predominant one listed first.

^b Same *P. tritricina* isolates used by Pretorius et al. (25).

^c A line was considered positive (+) for *Lr13* if at least one of the isolates produced a high infection type at 18.1°C and a low infection type at 25.5°C.

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