Genetics of Leaf Rust Resistance in Brambling Wheat

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ABSTRACT


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 F1, F2, F4, F6, and F7 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. F1 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/Ocoronoi 86f/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 F1 and generations of F2, F3, F4, F5, and F7 RILs developed by the single-seed descent method were used in the field studies. The F5,7 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 *Lr8*, *Lr10*, *Lr14a*, and *Lr18*. Races BBBB (isolate name: Race 1), THBJ (99 ND 588-1), MHDS (03 OH 237), SBDG (Race 9), and PBLR (MI...
Infection typea

91-2) were used in testing the F5:7 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 F5:7 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, Lr50, and Lr8 were tested with each isolate.

Seeds were planted in 3.5-cm2 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 seedlings 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 F2* RILs with low ITs were classified as homoygous resistant, RILs with high ITs were classified as homoygous susceptible, and RILs segregating for high and low ITs were classified as segregating. The ratio of homozygous resistant, 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 cslLV34 (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 isolate for Lr34) were included as checks. DNA was extracted from leaf segments of five random plants of each check and F5:7 lines 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 cslLV34 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 F2*3 and F4*4, F4*6, and F5*7 RILs, each plot was planted with approximately 100 seeds.

In the 2002–2003 crop season, F1, 237 F2*3 lines, and 133 F4*5 RILs were planted at Ciudad Obregon. Each generation was planted as different experiments. The F1 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 F2*3 and F4*5 lines as standard checks. In 2004, F4*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 F5*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 F1 to F4 derived (F4*5 and F4*6) generations were planted with one replication. Experimental design for F5*7 RILs was a randomized complete block with two replications in each year and location.

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**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

<table>
<thead>
<tr>
<th>Wheat lines</th>
<th>Greenhouse isolates</th>
<th>Growth chamber isolates and temperatures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BBBD</td>
<td>THBJ</td>
</tr>
<tr>
<td>Brabbling</td>
<td>.123</td>
<td>.22*</td>
</tr>
<tr>
<td>Jupateco 73S</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thatcher</td>
<td>3*</td>
<td>3*</td>
</tr>
<tr>
<td>Tclr14a RL 6013</td>
<td>1</td>
<td>.2</td>
</tr>
<tr>
<td>Tclr17a RL 6008</td>
<td>1</td>
<td>.2</td>
</tr>
<tr>
<td>Tclr23 RL 6012</td>
<td>2*3</td>
<td>.2*3</td>
</tr>
<tr>
<td>Tclr14a RL 6058</td>
<td>3*</td>
<td>3*</td>
</tr>
</tbody>
</table>

*a Infection types follow Long and Kolmer (17).

*b ... Indicates data not available.

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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 MCI/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 MBBJ (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 MBBJ 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 F1 and F2, F2, F2, and F2, 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 χ² 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 χ² analysis. Analysis of variance of the mean terminal leaf rust severity in the F2, trials with two replications were conducted, treating each year and location combination as one environment. Pearson correlation coefficients of disease severity of the F2, 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 F2, RIL in each environment.

**RESULTS**

**Seedling leaf rust resistance genes in Brambling and Jupateco 73S.** Jupateco 73S had low ITs 0; to 123 to isolate BBBD, THBJ, PBLR, TLGF, and high IT of 3+ to isolates MHDS, SBGD, 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 23+ to all isolates tested in Table 1, except for high IT to isolate MBDS (00 SD 520). Isolates BBBD, THBJ, MBDS, and TLGF (00 SD 218) were virulent to the Thatcher line with Lr14a (RL6013). Isolates SBGD 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 F5, 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 F5, with two independent dominant genes for a trait (Table 2). The F5, 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 MBDS. The F5, RILs segregated to MBDS in a ratio not different from the expected 0.4688:0.4688:0.0625 ratio for F5, for a single dominant trait that suggested that one gene in Brambling conditioned resistance to MBDS. Isolate SBGD had low ITs to Thatcher lines with Lr14a and Lr23 and the F5, RILs segregated 88:29:14 for resistant, susceptible, and segregating families, which conformed to a 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 F5, RILs in resistant, susceptible, and segregating categories was in accordance with a 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 SBGD 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 SBGD 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 SBGD. RIL 92 was postulated to have Lr23 because it had IT 123 to isolate BBBD, IT 23+ to THBJ, and; to isolates MHDS and SBGD and high IT to PBLR. RIL 14 was postulated to have Lr14a and Lr17a since it had IT 0; to isolates BBBD, THBJ, SBGD, 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 isolate BBBD, IT 23+ to THBJ, and Lr14a only because these lines had IT 0; to isolates BBBD, THBJ, SBGD, PBLR, and IT 33+ to MHDS. RIL 93 and RIL 113 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

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**Table 2. Segregation of a DNA marker diagnostic for Lr34 and leaf rust resistance in greenhouse tests of seedling plants of F5 recombinant inbred lines from the cross Jupateco 73S/Brambling**

<table>
<thead>
<tr>
<th>Leaf rust races/molecular marker</th>
<th>Gene(s) detected</th>
<th>No. of lines</th>
<th>Res.</th>
<th>Sus.</th>
<th>Seg.</th>
<th>Expected ratio</th>
<th>χ² value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>csLV34</td>
<td>Lr34</td>
<td></td>
<td>68</td>
<td>54</td>
<td>13</td>
<td>0.4688:0.4688:0.0625</td>
<td>4.20</td>
<td>0.12</td>
</tr>
<tr>
<td>BBBD</td>
<td>Lr17a, Lr23</td>
<td>87</td>
<td>34</td>
<td>11</td>
<td></td>
<td>0.7178:0.2197:0.0625</td>
<td>2.50</td>
<td>0.29</td>
</tr>
<tr>
<td>THBJ</td>
<td>Lr17a, Lr23</td>
<td>86</td>
<td>33</td>
<td>11</td>
<td></td>
<td>0.7178:0.2197:0.0625</td>
<td>2.28</td>
<td>0.32</td>
</tr>
<tr>
<td>MHDS</td>
<td>Lr23</td>
<td>53</td>
<td>70</td>
<td>8</td>
<td></td>
<td>0.4688:0.4688:0.0625</td>
<td>2.36</td>
<td>0.31</td>
</tr>
<tr>
<td>SBGD</td>
<td>Lr14a, Lr23</td>
<td>88</td>
<td>29</td>
<td>14</td>
<td></td>
<td>0.7178:0.2197:0.0625</td>
<td>4.45</td>
<td>0.10</td>
</tr>
<tr>
<td>PBLR</td>
<td>Lr14a, Lr17a</td>
<td>94</td>
<td>29</td>
<td>12</td>
<td></td>
<td>0.7178:0.2197:0.0625</td>
<td>1.61</td>
<td>0.45</td>
</tr>
</tbody>
</table>

* 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.*

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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 2005 than in 2006 (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 non-significant 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

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**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*

<table>
<thead>
<tr>
<th>Linesa</th>
<th>Infection typeb</th>
<th>Field leaf rust severity (%)b</th>
<th>Field environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBBD</td>
<td>THBJ</td>
<td>MHDS</td>
<td>SBDG</td>
</tr>
<tr>
<td>Th却cher</td>
<td>33+</td>
<td>3</td>
<td>33+</td>
</tr>
<tr>
<td>Jupateco 73S</td>
<td>0;</td>
<td>0;</td>
<td>3+</td>
</tr>
<tr>
<td>Brambling</td>
<td>123</td>
<td>22+</td>
<td>;1</td>
</tr>
<tr>
<td>RIL6013 Lr14a</td>
<td>33+</td>
<td>3</td>
<td>3+</td>
</tr>
<tr>
<td>RIL6008 Lr17a</td>
<td>;2+</td>
<td>2</td>
<td>3+</td>
</tr>
<tr>
<td>RIL6012 Lr23</td>
<td>;2+</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td>RIL6058 Lr34</td>
<td>3+</td>
<td>33+</td>
<td>3+</td>
</tr>
<tr>
<td>RIL117</td>
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<td>0;</td>
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<tr>
<td>RIL92</td>
<td>;123</td>
<td>2+</td>
<td>3</td>
</tr>
<tr>
<td>RIL14</td>
<td>0;</td>
<td>0;</td>
<td>3+</td>
</tr>
<tr>
<td>RIL57</td>
<td>;123</td>
<td>2+</td>
<td>3</td>
</tr>
<tr>
<td>RIL104</td>
<td>;123</td>
<td>2+</td>
<td>3</td>
</tr>
<tr>
<td>RIL93</td>
<td>0;</td>
<td>0;</td>
<td>0;</td>
</tr>
<tr>
<td>RIL113</td>
<td>0;</td>
<td>0;</td>
<td>0;</td>
</tr>
<tr>
<td>RIL34</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>RIL82</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>RIL106</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
</tr>
</tbody>
</table>

* Infection type follows Long and Kolmer (17).
* Leaf rust severity follows Peterson et al. (26).
* None of these RILs contain Lr34 according to our csLV34 marker results.
* Indicates data not available.
* 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$_2$-derived susceptible lines with 100 S in 2003 were 100 S in 2004. The other nine F$_2$-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 lrr23lrr23lrr34lrr34 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 (lrr23lrr23lrr34lrr34) genotype tested in four environments

<table>
<thead>
<tr>
<th>Environment</th>
<th>Genotype</th>
<th>St. Paul 2006a</th>
<th>Obregon, Mexico b 2005</th>
<th>El Batan, Mexico b 2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Paul 2005a</td>
<td>All RILs</td>
<td>0.83**</td>
<td>0.63***</td>
<td>0.63***</td>
</tr>
<tr>
<td></td>
<td>lrr23lrr23lrr34lrr34</td>
<td>0.73***</td>
<td>0.84***</td>
<td>0.85***</td>
</tr>
<tr>
<td>St. Paul 2006b</td>
<td>All RILs</td>
<td>0.45***</td>
<td>0.45**</td>
<td>-</td>
</tr>
<tr>
<td>Obregon 2005</td>
<td>All RILs</td>
<td>0.46***</td>
<td>0.47***</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>lrr23lrr23lrr34lrr34</td>
<td>0.99***</td>
<td>0.98***</td>
<td>-</td>
</tr>
</tbody>
</table>

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

![Table 5. Distribution and chi-square test of F2:3 to F5:7 generations of ‘Jupateco 73S/Brambling’ cross evaluated in seven field environments](image)

<table>
<thead>
<tr>
<th>Environment</th>
<th>Generation</th>
<th>No. of lines</th>
<th>Susceptible</th>
<th>Others</th>
<th>Estimated gene number*</th>
<th>$\chi^2$ value</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mexico</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obregon 2003</td>
<td>F$_{2:3}$</td>
<td>5</td>
<td>232</td>
<td>3</td>
<td>0.46</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Obregon 2003</td>
<td>F$_{4:5}$</td>
<td>18</td>
<td>115</td>
<td>2</td>
<td>2.70</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Obregon 2004</td>
<td>F$_{4:6}$</td>
<td>9</td>
<td>137</td>
<td>3</td>
<td>4.62</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Obregon 2005</td>
<td>F$_{5:7}$</td>
<td>13</td>
<td>122</td>
<td>3</td>
<td>36.77</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>El Batan 2006</td>
<td>F$_{5:7}$</td>
<td>13</td>
<td>122</td>
<td>3</td>
<td>0.93</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>St. Paul, MN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>F$_{4:6}$</td>
<td>7</td>
<td>139</td>
<td>3</td>
<td>31.39</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>F$_{5:7}$</td>
<td>9</td>
<td>127</td>
<td>3</td>
<td>7.19</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>F$_{5:7}$</td>
<td>7</td>
<td>123</td>
<td>3</td>
<td>0.13</td>
<td>0.72</td>
<td></td>
</tr>
</tbody>
</table>

*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.
bbling had dominant or strong partial domi-
nant effects. The Thatcher lines with
Lr23 had moderate levels of leaf rust resistance
in field plots at St. Paul. Our results indi-
cated that, when effective, gene Lr23 inter-
acted with the APR gene Lr34 and pro-
vided higher levels of APR than when those
genes acted alone. When ineffective against
concerning virulent races, Lr23 failed to inter-
act 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 viru-
ulent to Lr13 in the field, Lr13 did not en-
hance the resistance of Lr34 (30). Lr23 is
a common gene in U.S. spring wheat cul-
var. Spring wheat cultivars with the combina-
tion of Lr16, Lr23, and Lr34 have displayed
high levels of leaf rust re-

sistance in the United States (13). In our
study, the differential expression of Lr23 in
Mexico and St Paul was mainly due to the
to 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 nur-
series and in controlled environment at a
high temperature (R. P. Singh, unpublished
data). Lr23 was effective against one of the
isolates (THB3) used in St. Paul, MN.

Lr34 reduced leaf rust severities more in
Mexico than in St. Paul, MN. The resis-
tance conditioned by Lr34 was in accor-
dance with previous studies of 20 to 60% red-
uction in leaf rust severity (20,32). The
different effects of Lr34 in Mexico and St.
Paul were possibly due to the temperature
sensitivity of the gene. Lr34 is reported to
condition more resistance at low tempera-
tures than at high temperatures (6,33).

Comparing 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 F1 of Bram-
bbling/Jupateco 73S had much lower dis-
 ease 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 F1, 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 propor-
tional 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 quan-
titative 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 × envi-
ronment interaction between Mexico and St.
Paul. Temperature differences might be
another factor that caused genotype × envi-
ronment interaction between St. Paul and
Mexico. Pyramiding slow-rusting resis-
tance genes will 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
during 2006. The resistance to leaf rust in
Mexico and St Paul was mainly due to the
temperature sensitivity. The resistance
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