Molecular Mapping and Improvement of Leaf Rust Resistance in Wheat Breeding Lines

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ABSTRACT

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Leaf rust, caused by *Puccinia triticina*, is the most common and widespread disease of wheat (*Triticum aestivum*) worldwide. Deployment of host-plant resistance is one of the strategies to reduce losses due to leaf rust disease. The objective of this study was to map genes for adult-plant resistance to leaf rust in a recombinant inbred line (RIL) population originating from MN98550-5/MN99394-1. The mapping population of 139 RILs and five checks were evaluated in 2005, 2009, and 2010 in five environments. Natural infection occurred in the 2005 trials and trials in 2009 and 2010 were inoculated with leaf rust. Four quantitative trait loci (QTL) on chromosomes 2BS, 2DS, 7AL, and 7DS were detected. The

QTL on 2BS explained up to 33.6% of the phenotypic variation in leaf rust response, whereas the QTL on 2DS, 7AL, and 7DS explained up to 15.7, 8.1, and 34.2%, respectively. Seedling infection type tests conducted with *P. triticina* races BBBB and SBDG confirmed that the QTL on 2BS and 2DS were *Lr16* and *Lr2a*, respectively, and these genes were expressed in the seedling and field plot tests. The *Lr2a* gene mapped at the same location as *Sr6*. The QTL on 7DS was *Lr34*. The QTL on 7AL is a new QTL for leaf rust resistance. The joint effects of all four QTL explained 74% of the total phenotypic variation in leaf rust severity. Analysis of different combinations of QTL showed that the RILs containing all four or three of the QTL had the lowest average leaf rust severity in all five environments. Deployment of these QTL in combination or with other effective genes will lead to successful control of leaf rust.

Leaf rust, caused by *Puccinia triticina* Erikss., is the most common and widespread disease of wheat (*Triticum aestivum* L.) worldwide. Development of wheat cultivars resistant to leaf rust is the most practical method to control this disease (18,24). However, it has been difficult to achieve highly effective, durable resistance to leaf rust, mainly due to the high degree of virulence variation in the *P. triticina* population and the rapid increase of races with virulence to leaf rust genes in wheat cultivars (1,9). In North America, commercial wheat cultivars with *Lr21* were effectively resistant to leaf rust until a race with virulence to the gene was discovered in 2010 (7). As reported by Kolmer and Anderson (7), the new race with virulence to *Lr21* posed a threat to wheat production because >50% of spring wheat acreage in Minnesota and North Dakota relied on *Lr21* for effective resistance to leaf rust.

The release of improved cultivars with resistance to multiple diseases is emphasized in the University of Minnesota wheat breeding program. One of the most effective strategies for durable resistance has been the deployment of multiple resistance genes to increase broad-spectrum resistance to several races and the use of race non-specific genes that provide adult-plant resistance (APR) (20,22). For leaf rust, Kolmer et al. (9) reported that hard red spring wheat cultivars with resistance genes *Lr16*, *Lr23*, and *Lr34* remained highly resistant for several years in Minnesota and the Dakotas. To date, these genes still provide highly effective resistance when deployed together. The continual selection of gene combinations in a single genotype was difficult or even impossi-

ble when one or more genes in the background are highly effective against many races of the pathogen. Spring wheat cultivars with *Lr21* were highly resistant before the increase of races with virulence to this gene. The *Lr21* resistance precluded expression of any other effective resistance genes. To overcome this challenge of selecting for multiple genes, marker-assisted selection is being used for several disease resistance genes (<http://maswheat.ucdavis.edu/>).

Both MN98550-5 and MN99394-1 are hard red spring wheat breeding lines developed at the University of Minnesota and have different levels of resistance to leaf rust under natural field conditions. The objective of this study was to map genes for APR to leaf rust using the recombinant inbred line (RIL) population originating from MN98550-5 or MN99394-1.

MATERIALS AND METHODS

Plant materials. A population of 139 RILs ($F_{6,8}$) was developed from a single F_1 plant derived from a cross between the two University of Minnesota breeding lines MN98550-5 and MN99394-1 (28). The primary purpose of creating this RIL population was to map quantitative trait loci (QTL) for end-use quality traits. However, this population also has shown good segregation for APR to leaf rust. Parents, RILs, and three checks were evaluated for leaf rust resistance in five Minnesota field environments in 2005 (Crookston and Morris), 2009 (St. Paul), and 2010 (Crookston and St. Paul). The parents and checks were replicated four times in each environment and a single replication of each RIL was evaluated. In 2005, plants were grown in yield plots 2.6 m² in size. In the 2009 and 2010 growing seasons, plants were grown in single-row plots of 2-m rows with 30 cm between plots and 2-m alleys. Trials at all locations were sown in April and evaluated for leaf rust reaction in July.

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Leaf rust evaluation at adult plant stage. Natural infection occurred in 2005 at both Crookston and Morris. In 2009 and 2010, leaf rust epidemics were initiated by controlled inoculation with susceptible rust spreaders planted as borders. Susceptible spreader rows of wheat were artificially inoculated with a mixture of *P. triticina* races that had common virulence phenotypes in the Great Plains region (10). Lines were descriptively scored for severity and response (e.g. 10R, 20MR,...,100S) using the modified Cobb scale (21), where numbers indicate the percent leaf area infected with leaf rust, rust response of R is for small uredinia surrounded by necrosis, MR is moderate size uredinia with necrosis, MS is medium size uredinia without necrosis, and S is large uredinia without necrosis. Plants were also scored using a quantitative scale of 0 to 10, representing highly resistant (scores 0 to 2), resistant (score 3), moderately resistant (scores 4 to 5), moderately susceptible (scores 6 to 7), susceptible (score 8), and highly susceptible (scores 9 to 10). The scores for quantitative scale were used for the analysis of variance, correlation, and QTL analyses, as described in the statistical analyses section.

Leaf rust evaluations at seedling stage. The seedling tests were conducted to determine whether the population segregated for race-specific resistance genes that are effective in seedling plants. The whole RIL population, parents, and check varieties were inoculated with *P. triticina* races BBBD (virulent to *Lr14*) and SBDG (virulent to *Lr1*, *Lr2a*, *Lr2c*, *Lr17*, and *Lr10*) based on the modified virulence nomenclature of Long and Kolmer (13) following previously described standard methods of growing seedlings, inoculation, and evaluation of infection types (8,10). Seedling infection types were scored 12 days after inoculation using a scale of 0 to 4, as described by Long and Kolmer (13), where infection types of 0 to 2 were considered resistant and infection types of 3 or 4 were susceptible.

Statistical analyses. Analysis of variance was performed using PROC GLM in SAS (version 9.1; SAS Institute Inc., Cary NC), with genotypes and environments as random effects. Because leaf rust evaluations were made on a single replication for the population in each of the five environments, the significance of the main effects of genotype and environment were tested using the genotype–environment mean square (MS_{ge}). The genotype–environment interaction was tested for significance using the error mean square that was estimated from five check genotypes that were replicated four times within environments according to an augmented design defined by Federer (3). Significant difference between the means of parents was tested using the Fisher's least significant difference (at 0.05) calculated from the error mean square. Variance components for genotype and genotype–environment interaction were used to obtain estimates of the broad-sense heritability h^2_B :

$$h^2_B = \frac{\sigma_g^2}{[\sigma_g^2 + (\sigma_{ge}^2/e) + (\sigma_e^2/re)]} \quad \text{or} \quad 1 - \frac{MS_{ge}}{MS_g}$$

where MS_g and MS_{ge} represent the genotype and genotype–environment mean squares, respectively, σ_g^2 is the genotypic variance = $(MS_g - MS_{ge})/(re)$, σ_{ge}^2 is the genotype–environment interaction variance = $(MS_{ge} - MS_e)/r$, σ_e^2 is the error variance = MS_e , r is number of replications, and e is number of environments. The phenotypic distribution of leaf rust ratings based on the mean of five environments was tested for normality using the Shapiro-Wilk statistic (23). Correlation analysis was performed using linear correlation coefficient (r) which was calculated between environments based on the values of each environment.

The QTL analysis for individual environments was performed using composite-interval mapping (CIM) in WinQTL Cartographer (30). A genetic linkage map with 531 simple-sequence repeat (SSR) and diversity array technology (DARt) marker loci that covered the whole genome was used. Details of molecular marker screening and genetic map construction in this population were previously described by Tsilo et al. (26). The locus for the stem rust resistance gene *Sr6* was included in the map (27). The initial CIM step was run by SrMapQtl for QTL discovery using stepwise regression, which was followed by a final CIM step performed by ZMapQtl with a walk speed of 2 centimorgans (cM). Data for each environment were analyzed separately. In all, 300 permutations were performed with a significance level of 0.05; however, to avoid obvious type II error, a putative QTL was declared when the logarithm of odds (LOD) score was ≥ 2.5 in at least two of the five environments. The QTL were named following the nomenclature described in the Catalogue of Gene Symbols for Wheat (<http://wheat.pw.usda.gov/GG2/Triticum/wgc/2008/>). The seedling infection types of RILs were classified as resistant or susceptible to races BBBD and SBDG and the χ^2 distribution analyses were used to test whether the observed segregation ratios conformed to expected Mendelian ratios. Segregating seedling resistance genes were mapped using Mapmaker computer program version 3.0b (12).

RESULTS AND DISCUSSION

Phenotypic distribution analysis of leaf rust severity on adult plants. Trait distribution for leaf rust infection showed transgressive segregation in all environments with a mean of 4.7, a minimum of 1.0, and maximum of 9.0 for values averaged across five environments (Table 1), suggesting more than one gene conditioned resistance and also indicating that alleles with positive effects were contributed from both parents. Under natural infection, the level of leaf rust infection was low and both parents showed resistance in 2005 locations (Morris and Crookston), with MN98550-5 more resistant than MN99394-1. The population segregated for resistance to leaf rust, with maximum susceptible ratings of 8 and 7 for Morris 2005 and Crookston 2005, respectively (Table 1). However, the maximum susceptible rating reached 10 in all three environments that were inoculated that allowed separation of moderately susceptible from highly susceptible

TABLE 1. Average phenotypic distribution of adult plant resistance to leaf rust in 139 recombinant-inbred lines (RILs) of MN98550-5 and MN99394-1 evaluated in five environments in Minnesota

Environment	RIL population ($n = 139$)			Normality ^x	Parental lines ^y	
	Mean	Minimum	Maximum		MN99394-1	MN98550-5
2005 Morris	4.5	2.0	8.0	$P < 0.001$	4.8 b	3.6 a
2005 Crookston	3.2	1.0	7.0	$P < 0.001$	4.0 b	2.8 a
2009 St. Paul	6.3	2.0	10.0	$P < 0.001$	4.1 a	6.3 b
2010 St. Paul	4.7	0.0	10.0	$P < 0.001$	3.5 a	6.8 b
2010 Crookston	4.8	0.0	10.0	$P < 0.001$	3.5 a	6.5 b
All ^z	4.7	1.0	9.0	$P < 0.001$	4.0 a	5.2 b

^x Phenotypic distributions were tested for normality using the Shapiro-Wilk statistic and P values are given.

^y Means of parents are significantly different if followed by different letters (least significant difference at 0.05).

^z Average scores from all environments.

lines. The minimum leaf rust rating scores were 0, 1, and 2, which are considered resistant. Under high infection pressure in St Paul (2009 and 2010) and Crookston (2010), the parent MN99394-1 was more resistant than MN98550-5, with a descriptive rating of 40R (resistance), which falls within quantitative ratings of 3 and 4 (Table 1). The M99394-1 line remained resistant in all five environments, with a mean rating of 4, while the MN98550-5 line had a mean rating of 5.2. Based on the analysis of variance, the mean squares for genotype (16.7) and environment (165.5) were highly significant at $P \leq 0.001$, and for genotype-environment interaction (1.7) was significant at $P \leq 0.01$. Heritability estimate for leaf rust severity was 0.90, indicating that resistance in this population was highly heritable and consistent across environments. Also, correlations among all five environments were highly significant ($P \leq 0.001$) and ranged from 0.63 to 0.81 (Table 2). Correlation values were higher among inoculated trials than naturally infected trials, meaning the leaf rust scores in all three environments under high infection pressure were more similar.

QTL analysis and gene identification for leaf rust resistance. Adult plant leaf rust severity scores from five environments were used for QTL mapping and analysis. Four QTL influenced leaf rust ratings and were detected on chromosomes 2BS, 2DS, 7AL, and 7DS (Table 3; Fig. 1A to D). The QTL on 2BS explained up to 33.6% of the phenotypic variation in leaf rust response, whereas the QTL on 2DS, 7AL, and 7DS explained up to 15.7, 8.1, and 34.2%, respectively.

The 7DS QTL mapped within the marker interval of *Lr34* (25,31). The sequence tagged site (STS) marker csLV34 linked to *Lr34* (11) was included on the map and it confirmed that the QTL on 7DS was *Lr34*. The 7AL QTL was linked to the *XwPt1601* marker loci and this region has not been identified previously as having QTL for leaf rust resistance. The resistance alleles for the 7AL QTL and *Lr34* were contributed by MN98550-5 and the additive allele effects of these two genes were influenced by environmental effects, because the total phenotypic variation explained by each QTL was not the same in all environments.

The QTL on 2BS and 2DS mapped to chromosome regions that were known to genes *Lr16* (15) and *Sr6* (27), respectively. *Sr6* is known to be linked to the *Lr2* locus and *Lr15* (16). To confirm that the identity of the genes on 2BS and 2DS were *Lr16* and an

allele at the *Lr2* locus, a seedling infection analysis was performed with two leaf rust races SBDG and BBBD. Both isolates are avirulent on *Lr16* and SBDG is virulent and BBBD is avirulent to alleles *Lr2a*, *Lr2b*, and *Lr2c* (8,13).

Seedling infection analysis of leaf rust infection types. The MN98550-5 line had high seedling infection types of 3⁺ and 4 to both isolates that were tested (Table 4). The MN99394-1 line had low seedling infection types of 0 (no pustules on the leaves) and ;12 (flecks with small to medium size pustules surrounded by necrosis) to isolates BBBD and SBDG, respectively (Table 4). The segregation ratio of resistant and susceptible RILs to race BBBD fitted the two-gene segregation ratio of 3:1. The segregation ratio to SBDG conformed to the one-gene segregation ratio of 1:1. When using Mapmaker 3.0b to map seedling resistance, the gene that gave resistance to both BBBD and SBDG was confirmed to be *Lr16*. Based on the map position on 2BS, this gene was flanked by *Xwpt7191* and *Xwmc382a* at distances of 14.0 and 3.1, respectively. McCartney et al. (15) mapped *Lr16* as the terminal locus at the distal end of 2BS in three mapping populations. The map position of *Lr16* in the current study was in agreement with McCartney et al. (15), and it mapped distal of all the SSR markers; however, the addition of DArT markers in the current map implied that *Lr16* was not the terminal locus on 2BS.

The gene that gave seedling resistance to BBBD but not to SBDG was confirmed to be an allele at the *Lr2* locus (Table 4). The *Lr2* locus, *Lr15*, and *Sr6* are known to be linked (16). *Lr15* was recently mapped in the vicinity of *Sr6* between *Xgwm4562* and *Xgwm102* by Dholakia et al. (2), and *Lr2* has not been mapped. The allele on 2DS in MN99394-1 is likely *Lr2a* because this gene is present in U.S. hard red spring wheat (18,19). MN99394-1 and the RILs with resistance on 2DS also had very low infection types of 0; which is a characteristic of *Lr2a* (13). The presence of an allele at the *Lr2* locus in this material was confirmed using the *Lr2a* virulent race SBDG with a 1:1 segregation, as expected for *Lr2a* ($\chi^2 = 1.03$, $P = 0.31$), in all the RILs that did not have *Lr16*. When using Mapmaker 3.0b to map the seedling resistance of *Lr2a*, the locus mapped to the same locus with *Sr6* and both were flanked by *Xwmc453* and *XwPt0330* at 1.1 and 2.8 cM, respectively. These results suggest that the *Lr2* locus and *Sr6* could be allelic or closely linked genes because no recombination was observed between the two genes. Also, in the same population, these two genes mapped 6.2 cM from *Xgwm102*, a marker that mapped 9.3 cM from *Lr15* based on the map of Dholakia et al. (2). With molecular mapping results of *Lr2*, *Sr6*, and *Lr15*, the 2DS region has multiple alleles for rust resistance and could be a candidate region for further studies. With a limited population size in this study, a larger population size would be required for fine mapping and resolving mapping distances between markers and QTL or genes, and also for detecting and separating QTL and genes that could be segregating or closely linked within a QTL peak.

In the current study, the resistance alleles for both *Lr2a* and *Lr16* were contributed by MN99394-1 and this line was highly

TABLE 2. Correlation coefficients of leaf rust infection on 139 recombinant-inbred lines of MN98550-5 and MN99394-1 evaluated in five environments in Minnesota²

Environment	2005 Morris	2005 Crookston	2009 St. Paul	2010 St. Paul
2005 Morris	1	...	...	...
2005 Crookston	0.72***	1	...	...
2009 St. Paul	0.64***	0.66***	1	...
2010 St. Paul	0.70***	0.66***	0.77***	1
2010 Crookston	0.63***	0.74***	0.81***	0.80***

² Asterisks (***) indicate significance at $P \leq 0.001$.

TABLE 3. Quantitative trait loci (QTL) and their closely linked marker loci identified for adult plant resistance to leaf rust in 139 recombinant-inbred lines of MN98550-5 and MN99394-1 evaluated in five environments in Minnesota²

Environment	<i>QLr.mna-2BS (Xwmc382a)</i>			<i>QLr.mna-2DS (Xwmc453)</i>			<i>QLr.mna-7AL (XwPt1601)</i>			<i>Lr34 (Xbarc92)</i>		
	LOD	$R^2 \times 100$	Add	LOD	$R^2 \times 100$	Add	LOD	$R^2 \times 100$	Add	LOD	$R^2 \times 100$	Add
2005 Morris	3.3	7.9	-0.47	2.7	6.1	-0.39	1.9	5.7	0.39	4.3	14.2	0.61
2005 Crookston	2.9	5.4	-0.26	1.8	2.8	-0.19	4.3	8.1	0.31	16.8	34.2	0.66
2009 St. Paul	9.6	24.6	-1.13	10.2	15.7	-0.84	3.9	6.9	0.56	10.1	16.4	0.89
2010 St. Paul	13.9	22.4	-1.21	6.3	9.0	-0.74	1.7	2.3	0.38	11.1	22.7	1.21
2010 Crookston	19.4	33.6	-1.98	5.1	5.5	-0.74	1.7	2.2	0.47	19.1	29.5	1.79
All ²	14.0	17.9	-0.85	9.1	10.7	-0.62	3.9	5.1	0.43	17.4	27.9	1.04

² LOD = logarithm of odds and Add = additive allele effects for the QTL. Positive and negative additive allele effects indicate that the favorable QTL alleles were contributed by MN98550-5 and MN99394-1, respectively.

² Average scores from all environments.

resistant at the seedling stage to races SBDG and BBBB, with infection types of 0 and 1, respectively. The resistance alleles for the 7AL QTL and *Lr34* were contributed by MN98550-5 and this parent was highly susceptible in the seedling stage, with infection types of 3⁺ and 4 against both races, and the same infection types were observed on RILs that had only the 7AL QTL or *Lr34*, indicating that the two genes were only expressed as APR genes and

were not expressed at the seedling stage. The multiple regression analysis of all the QTL for leaf rust showed that their joint effects explained $\approx 74\%$ of the phenotypic variation in leaf rust severity (Table 5).

The joint effects of these QTL were also clearly observable because most RILs that combined all four QTL and genes were highly resistant in most environments, with leaf rust ratings of 0,

Fig. 1. Logarithm of odds score curves on chromosome regions where quantitative trait loci (QTL) were detected. Each curve represents environment where QTL were detected: Mo = Morris, Cr = Crookston, St = St. Paul, and All = average scores from all five environments. **A**, Short arm of chromosome 2B; **B and C**, short arm of chromosome 2D and long arm of chromosome 7A; and **D**, short arm of chromosome 7D. QTL positions are marked black on the chromosomes.

1, and 2, and only a few of these RILs had scores of 3 and 4. When averaged across five environments and across a number of RILs per QTL combination, the leaf rust reaction of RILs with different combinations of these QTL was still significantly lower when compared with RILs with one or no QTL present (Table 6). However, the joint effects of 7AL QTL in addition to other QTL only became significant in the presence of 2DS QTL; for example, the average ratings were 2.0 versus 2.9 and 3.3 versus 5.0. In the absence of 2DS QTL, the joint effects of QTL combinations with or without 7AL were the same; for example, the average ratings were 3.2 versus 3.2 and 5.4 versus 5.4 (Table 6). The resistance-enhancing effects of *Lr34* on seedling and APR genes was also described by German and Kolmer (4), in which the Thatcher line with *Lr16* and *Lr34* had better resistance than the Thatcher isogenic lines with only one of these two genes. Oelke and Kolmer (19) showed that 'Norm' wheat with *Lr16*, *Lr23*, and *Lr34* was highly resistant to leaf rust. Vanzetti et al. (29) also found that the combinations of seedling resistance genes such as *Lr16*, *Lr47*, *Lr19*, *Lr41*, *Lr21*, *Lr25*, and *Lr29* with APR gene such as *Lr34*, *SV2*, and *Lr46* provided durable resistance to leaf rust in Argentina. Moullet et al. (17) pyramided two leaf rust resistance genes (*Lr9* and *Lr24*) using marker-assisted selection and showed that lines with combinations of these genes were highly resistant to leaf rust. Based on the current study and other literature, high levels of APR can be achieved using a combination of genes that, when deployed, singly offer little resistance. Marker-assisted selection can facilitate the identification of lines having effective combinations of such genes. Several APR genes

were previously reported for leaf rust and their linked markers are available to initiate pyramiding schemes. These include *Lr46* on chromosome 1BL (14), *Lr67* on chromosome 4DL (6), and *Lr68* on chromosome 7BL (5).

In this study, high leaf rust infection pressure imposed severe epidemics and facilitated excellent phenotyping which, when combined with multi-environment analysis, allowed the identification of stable QTL. Four QTL influenced resistance to leaf rust in this population of hard red spring wheat. Two QTL were confirmed to be *Lr2a* and *Lr16* based on seedling tests and the resistance was expressed at all stages of plant growth. *Lr2a* mapped at the same location as *Sr6* and the two genes are allelic or closely linked. *Lr16* mapped within two flanking markers. Lines with combinations of desirable alleles for the QTL showed a high level of resistance to leaf rust. These lines and QTL will be useful sources of breeding material and for pyramiding schemes that are aimed at increasing durable leaf rust resistance in wheat. With molecular markers available for most effective leaf rust resistance genes, it is possible to select or pyramid all genes together or in combination with other effective genes.

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TABLE 4. Segregation for leaf rust resistance in seedling tests of 139 recombinant-inbred lines (RILs) of MN98550-5 and MN99394-1

Leaf rust isolates	Number of RILs (infection types)		Ratio ^y	χ^2	P	Detected ^z	Parental lines	
	Resistant	Susceptible					MN99394-1	MN98550-5
BBBD	89 (:,0,1,2)	31 (3+,4)	3:1	0.01	0.92	<i>Lr2a</i> , <i>Lr16</i>	0	3+/4
SBDG	71 (:,0,1,2)	54 (3+,4)	1:1	2.04	0.15	<i>Lr16</i>	:1/2	3+/4

^y Expected ratio.

^z *Lr* genes detected.

TABLE 5. Total phenotypic variation explained by the joint-effects of quantitative trait loci (QTL) using multiple regression analysis on trait values of 139 recombinant inbred lines of MN98550-5 and MN99394-1 averaged across five environments

QTL	Position	Markers in the model	<i>b</i> ^z	<i>R</i> ²
<i>QLr.mna-2BS</i>	16.7 (2BS)	wmc382a	-2.05***	74.0
<i>QLr.mna-2DS</i>	5.0 (2DS)	wmc453	-1.14***	...
<i>QLr.mna-7AL</i>	0.01 (7AL)	wPt1601	0.71**	...
<i>Lr34</i>	55.9 (7DS)	barc92	1.60***	...

^z Regression coefficient for every QTL in the model. Positive regression coefficient indicates that the QTL alleles that increased the regression were contributed by MN98550-5 and negative means that the QTL alleles that increased the regression were contributed by MN99394-1. The percent phenotypic variation ($R^2 \times 100$) explained by joint-effects of QTL was provided; *** indicates significance at $P \leq 0.001$ and ** indicates significance at $P \leq 0.01$.

TABLE 6. Effects of multiple quantitative trait loci (QTL) on average leaf rust rating of MN98550-5 and MN99394-1 recombinant inbred lines (RILs) evaluated in five environments

QTL and their combinations	Number of RILs	Average leaf rust rating ^z
<i>Lr34</i> , <i>QLr.mna-2BS</i> , <i>QLr.mna-2DS</i> , and <i>QLr.mna-7AL</i>	9	2.0 a
<i>Lr34</i> , <i>QLr.mna-2BS</i> and <i>QLr.mna-2DS</i>	7	2.9 ab
<i>Lr34</i> , <i>QLr.mna-2BS</i> and <i>QLr.mna-7AL</i>	13	3.2 bc
<i>Lr34</i> and <i>QLr.mna-2BS</i>	11	3.2 bc
<i>QLr.mna-2BS</i> , <i>QLr.mna-2DS</i> , and <i>QLr.mna-7AL</i>	14	3.3 bcd
<i>Lr34</i> and <i>QLr.mna-2DS</i>	7	4.0 cde
<i>QLr.mna-2BS</i> and <i>QLr.mna-2DS</i>	6	5.0 ef
<i>QLr.mna-2BS</i> and <i>QLr.mna-7AL</i>	9	5.4 f
<i>QLr.mna-2BS</i>	15	5.4 f
<i>Lr34</i>	5	5.6 fg
<i>QLr.mna-2DS</i>	11	6.4 gh
<i>QLr.mna-7AL</i>	10	6.5 gh
Without QTL	17	7.0 h

^z Means of parents are significantly different if followed by different letters (least significant difference at 0.05). For means separation of QTL and QTL combinations, only those with ≥ 5 RILs were included in the analysis.

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