Genetics of Leaf Rust Resistance in Brambling Wheat

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ABSTRACT

Zhang, J. X., Singh, R. P., Kolmer, J. A., Huerta-Espino, J., Jin, Y., and Anderson, J. A. 2008. Genetics of leaf rust resistance in Brambling wheat. Plant Dis. 92:1111-1118.

The CIMMYT-developed spring wheat 'Brambling' has a high level of adult-plant resistance (APR) to leaf rust caused by *Puccinia triticina*. Our objectives were to determine the genetic basis of resistance in seedlings and adult plants and the magnitude of genotype × environment effects on the expression of APR. Brambling was crossed with spring wheat 'Jupateco 73S' that is highly susceptible to current predominant *P. triticina* races in Mexico and the United States. The F_1, $F_{2:3}$, $F_{4:5}$, $F_{4:6}$, and $F_{5:7}$ recombinant inbred lines (RILs) were evaluated under artificial field epidemics in Mexico and St. Paul, MN. The RILs also were tested with five races of *P. triticina* in greenhouse seedling experiments. A DNA marker was used to postulate the presence of slow-rusting gene *Lr34* in the RILs. F_1 data suggested strong dominant effect of the APR genes in Brambling. The proportion of homozygous susceptible lines in each generation indicated the presence of three effective resistance genes in adult plants of Brambling in tests in Mexico and three or four genes in tests in St. Paul. The RILs segregated for seedling genes *Lr14a* and *Lr23* and adult-plant slow-rusting gene *Lr34* derived from Brambling and *Lr17a* from Jupateco 73S. Gene *Lr23* conditioned APR to *P. triticina* races present in the St. Paul nursery and accounted for the additional effective gene at this location. Expression of APR was influenced by the environment in the RILs, even though Brambling displayed a consistent response, indicating that stability of APR can be achieved by combinations of slow-rusting resistance genes.

Additional keywords: durable resistance, temperature sensitivity

Leaf or brown rust, caused by the fungus *Puccinia triticina* Eriks., is the most common disease of wheat (*Triticum aestivum* L.) in North America (3). Although more than 50 leaf rust resistance genes have been catalogued (21), most of those genes confer seedling resistance, are race specific, and vulnerable to selection for new virulent races. Some leaf rust resistance genes express resistance optimally in adult plants and are referred to as adult-plant resistance (APR) genes. Genes *Lr12* and *Lr13* are race-specific APR genes that have been rendered largely ineffective due to selection of virulent rust races (11,12). While almost all seedling resistance genes and many APR genes condition a hypersensitive response (HR), some APR genes condition a response of fewer and smaller uredinia compared with susceptible check genotypes, which has been referred to as slow rusting (2) or partial (25) resistance.

Two slow-rusting resistance genes, *Lr34* and *Lr46*, condition nonrace-specific APR and have provided durable leaf rust resistance (4,19,27,36). It was estimated that approximately a dozen slow-rusting genes were present among cultivars developed at the International Maize and Wheat Improvement Center (CIMMYT) (34). Combinations of three to four such slow-rusting genes resulted in resistance that can approach immunity (30). Currently however, only *Lr34* and *Lr46* have been well characterized.

Genetic characterization of additional APR genes is essential for parental selection for breeders to alter selection strategies and introduce genetic diversity in breeding for durable leaf rust resistance. Moreover, the addition of CIMMYT APR into the U.S. wheat breeding germplasm could result in more durable leaf rust resistance in U.S. wheat cultivars. Comparing the performance of genotypes with APR in CIMMYT and U.S. wheat environments can determine the extent of environmental effects on the expression of APR.

Brambling is a wheat breeding line developed at CIMMYT and is highly resistant to leaf rust in Mexico and the United States. On the basis of the pedigree and observation of leaf rust resistance, it might carry other APR genes in addition to *Lr34* and/or *Lr46*. Our objectives were to: i) identify race-specific resistance genes in the cross of Jupateco 73S × Brambling by evaluating recombinant inbred lines (RILs) in the seedling stage with *P. triticina* races; ii) determine the genetic basis of slow-rusting APR present in Brambling through field trials in Mexico and St. Paul, MN; and iii) study the magnitude of genotype × environment effects of APR in the Mexico and St. Paul environments.

MATERIALS AND METHODS

Plant materials. Brambling, a spring wheat line developed by CIMMYT with the pedigree Weaver/Ocoroni 86//Borlaug 95 (cross number and selection history: CGSS96B00145T-099B-021Y-099M-19Y-0B-1B) has stable, near immune levels of APR to leaf rust. It was crossed as the male parent with the CIMMYT-derived spring wheat line 'Jupateco 73S' that is highly susceptible to the *P. triticina* races predominant in North America. Jupateco 73S was a reselection from the Mexican spring wheat cultivar Jupateco 73 for leaf rust susceptibility (29). The F_1 and generations of $F_{2:3}$, $F_{4:5}$, $F_{4:6}$, and $F_{5:7}$ RILs developed by the single-seed descent method were used in the field studies. The $F_{5:7}$ RILs were used in the seedling greenhouse experiments.

Seedling studies. Seven isolates of *P. triticina* were used in various seedling studies (Table 1). These isolates were collected from wheat in the United States and Canada and were designated for virulence phenotype following the three letter code system of Long and Kolmer (17), with an additional fourth letter that described the high or low infection type (IT) to Thatcher wheat lines with genes *LrB*, *Lr10, Lr14a*, and *Lr18.* Races BBBD (isolate name: Race 1), THBJ (99 ND 588-1), MHDS (03 OH 237), SBDG (Race 9), and PBLR (MI

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Accepted for publication 14 March 2008.

doi:10.1094/PDIS-92-7-1111

91-2) were used in testing the $F_{5:7}$ RILs of the Jupateco 73S/Brambling cross. These five isolates were selected on the basis of their low IT to at least one of the parents in the cross and low IT to specific leaf rust resistance genes common in CIMMYT germplasm. Approximately 14 seeds from each of the 135 $F_{5:7}$ RILs, Brambling, Jupateco 73S, the leaf rust susceptible wheat Thatcher, and 17 near-isogenic lines of Thatcher wheat with single resistance genes *Lr1*, *Lr2a*, *Lr2c*, *Lr3*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a*, *Lr16*, *Lr17a*, *Lr18*, *Lr23*, *Lr24*, *Lr26*, *Lr30*, and *LrB* were tested with each isolate.

Seeds were planted in 3.5-cm^2 plastic pots in vermiculite as described by Oelke and Kolmer (23). Plants were grown in a greenhouse at 18 to 22°C with 16 h of supplemental light. Approximately 8 days after planting, when the first leaf was fully expanded, the seedlings were inoculated by spraying urediniospores of the individual isolate suspended in Soltrol 170 oil. After air drying for 30 min, the inoculated seedling plants were incubated in a mist chamber for 16 to 24 h at 18°C and 100% relative humidity and then moved back to the greenhouse for further incubation.

On the basis of the greenhouse seedling experiments, we postulated that *Lr23* was present in Brambling. In our greenhouse studies, the Thatcher *Lr23* line had ITs of ;2^+3 to isolates BBBD and THBJ. Most of the RILs had the same IT to isolates BBBD and THBJ. However, nine RILs differed for IT to isolates BBBD and THBJ. The seedling ITs of those lines were intermediate to high. The uncertainty and disagreement between BBBD and THBJ in those RILs could be due to temperature sensitivity of *Lr23* since this gene is more effective at higher temperatures (5). To validate the postulation of *Lr23* in Brambling, the nine RILs with different ITs to isolates BBBD and/or THBJ, along with Brambling, Jupateco 73S, Thatcher, Thatcher near-isogenic lines with *Lr17a*, *Lr14a*, and *Lr23* were evaluated for reaction to isolates BBBD and THBJ. After incubation in the dew chamber, the plants were placed in growth cabinets at 20 or 25°C with a mixture of florescent and incandescent light with a 16-h day. Planting, inoculation, and dew chamber incubation procedures of the growth cabinet study were the same as the greenhouse experiment. Infection types were recorded 12 days after inoculation in the greenhouse and 20°C growth cabinet tests and 8 days after inoculation in the 25°C growth cabinet experiment. The ITs were classified as 0 to 4 following Long and Kolmer (17) in which 0 = no uredinia or hypersensitive flecks, ; = no uredinia but hypersensitive necrotic or chlorotic flecks, 1 = small uredinia surrounded by distinct necrosis, 2 = small to medium uredinia surrounded by necrosis or chlorosis, and 3 = moderate size uredinia without chlorosis. Designations of "+" or "–" indicate larger than normal uredinia and smaller uredinia, respectively. ITs of 0 to 2^+ were classified as low infection type and ITs of 3 to 4 were classified as high. Infection type combining different numbers and/or symbols indicated that the infected leaf had uredinia of different sizes and were classified as low if ITs of 0 to 2^+ were present in the combination. The $F_{5:7}$ RILs with low ITs were classified as homozygous resistant, RILs with high ITs were classified as homozygous susceptible, and RILs segregating for high and low ITs were classified as segregating. The ratio of homozygous resistance, homozygous susceptible, and segregating RILs was used to determine the number of seedling resistance genes that were effective against each isolate. Probable genes in each RIL were postulated by comparing with the ITs of the Thatcher near-isogenic lines with individual leaf rust resistance genes.

Molecular marker for *Lr34*. The sequence-tagged site DNA marker *csLV34* (14) was used to screen for *Lr34* in Brambling. This marker is 0.4 centimorgans (cM) distant from *Lr34* and is diagnostic for *Lr34* of wheat cultivars from different parts of the world (14). Jupateco 73S, Thatcher, and RL 6058 (Thatcher isoline for *Lr34*) were included as checks. DNA was extracted from leaf segments of five random plants of each check and $F_{5:7}$ line following Liu et al. (16). Polymerase chain reactions (PCR) were performed in 10-µl reactions containing 3-µl of genomic DNA (30 to 45 ng). After an initial denaturing step for 3 min at 94°C, 35 cycles were performed with 1 min at 94°C, 1 min at 58°C, 2 min at 72°C, followed by a final extension step of 10 min at 72°C. PCR products of *csLV34* were run on polyacrylamide gels containing 32% (v/v) formamide (15). The gels were visualized by silver staining (1).

Adult-plant field studies. Field evaluations were conducted at Ciudad Obregon, State of Sonora, Mexico; El Batan, State of Mexico, Mexico; and St. Paul, Minnesota during various crop seasons from 2003 to 2006. The crop season at Ciudad Obregon is from November to April, whereas at El Batan, the cycle runs from May to October and at St. Paul, from April to August.

Field plots at Ciudad Obregon and El Batan, Mexico, were established on 80 cm wide raised beds and consisted of two 1-m rows, 20 cm apart, on top of the beds with 0.5-m alleys. The field plots at St. Paul, MN consisted of single 2-m rows with 30 cm between plots and 2-m alleys. In the studies of the $F_{2:3}$ and $F_{4:5}$, $F_{4:6}$, and $F_{5:7}$ RILs, each plot was planted with approximately 100 seeds.

In the 2002–2003 crop season, F_1, 237 $F_{2:3}$ lines, and 133 $F_{4:5}$ RILs were planted at Ciudad Obregon. Each generation was planted as different experiments. The F_1 was space sown in two plots with 20 seeds per plot. Brambling and Jupateco 73S were planted in the beginning, middle, and end of the $F_{2:3}$ and $F_{4:5}$ lines as standard checks. In 2004, $F_{4:6}$ RILs were grown in Ciudad Obregon, Mexico and St. Paul, MN with one replication at each location. In Mexico and St. Paul, MN in 2005 and 2006, 137 $F_{5:7}$ RILs were evaluated. In 2005, Mexican leaf rust evaluation was conducted at Ciudad Obregon, whereas the 2006 Mexican nursery was planted at El Batan. The F_1 to F_4 derived ($F_{4:5}$ and $F_{4:6}$) generations were planted with one replication. Experimental design for $F_{5:7}$ RILs was a randomized complete block with two replications in each year and location

Table 1. Seedling infection types of 'Jupateco 73S', 'Brambling', and four 'Thatcher' near-isogenic lines for leaf rust resistance genes to races of *Puccinia triticina* used in greenhouse and growth chamber environments

	Infection type[a]										
	Greenhouse isolates							Growth chamber isolates and temperatures			
Wheat lines	BBBD	THBJ	MHDS	SBDG	PBLR	MBDS	TLGF	BBBD 20°C	BBBD 25°C	THBJ 20°C	THBJ 25°C
Brambling	;123	22^+	;1	0;	;1^-	3	;2^+3	0;	0;	;2^+	0;
Jupateco 73S	0;	0;	3^+	3^+	123	3^+	;1	0;	0;	0;	0;
Thatcher	33^+	3	33^+	33^+	3	3^+	33^+	3	3^+	3	3^+
Tc*Lr14a* RL 6013	33^+	3^+	3^+	0;	;123	3^+	3^+	3^+	3^+	3	3
Tc*Lr17a* RL 6008	;1	;2	3^+	3^+	;12	3^+	;1	0;1^-	0;$^-$	0;	0;1^-
Tc*Lr23* RL 6012	;2^+3	;2^+3	0;	;1^-	33^+	33^+	2^+3	;1	0;	0;	0;1^-
Tc*Lr34* RL 6058	3^+	33^+	3^+	33^+	…[b]	…	…	3	3	3	3

[a] Infection types follow Long and Kolmer (17).
[b] … Indicates data not available.

except one replication in the 2005 St. Paul field test.

Leaf rust epidemics in each environment (one environment = one year and one location) were initiated by artificial inoculations of susceptible spreader rows. The Mexican locations were inoculated with the Mexican race MCJ/SP (28) that has avirulence/virulence formula of *Lr2a*, *2b*, *2c*, *3ka*, *9*, *16*, *19*, *21*, *24*, *25*, *28*, *29*, *30*, *32*, *33*, *34/1*, *3*, *3bg*, *10*, *11*, *12*, *13*, *14a*, *14b*, *15*, *17*, *18*, *20*, *22b*, *23*, *26*, and *27+31*. Seedlings of Jupateco 73S and Brambling had high ITs to this race. Approximately 4 and 6 weeks after planting at El Batan and Obregon, respectively, the spreader rows of the highly susceptible cultivar Morocco, planted as hills on one side of the plots in the middle of the pathway and around the experimental block, were sprayed with urediniospores suspended in a mineral oil once a day for 3 days. The St. Paul location was spray inoculated with isolates THBJ, MCDS (520), and MBRJ (16-2B) when the earliest plants in the spreader row (leaf rust susceptible wheat cultivars LMPG-6, Morocco, and Thatcher) reached heading stage. Races THBJ and MCDS were common in the recent leaf rust population (11) and MBRJ was common in the 1990s (18).

Leaf rust severity was recorded on the basis of the modified Cobb scale (26) when all plants in the plots of susceptible parent Jupateco 73S just reached 100% severity and the leaves had not yet senesced. The F_1 and $F_{2:3}$, $F_{4:5}$, $F_{4:6}$, and $F_{5:7}$ lines were assessed on the basis of the average rust severity on the flag leaves of 5 to 10 plants. Lines in various generations were grouped into two phenotypic categories for χ^2 analyses: i) homozygous susceptible (lines with severity response similar to the susceptible parent, Jupateco 73S); and ii) others (lines homozygous for low to intermediate disease severities or segregating for low/intermediate and high severities).

The goodness of fit of the observed and expected phenotypic groups of the RILs was tested by χ^2 analysis. Analysis of variance of the mean terminal leaf rust severity in the $F_{5:7}$ trials with two replications were conducted, treating each year and location combination as one environment. Pearson correlation coefficients of disease severity of the $F_{5:7}$ lines were estimated using the mean disease severity of each line in each environment. A Student's *t*-test was conducted between lines of different gene combinations using the means of each $F_{5:7}$ RIL in each environment.

RESULTS

Seedling leaf rust resistance genes in Brambling and Jupateco 73S. Jupateco 73S had low ITs 0; to 123 to isolates BBBD, THBJ, PBLR, TLGF and high IT of 3^+ to isolates MHDS, SBDG, and MBDS (Table 1). ITs displayed by Jupateco 73S to BBBD and THBJ were not affected by temperature. The low and high IT pattern of Jupateco 73S to the isolates were the same as the Thatcher line with *Lr17a* (RL6008), which indicated that *Lr17a* was likely present in Jupateco 73S. In greenhouse tests, Brambling had low ITs 0; to ;2^+3 to all isolates tested in Table 1, except for high IT to isolate MBDS (00 SD 520). Isolates BBBD, THBJ, MHDS, MBDS, and TLGF (00 SC 218) were virulent to the Thatcher line with *Lr14a* (RL6013). Isolates SBDG and PBLR were avirulent to the Thatcher line with *Lr14a*. Among the isolates tested, only PBLR and MBDS were virulent to the Thatcher line with *Lr23* (RL6012). The ITs of Brambling to BBBD and THBJ were affected by temperature. In greenhouse tests, Brambling had IT ;123 to isolate BBBD and 22^+ to isolate THBJ. In the growth cabinet, Brambling had IT 0; to BBBD at 20 and 25°C. Brambling had lower IT to THBJ at 25°C than at 20°C. The Thatcher line with *Lr23* also had lower IT in the growth cabinet at 25°C than at 20°C to isolate THBJ. On the basis of these results, Brambling was postulated to carry resistance genes *Lr14a* and *Lr23*.

In greenhouse tests with races BBBD and THBJ, the $F_{5:7}$ RILs segregated for resistant, susceptible, and segregating lines in a ratio not significantly different from the 0.7178:0.2197:0.0625 ratio expected for $F_{5:7}$ with two independent dominant genes for a trait (Table 2). The $F_{5:7}$ RILs had the same resistant, susceptible, or segregating patterns to BBBD and THBJ, which indicated that the same two genes controlled seedling resistance to these races. The Thatcher line with *Lr23* had IT 0; to isolate MHDS. The $F_{5:7}$ RILs segregated to MHDS in a ratio not different from the expected 0.4688:0.4688:0.0625 ratio for $F_{5:7}$ for a single dominant trait that suggested that one gene in Brambling conditioned resistance to MHDS. Isolate SBDG had low ITs to Thatcher lines with *Lr14a* and *Lr23* and the $F_{5:7}$ RILs segregated 88:29:14 for resistant, susceptible, and segregating families, which conformed to a 0.7178:0.2197:0.0625 ratio expected for two genes in Brambling that conferred resistance to this isolate. To isolate PBLR, which had low ITs on *Lr14a* and *Lr17a*, the segregation ratio of $F_{5:7}$ RILs in resistant, susceptible, and segregating categories was in accordance with a 0.7178: 0.2197:0.0625 ratio, which suggested that two genes conditioned resistance to this isolate.

Table 3 lists a subset of lines that do not contain *Lr34* based on lack of the diagnostic linked marker *csLV34*, but were postulated to have individual resistance genes or a combination of seedling resistance genes. RIL 117 was postulated to have *Lr14a* because it had high ITs to isolates BBBD, THBJ, and MHDS, but low ITs to SBDG and PBLR. RIL 128 was postulated to segregate for *Lr14a* because it had high ITs to isolates BBBD, THBJ, and MHDS and segregated for low, and high ITs to isolates SBDG and PBLR. The RILs 105, 119, and 144 were postulated to have *Lr17a* only because these lines had IT 0; to isolates BBBD, THBJ, and PBLR, and IT 33^+ to MHDS and SBDG. RIL 92 was postulated to have *Lr23* because it had IT ;123 to isolate BBBD, IT 2^+3 to THBJ, and 0; to isolates MHDS and SBDG and high IT to PBLR. RIL 14 was postulated to have *Lr14a* and *Lr17a* since it had IT 0; to isolates BBBD, THBJ, SBDG, PBLR and IT 33^+ to MHDS. RIL 57 and RIL 104 were postulated to have *Lr23* and *Lr14a* because those lines had IT ;123 to BBBD, IT 2^+3 to THBJ, and IT 0; to MHDS, SBDG, and PBLR. When *Lr17a* and *Lr23* were postulated to be present, the line had low ITs to all five isolates in Table 3. Restricted by the available isolates that were used, we could not postulate the presence or absence of *Lr14a* in those lines, e.g., RIL 93 and RIL 113 in Table 3. The RILs

Table 2. Segregation of a DNA marker diagnostic for *Lr34* and leaf rust resistance in greenhouse tests of seedling plants of $F_{5:7}$ recombinant inbred lines from the cross 'Jupateco 73S/Brambling'

Leaf rust races/molecular marker	Gene(s) detected	No. of lines[a]			Expected ratio	χ^2 value	*P* value
		Res.	Sus.	Seg.			
csLV34	**Lr34**	68	54	13	0.4688:0.4688:0.0625	4.20	0.12
BBBD	*Lr17a*, *Lr23*	87	34	11	0.7178:0.2197:0.0625	2.50	0.29
THBJ	*Lr17a*, *Lr23*	86	33	11	0.7178:0.2197:0.0625	2.28	0.32
MHDS	*Lr23*	53	70	8	0.4688:0.4688:0.0625	2.36	0.31
SBDG	*Lr14a*, *Lr23*	88	29	14	0.7178:0.2197:0.0625	4.45	0.10
PBLR	*Lr14a*, *Lr17a*	94	29	12	0.7178:0.2197:0.0625	1.61	0.45

[a] Res. = homozygous for the resistance allele or resistant to the race; Sus. = homozygous for the alternative allele or susceptible to the race; and Seg. = segregating for resistance and alternative allele or resistant and susceptible plants.

34, 82, and 106 had high ITs to all the isolates, which indicated these did not have any seedling resistance genes.

***Lr34* genotyping.** Marker *csLV34* produced an allele of approximately 155 bp in the Thatcher line with *Lr34* and in Brambling and an alternative allele of approximately 250 bp in Thatcher and Jupateco 73S, which indicated that Brambling has the *Lr34* APR gene. The $F_{5:7}$ lines segregated 68:54:13 for lines homozygous for the *Lr34* associated allele, homozygous for the alternative allele, and segregating for the *Lr34* and the alternative allele, respectively; these results fit the 0.4688:0.4688:0.0625 expected ratio for a single locus (Table 2). Across the four environments, the field leaf rust severity of the 68 lines postulated to have *Lr34* ranged from 1 to 70%. Some RILs without the *Lr34* gene were highly susceptible to leaf rust (leaf rust severity = 100%) (Table 3). The lack of complete susceptibility of the RILs containing *Lr34* marker allele indicated that *csLV34* is diagnostic of *Lr34* in the Jupateco 73S/Brambling cross, and recombination between *csLV34* and *Lr34* did not occur or it was a rare event in this population.

Field APR in Brambling. Brambling had rust severity and response of 0 to 1 MSS (moderately susceptible to susceptible) in all the environments except in 2006, St. Paul, where it was 10 MS. Jupateco 73S had 100 S (susceptible) severity and response in all environments except 50 to 70 S in 2006 St. Paul. The relatively lower rust severity for Jupateco 73S and higher leaf rust severity for Brambling in 2006 St. Paul nursery was likely because of abnormally dry and hot weather immediately after planting until the first week of July. From May until early July in 2006 St. Paul, the dew point for most nights was lower than the night temperature, which indicated that moisture was probably not adequate for leaf rust infection. In early July, when the environment became conducive for leaf rust infection, Jupateco 73S had already reached the anthesis stage. Thus, the leaf rust severity of Jupateco 73S in this environment was lower than normal. Generally, Brambling reached the anthesis stage 2 days later than Jupateco 73S. Reduced effectiveness of possible temperature-sensitive APR gene(s) in Brambling therefore could have resulted in higher levels of leaf rust in Brambling in the 2006 St. Paul nursery. The effect of drought and heat was also observed in the $F_{5:7}$ RILs in this environment. Disease severities of highly susceptible lines were lower in 2006 than in 2005 (Table 3), and lines with very low rust in 2005, tended to have similar or higher disease than in 2006.

Analysis of variance for leaf rust severity of the $F_{5:7}$ RILs in Ciudad Obregon in 2005 and El Batan and St. Paul in 2006 revealed highly significant effects of RIL, environment, and RIL × environment interaction ($P < 0.001$). However, analysis of variance using only two environments indicated that RIL × environment effect was due to the difference between the St. Paul and Mexican locations. The RIL × environment interaction was significant when 2006 St. Paul and 2006 El Batan ($P < 0.001$) or 2006 St. Paul and 2005 Obregon were analyzed ($P < 0.001$), but nonsignificant in analyzing El Batan and Obregon ($P = 0.43$). The significant difference between 2006 St. Paul and the Mexican environments is further supported by the moderate correlation coefficients of the means of rust severity of the RILs between 2006 St. Paul and 2006 El Batan ($r = 0.45$, $P < 0.001$; Table 4) and 2006 St. Paul and 2005 Obregon ($r = 0.45$, $P < 0.001$) and very high correlation between 2005 Obregon and 2006 El Batan ($r = 0.99$, $P < 0.001$). The correlation coefficient of leaf rust severity between 2005 St Paul and means of rust severity in the Mexican locations was higher ($r = 0.63$, $P < 0.001$) than between 2006 St. Paul and the two Mexican locations. The correlation coefficient of leaf rust severity between 2005 St. Paul and mean of 2006 St. Paul nursery was high ($r = 0.83$, $P < 0.001$).

There was a continuous distribution of leaf rust severity from 0 to 100% for lines

Table 3. Greenhouse seedling infection types and field leaf rust severities of 14 'Jupateco 73S/Brambling' $F_{5:7}$ recombinant inbred lines (RILs) and three 'Thatcher' near-isogenic lines for leaf rust resistance to five races of *Puccinia triticina*

	Infection type[a]					Leaf rust severity (%)[b]				
	Isolates					Field environment				
Lines[c]	BBBD	THBJ	MHDS	SBDG	PBLR	Obregon 2005	El Batan 2006	St. Paul 2005	St. Paul 2006	Seedling genes
Thatcher	33^+	3	33^+	33^+	3	…[d]	…	40–80	…	
Jupateco 73S	0;	0;	3^+	3^+	123	100	100	100	50–70	*Lr17a*
Brambling	123	22^+	;1	0;	$;1^-$	0–1	0–1	0–1	5–10	*Lr14a*, *Lr23*
RL6013 *Lr14a*	33^+	3	3^+	0;	$;1^-$	…	…	50–70	…	
RL6008 *Lr17a*	$;2^+3$	;2	3^+	3^+	;123	…	…	20–70	…	
RL6012 *Lr23*	$;2^+3$	2^+3	0;	$;1^-$	33^+	…	…	5–10	…	
RL6058 *Lr34*	3^+	33^+	3^+	33^+	…	…	…	5–10	…	
RIL117	3^+	3^+	3^+	0;	0;	100	100	100	90	*Lr14a*
RIL128	3^+	3^+	3^+	$0;/3^+$	$;123/3^+$	100	100	100	90	*Lr14a* heterozygous
RIL105	0;	0;	3^+	3	0;	100	100	100	80	*Lr17a*
RIL119	0;	0;	3^+	3^+	0;	100	100	100	60	*Lr17a*
RIL144	0;	0;	3^+	3^+	0;	90	100	100	50	*Lr17a*
RIL92	;123	2^+3	0;	0;	3^+	5	5	10	18	*Lr23*
RIL14	0;	0;	3^+	0;	0;	100	100	100	60	*Lr17a*, *Lr14a*
RIL57	;123	2^+3	0;	0;	0;	100	100	20	15	*Lr23*, *Lr14a*
RIL104	;123	2^+3	0;	0;	0;	100	100	20	15	*Lr23*, *Lr14a*
RIL93	0;	0;	0;	0;	0;	100	100	50	20	*Lr17a, Lr23,* (*Lr14a*?)[e]
RIL113	0;	0;	0;	0;	0;	100	100	60	30	*Lr17a*, *Lr23*, (*Lr14a*?)[e]
RIL34	3^+	3^+	3^+	3	3^+	100	100	100	90	None
RIL82	3^+	3^+	3^+	3^+	3^+	100	100	100	90	None
RIL106	3^+	3^+	3^+	3^+	3^+	100	90	100	40	None

[a] Infection type follows Long and Kolmer (17).
[b] Leaf rust severity follows Peterson et al. (26).
[c] None of these RILs contain *Lr34* according to our *csLV34* marker results.
[d] … Indicates data not available.
[e] The presence or absence of *Lr14a* in the RIL cannot be postulated by the isolates used in this study.

in each segregating generation in the field nurseries (Fig. 1). However, the disease severity was significantly skewed toward lines with low leaf rust severities. The $F_{4:5}$ and $F_{4:6}$ lines were considered as the same genotypes. In Mexico, in the $F_{4:5}$ and $F_{4:6}$ lines, 23% had 0 to 1% disease severity, which is similar to Brambling (Fig. 1A). Sixty percent of the lines had average rust severity of 0 to 10%. In the 2005–2006 Mexico environment, 14% of $F_{5:7}$ RILs had disease severity of 0 to 1% and 64% of the RILs had average rust severity of 0 to 10%. The same trend was observed in the $F_{4:6}$ and $F_{5:7}$ RILs grown in St. Paul (Fig. 1B). In St. Paul, 28% of the $F_{4:6}$ RILs and 15% of the $F_{5:7}$ RILs had rust severity of 0 to 5%, in the range of Brambling, and 40% of the $F_{4:6}$ RILs and 30% of $F_{5:7}$ RILs had rust severity of 0 to 10%. The F_1 had an average of 5% rust severity. The skewness toward low rust severity and F_1 severities suggested that Brambling had APR gene(s) of partial dominance and large effects to reduce rust severity. A chi-squared test was performed using the ratio of lines phenotypically similar to the susceptible parent Jupateco 73S and lines with some field resistance. In Mexico, the ratio of number of susceptible lines (100% leaf rust) to number of lines with some field resistance in $F_{2:3}$, $F_{4:6}$, and $F_{5:7}$ fit a three-gene segregation ratio (Table 5). The segregation ratio in $F_{4:5}$ fit a two-gene model better than a three-gene model. The high number of susceptible lines in $F_{4:5}$ tested in 2003 was likely because of higher than normal temperature in the rust development period in Obregon in 2003. In Mexico, the temperature during rust development period (1 February to 15 March in Obregon and 1 July to 15 August in El Batan) ranged from 9 to 25°C, with an average of 16°C. In 2003, the maximum temperature from 1 March to 15 March was above 30°C. In hot weather, leaf rust infected leaves became necrotic in a short period of time and moderately susceptible plants tend to resemble plants with high terminal disease severity. Nine F_4-derived susceptible lines with 100 S in 2003 were 100 S in 2004. The other nine F_4-derived lines scored as 100 S in 2003 had 40 to 90 MSS severity and response in 2004. Those nine lines apparently had gene(s) that were not very effective under high temperature. At the Mexico locations, on the basis of leaf rust severity of $F_{2:3}$, $F_{4:5}$, $F_{4:6}$, and $F_{5:7}$ RILs obtained from 2003–2005, we conclude that leaf rust resistance in Brambling was due to three genes. In St. Paul, the ratio of susceptible lines to lines with some resistance in $F_{4:6}$ and $F_{5:7}$ fit both three and four independent genes segregation models with a higher probability for four genes (Table 5).

Eight of the nine $F_{5:7}$ RILs that scored as 100 S in 2005 at St. Paul also had 100 S severity and response in Mexico (Table 3). The other four $F_{5:7}$ RILs that were 100 S in Mexico, but had lower leaf rust severities in St. Paul, were postulated to carry *Lr23* (Table 3). Leaf rust severities of lines that lacked *Lr34* in Table 3 indicated that *Lr17a* and *Lr14a* were not effective in St. Paul or Mexico. On the basis of the seedling and field results, we concluded that adult-plant leaf rust resistance in Brambling was due to three genes in Mexico, and most likely due to four genes in St. Paul field nurseries. The extra effective gene in St. Paul was likely *Lr23*.

Since *Lr23* was effective in St. Paul, the effect of the two APR genes could be studied in lines without *Lr23* and *Lr34*. The correlation coefficient of rust severity of *lr23lr23lr34lr34* $F_{5:7}$ RILs in 2005 at St. Paul with rust severity of the other environments was higher with the Mexican environments (r = 0.84 - 0.85; Table 4) than with the 2006 St. Paul nursery (r = 0.73). In St. Paul, the same leaf rust isolates were used in 2005 and 2006. The lower correlation between the two years in

Table 4. Correlation coefficients for leaf rust severity of 136 $F_{5:7}$ recombinant inbred lines (RILs) and a subset of the $F_{5:7}$ RILs lacking *Lr23* and *Lr34* (*lr23lr23lr34lr34*) genotype tested in four environments

Environment	Genotype	St. Paul 2006[a]	Obregon, Mexico[a] 2005	El Batan, Mexico[a] 2006
St. Paul 2005[b]	All RILs	0.83***[c]	0.63***	0.63***
	lr23lr23lr34lr34	0.73***	0.84***	0.85***
St. Paul 2006	All RILs		0.45***	0.45***
	lr23lr23lr34lr34		0.46***	0.47***
Obregon 2005	All RILs			0.99***
	lr23lr23lr34lr34			0.98***

[a] Using mean leaf rust severity of two replications.
[b] Using leaf rust severity of one replication.
[c] *** Indicates significant at $P < 0.001$.

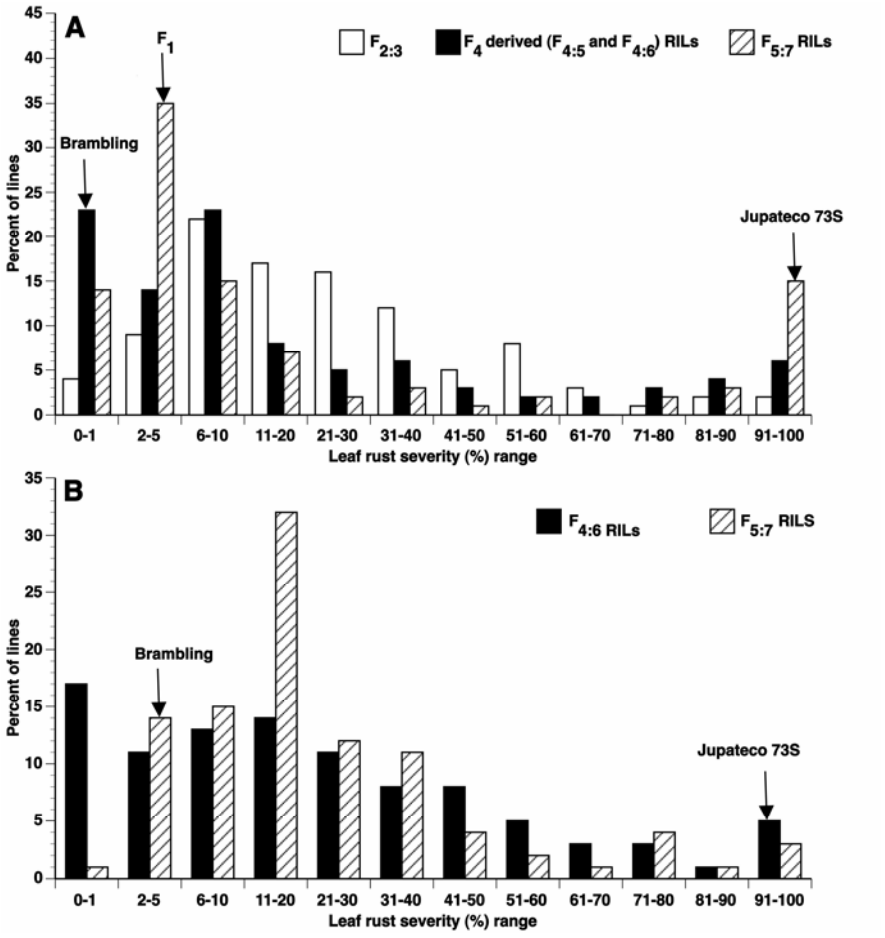

Fig. 1. Frequency distribution of leaf rust severity of $F_{2:3}$ lines, F_4-derived ($F_{4:5}$ and $F_{4:6}$), and $F_{5:7}$ recombinant inbred lines (RILs) of Jupateco 73S/Brambling evaluated in **A,** Mexico and **B,** St. Paul, MN. Data of F_4-derived RILs planted in Mexico were based on means of $F_{4:5}$ in 2003 and $F_{4:6}$ in 2004 in Ciudad Obregon. Data of $F_{5:7}$ RILs in Mexico were based on means of Ciudad Obregon in 2005 and El Batan in 2006. Data of $F_{5:7}$ RILs in St. Paul, MN were based on means of 2005 and 2006.

St. Paul was most likely because of the negative effect of high temperature and dry weather that occurred in 2006. This was also supported by the moderate correlation coefficients between 2006 St. Paul and the two Mexican locations ($r = 0.46, 0.47$).

In Mexico, the $F_{5:7}$ RILs without *Lr34* displayed continuous distribution of leaf rust severity with most lines skewed toward either of the parents (Fig. 2A). Ten of those 53 lines that lacked *Lr34* had a level of resistance similar to Brambling in Mexico (Fig. 2A). The high leaf rust resistance in those lines was most likely due to the combination of the two APR genes. The presence of lines with intermediate low to intermediate high leaf rust severities also indicated that those two genes might have unequal effects in reducing leaf rust severity. In St. Paul, the RILs that lacked *Lr23* and *Lr34* also displayed a continuous distribution of leaf rust severity (Fig. 2B).

DISCUSSION

The seedling and adult-plant field rust studies and use of the molecular marker *csLV34* allowed us to postulate that Jupateco 73S has leaf rust resistance gene *Lr17a* and Brambling has genes *Lr14a*, *Lr23*, *Lr34*, and two unknown APR genes that had an unequal effect on resistance. Using a collection of Mexican leaf rust isolates, Singh and Rajaram (37) postulated that Jupateco 73S had *Lr17a* and complementary genes *Lr27+Lr31* (35). The leaf rust isolates used in our seedling study were virulent to *Lr27+Lr31*. Jupateco 73S was highly susceptible at both field sites in Mexico where race MCJ/SP was used to initiate rust epidemics. It was also susceptible to a mixture of isolates used at St. Paul, MN nurseries. Thus, *Lr17a* and *Lr27+Lr31* in Jupateco 73S were not effective against the isolates used in Mexico and St. Paul. Gene *Lr14a* identified in Brambling also was not effective against races in our field studies.

The near-immune level of APR in Brambling was due to the combination of slow-rusting gene *Lr34* with two other APR genes at both Mexico and St. Paul with additional resistance conditioned by *Lr23* at St. Paul only. The APR genes in Bram-

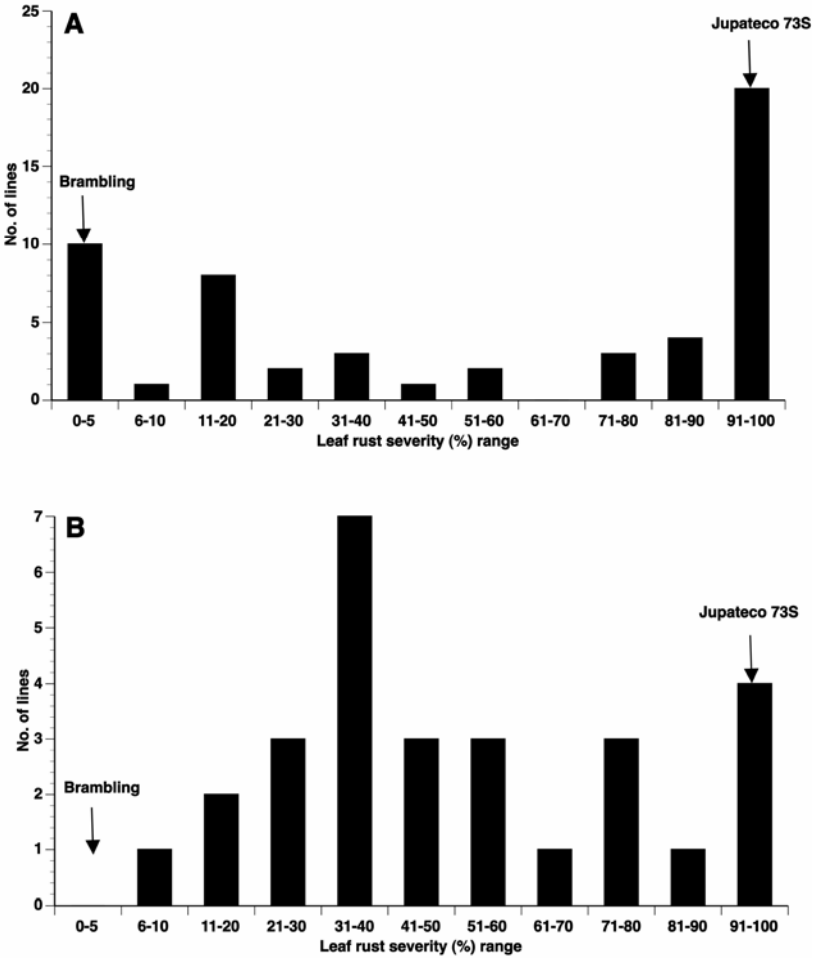

Fig. 2. Distribution of adult-plant leaf rust severity of 54 $F_{5:7}$ lines lacking *Lr34* evaluated in **A,** Mexico and 28 lines lacking *Lr23* and *Lr34* evaluated in **B,** St. Paul, MN. Mexico data was based on means of Ciudad Obregon in 2005 and El Batan in 2006. The St. Paul data was based on means of field trials in 2005 and 2006.

Table 5. Distribution and chi-square test of $F_{2:3}$ to $F_{5:7}$ generations of 'Jupateco 73S/Brambling' cross evaluated in seven field environments

Environment	Generation	No. of lines		Estimated gene number[a]	χ^2 value	*P* value
		Susceptible	Others			
Mexico						
Obregon 2003	$F_{2:3}$	5	232	3	0.46	0.50
Obregon 2003	$F_{4:5}$	18	115	2	2.70	0.10
				3	4.62	0.03
				4	36.77	<0.01
Obregon 2004	$F_{4:6}$	9	137	3	0.93	0.34
				4	31.39	<0.01
Obregon 2005	$F_{5:7}$	13	122	3	0.03	0.86
				4	7.19	0.01
El Batan 2006	$F_{5:7}$	13	122	3	0.03	0.86
				4	7.19	0.01s
St. Paul, MN						
2004	$F_{4:6}$	7	139	3	2.43	0.12
				4	0.53	0.47
2005	$F_{5:7}$	9	127	3	2.51	0.11
				4	1.04	0.31
2006	$F_{5:7}$	7	123	3	3.18	0.07
				4	0.13	0.72

[a] The ratio used for $F_{2:3}$ was 1 susceptible:63 others for three genes. The ratios used in $F_{4:5}$ and $F_{4:6}$ were 0.1914 susceptible:0.8086 others for two genes, 0.0837 susceptible:0.9163 others for three genes, and 0.0366 susceptible:0.9634 others for four genes. The ratios used in $F_{5:7}$ were 0.101 susceptible:0.899 others for three genes and 0.0473 susceptible:0.9527 others for four genes.

bling had dominant or strong partial dominant effects. The Thatcher lines with *Lr23* had moderate levels of leaf rust resistance in field plots at St. Paul. Our results indicated that, when effective, gene *Lr23* interacted with the APR gene *Lr34* and provided higher levels of APR than when those genes acted alone. When ineffective against corresponding virulent races, *Lr23* failed to interact with *Lr34*. Thus, there was not a residual effect of gene *Lr23*. A similar example of interaction where *Lr34* enhances the effect of *Lr13* and other adult-plant race-specific leaf rust resistance genes was reported earlier (7,8,10). In Mexico, when tested against races virulent to *Lr13* in the field, *Lr13* did not enhance the resistance of *Lr34* (30). *Lr23* is a common gene in U.S. spring wheat cultivars (13,24). Spring wheat cultivars with the combination of *Lr16*, *Lr23*, and *Lr34* have displayed high levels of leaf rust resistance in the United States (13). In our study, the differential expression of *Lr23* in Mexico and St Paul was mainly due to the race specificity of *Lr23* rather than temperature sensitivity. The resistance gene *Lr23* was not effective against the race used in Mexico at the seedling stage and at the adult-plant stage in Mexican field nurseries and in controlled environment at a high temperature (R. P. Singh, *unpublished data*). *Lr23* was effective against one of the isolates (THBJ) used in St. Paul, MN.

Lr34 reduced leaf rust severities more in Mexico than in St. Paul, MN. The resistance conditioned by *Lr34* was in accordance with previous studies of 20 to 60% reduction in leaf rust severity (20,32). The different effects of *Lr34* in Mexico and St. Paul were possibly due to the temperature sensitivity of this gene. *Lr34* is reported to condition more resistance at low temperatures than at high temperatures (6,33). Compared with Mexican locations, the rust development period at St. Paul was much shorter and the daily temperature rose at a faster rate (daily temperature 16 to 20°C, average 22°C). The resistance of lines with *Lr34* in 2005 was typical of this gene at St. Paul and the rest of the U.S. spring wheat region (23). The higher rust severities on lines with *Lr34* in 2006 compared with 2005 at St. Paul was due to the abnormally hot, dry weather in 2006.

In Obregon, the F_1 of Brambling/Jupateco 73S had much lower disease severity than the mid parent value. The two APR genes combined with *Lr34* skewed the segregation of progeny toward Brambling phenotype. Similar levels of rust in the F_1 because of partial dominance of slow-rusting genes were reported in genetic studies of lines carrying three to four slow-rusting genes (22,30). However, the frequency of homozygous parental type resistance and homozygous parental type of susceptible lines in segregating generations in those studies were proportional according to an additive gene model. Previous studies indicated that lines with two slow-rusting genes had approximately 5 to 30% leaf rust severity (9,36), and immune levels of resistance was reached by combining three to four genes (22,30,31). This is the first report that the combination of the two slow-rusting genes other than *Lr34* had immune levels of resistance in the Mexico environments. Further quantitative trait loci (QTL) analysis should provide the chromosome location of these genes in Brambling.

Differential expression due to race specificity of gene *Lr23* was one of the main factors causing genotype × environment interaction between Mexico and St. Paul. Temperature differences might be another factor that caused genotype × environment interaction between St. Paul and Mexico. Pyramiding slow-rusting resistance genes can achieve stable high levels of resistance. The dominant nature of some of the slow-rusting resistance genes in Brambling are of great interest in breeding for durable rust resistance. This type of resistance will be easier to select for in segregating generations as smaller population sizes would be required than when using lesser effects.

ACKNOWLEDGMENTS

We thank Jackie Rudd, Texas Agricultural Experiment Station, Texas A&M University for his support of this research at the early stage of the population development.

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