Genetic Analysis and Molecular Mapping of Leaf Rust Resistance Genes in the Wheat Line 5R618

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Abstract

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The wheat (*Triticum aestivum* L.) line 5R618, bred at the China Agricultural University, is resistant in the seedling stage to the majority of the current Chinese pathotypes of wheat leaf rust (*Puccinia triticina*). To identify and map the leaf rust resistance gene in the 5R618 line, F_2 plants and $F_{2:3}$ families from a cross between 5R618 and Zhengzhou5389 (susceptible) were inoculated in the greenhouse with the Chinese *P. triticina* pathotype THJP. Results from the F_2 and $F_{2:3}$ populations indicate that a single dominant gene, temporarily designated *Lr5R*, conferred resistance. Using the molecular marker method, *Lr5R* was located on the 3DL chromosome. It was closely linked to the markers *Xbarc71* and *OPJ-09* with genetic distances of 0.9 cM and 1.0 cM, respectively. At present only one designated gene (*Lr24*) is located on the 3DL chromosome. The genetic distance between *Lr5R* and *Lr24* confirms that *Lr5R* is a new leaf rust resistance gene.

Keywords: gene postulation; genetic mapping; molecular marker; wheat leaf rust

Wheat leaf rust is one of the main diseases affecting wheat production in China. It is adapted to a broad range of temperatures and occurs in most of China's wheat growing areas. Approximately 23.7 million hectares of wheat are planted in China each year, with a total output of about 109 million tons. Wheat leaf rust occurs on about 15 million hectares per year, especially in Western China, causing severe yield losses (Huerta-Espion *et al.* 2011). The use of resistant wheat varieties is the most effective method for reducing the harmful effects of leaf rust. China has a large number of wheat cultivars with leaf rust resistance, but little is known about the specific content of leaf rust resistance genes. Therefore, in this work, we explore resistant genes and, thus, contribute to the development of new cultivars. At present, more than 100 wheat leaf rust resistance genes have been found, among these 71 have been officially named (Singh *et al.* 2013). However, due to the continuous variation in leaf rust races and with large-scale cultivation of monogenic resistant wheat varieties, many leaf rust resistance genes have become ineffective. Thus, the exploration of new sources of wheat leaf rust resistance and the discovery of a tight chain of molecular markers is of great significance to disease-resistant genetic breeding research.

Molecular genetic markers are a direct reflection of the level of DNA polymorphism, and are widely used in gene mapping, genetic breeding, and identification of related species. A variety of molecular marker technologies has been applied to the study of leaf rust resistance genes. The technologies are optimized constantly with more stable and simpler molecular markers replacing the older more complicated markers with poor repeatability. Gold *et al.* (1999) transformed the ISSR marker of linkaging with *Lr35* into the SCAR marker. Prins *et al.* (2001) successfully used the AFLP marker for *Lr19*, and transformed it into an STS marker. Gupta *et al.* (2006) developed three SCAR markers co-segregating with *Lr24*. In a study of the location of the leaf rust gene (*LrZH84*), Zhou *et al.* (2013) developed the STS marker, *Hbsf-1*, to detect *LrZH84*

and *Lr26*. Simple sequence repeats (SSR) are a type of molecular marker; their advantages include high repeatability, lower DNA usage, and the ability to identify heterozygosity and homozygosity. In the study of wheat resistance genes, SSR marker linkage maps have been used increasingly as the key marker (Li *et al.* 2013). Zhao *et al.* (2008), Li *et al.* (2010), Zhang *et al.* (2011), Zhou *et al.* (2012) successfully used SSR molecular markers to map the leaf rust resistance gene.

The Chinese wheat line 5R618, developed by Yang Zuomin at China Agricultural University, resists leaf rust in the seedling stage. So far no reports of a leaf rust resistance gene in 5R618 have been published. The present work combines traditional hybridization, postulation, and molecular markers to map the leaf rust resistance gene in the Chinese wheat line 5R618 to provide a new source of resistance to molecular wheat breeding.

MATERIAL AND METHODS

Plant materials and *P. triticina* isolates. The resistant parent line 5R618, the susceptible parent Zhengzhou5389, and their F_2 plants and $F_{2:3}$ families were included in the genetic analysis. Thirty-six near-isogenic lines (Table 1) in the background of Thatcher with known resistance genes were kindly provided by the USDA-ARS Cereal Disease Laboratory, University of Minnesota, Saint Paul, USA. The fifteen *P. triticina* pathotypes used in gene postulation (Table 1) and genetic analysis are maintained at the Biological Control Center for Plant Diseases and Plant Pests of Hebei, Agricultural University of Hebei, China. The *P. triticina* races were named using the Prt-code System (Long *et al.* 1989).

Evaluation of seedling leaf rust responses. The 5R618, Zhengzhou5389, and 36 near-isogenic lines were inoculated with fifteen *P. triticina* pathotypes (Table 1) to compare the leaf rust reaction arrays. The 5R618 line, Zhengzhou5389, 254 F_2 plants and 236 $F_{2:3}$ families (20 seedlings each) were inoculated with Chinese *P. triticina* pathotype THJP (virulent on Zhengzhou5389 and avirulent on 5R618).

The seedlings were grown in a growth chamber. Inoculations were performed after the first leaf was fully expanded by brushing conidia from isolates of a fully infected susceptible genotype onto the seedlings. Inoculated seedlings were placed in plastic-covered cages and incubated at 18°C and 100% relative humidity (RH) for 24 h. They were then transferred to a growth chamber maintained with 12 h light/12 h darkness at 18–25°C with 70% RH. Infection types (IT) were scored 12–15 days after inoculation on a 0–4 scale (Bariana & McIntosh 1993). Plants with ITs of 0 to 2 were considered resistant, while those with ITs of 3 to 4 were considered susceptible. According to Dubin *et al.* (1989) the principle of gene postulation was proposed.

DNA extraction and bulk preparation. Genomic DNA was extracted from the seedlings of the $F_{2:3}$ family (bulked for each line) using the CTAB protocol (Sharp *et al.* 1988). The DNA was quantified with a UV spectrophotometer, and diluted to a final concentration of 30 ng/μl.

Bulked segregant analysis (Michelmore *et al.* 1991) was performed to identify the molecular marker putatively linked to the leaf rust resistance genes in 5R618. Genomic DNA from ten resistant and ten susceptible $F_{2:3}$ families was inoculated with THJP and mixed in equal amounts to form resistant and susceptible bulks. Samples of DNA from the two parents and bulks were screened for polymorphism in the molecular markers.

Molecular marker analyses. The 1021 wheat SSR loci surveyed included 341 gwm (Gatersleben wheat microsatellite) primer sequences described by Roder *et al.* (1998), 543 wmc primer sequences developed by the Wheat Microsatellite Consortium (wmc) (http://wheat.pw.usda.gov/ggpages/SSR/WMC), and 137 barc markers developed by Cregan associates (USDA-ARS Beltsville Agriculture Research Centre). All are listed at http://www.graingenes.org/. Three markers co-segregating with *Lr24* were surveyed, they included *STS24-16* (*STS24-16*F: 5'-CTTCGGACAGGAGGGTATGA-3', *STS24-16*R: 5'-GGACAGCTGTAAACGGGTTC-3') (Zhang *et al.* 2008), SCAR marker *OPJ-09* (*OPJ-09*F: 5'-TCTAGTCTGTACATGGGGGC-3', *OPJ-09*R: 5'-TGGCACATGAACTCCATACG-3') (Yuan *et al.* 2004), and SCAR marker *S1302-609* (*S1302-609*F: 5'-CGCAGGTTCCAAATACTTTTC-3', *S1302-609*R: 5'-CGCAGGTTCTACCTAATGCAA-3') (Gupta *et al.* 2006).

The SSR markers showing polymorphism between resistant and susceptible bulks were used to genotype individual $F_{2:3}$ families. With minor modifications, the microsatellite analysis followed the procedure described by Bryan *et al.* (1997). Polymerase chain reactions (PCR) were performed in volumes of 10 μl with 1.0 U *Taq* of DNA polymerase (Zexing Biotechnology Co. Ltd, Beijing, China); 1× PCR buffer (25mM KCl, 5mM Tris–HCl, 0.75mM $MgCl_2$, pH 8.3); 100μM each of dNTP, 3 pmol of each primer, and

Table 1. Seedling infection types in 5R618, Zhengzhou5389, and 36 near-isogenic lines with known leaf rust resistance to fifteen pathotypes of *Puccinia triticina*

Lr gene or line	Pathotype														
	PH JS	MH JS	FH DQ	FG BQ	FH BR	FH BQ	FG BR	TH JL	FH DR	FG DQ	FH DS	TH JP	TG TT	PH GN	TH JC
Lr1	4	4	;	;	;	;	;	4	0	;	0	4	4	4	4
Lr2a	;	;	1+	;	;	1	1	3	;	;	2	3	3	;	4
Lr2c	4	1	4	4	4	4	4	4	4	4	4	4	4	4	4
Lr3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Lr9	;	;	;	;	0;	0	0	;	0	0	;	;	;	0	;
Lr16	4	4	4	4	4	4	3+	3+	4	4	4	4	4	3	4
Lr24	;1	;	;	;	;	;	;	;	;	;	;	;	;	;	;
Lr26	4	4	4	1	4	4	;	4	4	1	4	4	2	4	4
Lr3ka	X	X	;	;	;	;	1	1	;	;	;	1	4	;	X
Lr11	4	4	1	;	;	1+	2	3+	1	1	2	4	3+	4	4
Lr17	4	3+	3+	2	2	2	2+	4	3+	4	4	4	4	2+	4
Lr30	3C	1	1	;	;	;	;	1	;	;	;	;	4	;	1
LrB	3+	4	4	4	3+	4	4	3+	4	4	4	4	4	4	X
Lr10	3	3	4	4	4	4	4	2	4	4	4	2+	4	1	X
Lr14a	4	4	X	X	X	X	X	X	X	2	4	4	4	3+	X
Lr18	1	1+	2	2	4	2	4	1+	4	2+	2	4	3+	3C	3
Lr2b	1	0;	4	;	3	3+	2	4	3	3+	3+	2	4	3C	4
Lr3bg	4	4	4	4	4	3+	4	4	4	4	4	4	4	4	4
Lr13	3	4	4	3	3	4	4	3	3	2	3+	4	4	4	4
Lr14b	4	4	4	4	4	4	4	4	4	4	4	X	4	X	4
Lr15	1	;	;	;	;	;	;	4	1	;	;	4	3+	4	4
Lr19	0	0	;	0	0	;	0	0	0	0	;	0	0	0	;
Lr20	4	4	;	;	;	;	0	;	;	;	;	4	1	4	;
Lr21	4	2	2	;	2+	3	2	;	1	;	1+	;	3	1	1
Lr23	4	4	4	3+	3+	4	3+	1	4	4	4	4	4	3+	4
Lr28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lr29	0	0	0	0	;	0	0	;	;	0	3+	4	;	0	0
Lr33	3	4	3+	3+	3+	4	2+	3C	3+	3	4	4	4	3+	3+
Lr36	4	2	1+	;	2	2	1	1	2	2+	3	2+	3+	2+	3+
Lr39	;	;1	;	;	;	;	;	;	;	;	;	;	;	;	;
Lr42	;	;	;	0	0	;	;	;	1	;	;	0	;	0	1
Lr44	1	;	4	4	4	4	4	1	4	4	4	;	1+	;1	1
Lr45	4	4	4	4	4	4	4	;	4	4	4	4	;	;	;
Lr47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lr51	;	;	;	;	1	;	0	;	;	;	;	0	;	;	;
Lr53	;	0	0	0	0	0	0	;	0	0	0	0	0	0	0
5R618	;	;	0	;	;	0	0	;	0	0	1+	;	0	;	0
Zhengzhou5389	4	4	4	4	3+	4	4	4	4	4	4	4	4	4	4

;, 0, 1, 2 – resistant; 3, 4 – susceptible; C – more chlorosis than normal for the infection type, + – uredinia somewhat larger than normal for the given infection type; X – small or large uredinia distributing on the leaf

Table 2. Segregation of seedling reactions to the pathotype THJP in the 5R618 and Zhengzhou5389 lines and their F_2 plants and $F_{2:3}$ families

Material	Total	Infection types			Chi-square tests
		resistant	susceptible	separate	
5R618	20	20			
Zhengzhou5389	20		20		
F_2 plants	254	183	71		$\chi^2_{3:1} = 1.03 < \chi^2_{0.05,\,1} = 3.84$
$F_{2:3}$ families	236	59	70	107	$\chi^2_{1:2:1} = 2.86 < \chi^2_{0.05,\,2} = 5.99$

30 ng of template DNA. The conditions of PCR were denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55–60°C (depending on the primer pair) for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR product was mixed with 2 ul of formamide loading buffer (98% formamide, 10mM EDTA, 0.25% bromophenol blue, 0.25% xylene cyanol, pH 8.0). The mixture was then loaded on 10% non-denaturing polyacrylamide gels or agarose electrophoresis.

Linkage analysis and genetic mapping. Phenotypic frequencies were tested for goodness-of-fit to postulated ratios using chi-squared tests. Linkage analysis was performed using MapManager QTXb20 software (Manly *et al.* 2001) and recombination values were converted to centiMorgans using the Kosambi mapping function (Kosambi 1944).

RESULTS

Inheritance of leaf rust resistance in 5R618. In the seedling test with the THJP race, 5R618 was resistant (IT: ;), whereas Zhengzhou5389 was susceptible (IT: 4). The 254 F_2 population segregated monogenically with 183 resistant plants (IT: ; to 2) and 71 susceptible plants (IT: 3 to 4) ($\chi^2_{3:1}$=1.03,1 df, $P > 0.05$), fitting a 3:1 ratio. When the 236 $F_{2:3}$ families from these F_2 plants were tested as seedlings, 59 lines were homozygous resistant, 107 were segregated, and 70 were homozygous susceptible ($\chi^2_{1:2:1}$=2.86, 2 df, $P > 0.05$), fitting a 1:2:1 ratio (Table 2). Results from the F_2 population and the $F_{2:3}$ families indicated that a single dominant gene conferred resistance to the leaf rust pathotype THJP in the 5R618 line.

Seedling resistance postulation. In seedling tests, 5R618 and thirty-six near-isogenic lines with known resistance genes were inoculated with fifteen *P. triticina* isolates (Table 1). The results showed that *Lr9*, *Lr24*, *Lr19*, *Lr28*, *Lr39*, *Lr42*, *Lr47*, *Lr51*, and *Lr53* resisted all of the leaf rust pathotypes. The 5R618 line also resisted all of the leaf rust pathotypes. Therefore, it can be preliminarily inferred that 5R618 may contain *Lr9*, *Lr24*, *Lr19*, *Lr28*, *Lr39*, *Lr42*, *Lr47*, *Lr51*, and *Lr53*, but further validation using molecular markers is needed.

Linkage analysis and genetic map. The seedling test on the F_2 plants and $F_{2:3}$ families indicated that the 5R618 line had a single dominant gene, tentatively designated *Lr5R*. Of the 1021 SSR markers, *Xbarc71* (Figure 1) on the 3DL chromosome showed polymorphisms between the resistant and susceptible bulks as well as between the parents. Three markers – *OPJ-09* (Figure 2), *STS24-16,* and *S1302-609* – co-segregated with *Lr24* on the 3DL chromosome; these three markers also showed polymorphisms between the resistant and susceptible bulks and between the parents. This indicates that *Lr5R* is located on the 3DL chromosome. The four polymorphic markers were then screened on DNA bulks from each of the 236 $F_{2:3}$ families previously tested with leaf rust. Resistance gene *Lr5R* was linked to the four molecular markers with genetic distances ranging from 0.9 cM

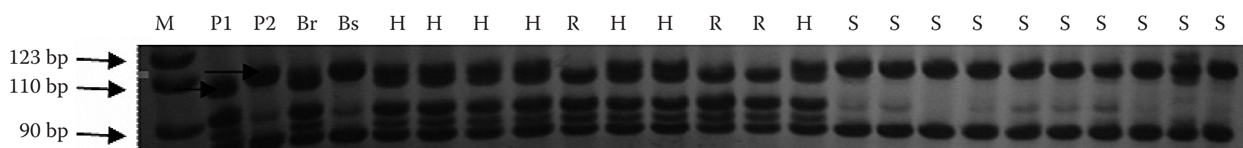

Figure 1. Specific PCR amplified fragments of the parents, resistant and susceptible bulks, and $F_{2:3}$ families with SSR marker *Xbarc71*; M – PBR322 marker; P1 – the resistant parent 5R618; P2 – the susceptible parent Zhengzhou5389; Br – the resistant bulk; Bs – the susceptible bulk; R – resistant plants in $F_{2:3}$ families; S – susceptible plants in $F_{2:3}$ families; H – resistant plants with heterozygous genotype

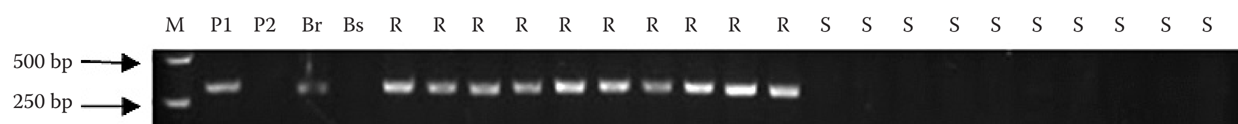

Figure 2. Specific PCR amplified fragments of the parents, resistant and susceptible bulks, and $F_{2:3}$ families with the STS marker *OPJ-09*; M – PBR322 marker; P1 – the resistant parent 5R618; P2 – the susceptible parent Zhengzhou5389; Br – the resistant bulk; Bs – the susceptible bulk; R – resistant plants in $F_{2:3}$ families; S – susceptible plants in $F_{2:3}$ families

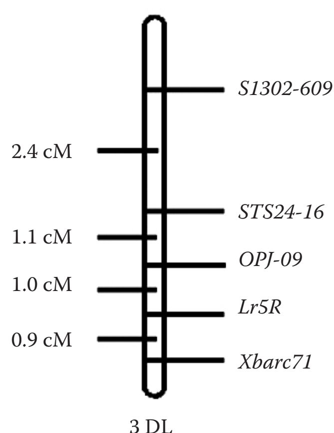

Figure 3. Linkage map of the leaf rust resistance gene *Lr5R*

to 4.5 cM (Figure 3). The two closest flanking molecular markers were *Xbarc71* and *OPJ-09* with genetic distances of 0.9 and 1.0 cM (Figure 3), respectively.

DISCUSSION

Depending on the experimental conditions and goals, researchers have chosen various methods of developing wheat leaf rust resistant genes, including traditional hybridization, genetic postulation, and molecular markers. These methods can be used alone or in combination. Traditional hybridization can accurately analyse the test material with resistance genes, their mode of inheritance and the number of genes involved, but the cycle is long, and labour intensive. While genetic postulation can test a large amount of material in a short time, the accuracy is low, and the results are easily influenced by temperature and pathotype toxins. The molecular marker method is unaffected by environmental conditions and gene expression, but it also has its limitations, such as the number and specificity of markers. The present trial combined traditional hybridization, genetic postulation, and molecular marker methods to make up for the individual limitations of each method and produce more accurate and reliable results.

Based on genetic postulation it was thought that 5R618 may contain *Lr9*, *Lr24*, *Lr19*, *Lr28*, *Lr39*, *Lr42*, *Lr47*, *Lr51*, *and Lr53*. Using molecular marker detection, *Lr5R* was located on the 3DL chromosome. The literature reports that the molecular markers *OPJ-09*, *STS24-16*, and *S1302-609* co-segregate with *Lr24*. However, in $F_{2:3}$ families originating from a cross between 5R618 and Zhengzhou 5389, the three markers that have commutations with *Lr5R* were detected; genetic linkage mapping found that *OPJ-09*, *STS24-16,* and *S1302-609* had genetic distances with *Lr5R* of 1.0 cM, 2.1 cM, and 4.5 cM, respectively. Therefore we speculate that *Lr5R* is unlike to *Lr24* and may be a new leaf rust resistance gene. To verify this, the relationship between *Lr5R* and *Lr24* will require further allelism tests.

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