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Genetic Analysis and Molecular Mapping of a Leaf Rust Resistance Gene in the Wheat Line 19HRWSN-129

Lingzhi SHI, Zaifeng LI, Xiaodong WANG, Zhanhai KANG, Lin ZHU, Zhikuan REN, Xing LI and Daqun LIU

College of Plant Protection, Biological Control Center of Plant Diseases and Plant Pests of Hebei, Agricultural University of Hebei, Baoding, P.R. China

Abstract

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The wheat (*Triticum aestivum* L.) line 19HRWSN-129 from CIMMYT showed resistance to the major *Puccinia triticina* (*Pt*) races collected in China. A 144 $F_{2:3}$ population was generated by crossing the resistant line 19HRWSN-129 with the susceptible line Zhengzhou5389. All the $F_{2:3}$ plants were phenotyped by inoculating with the *Pt* race PHGN in the greenhouse. Segregation analysis indicated the presence of a single dominant gene conferring resistance to leaf rust in the wheat line 19HRWSN-129, temporarily designated as *Lrshi*. Using molecular markers, we mapped this resistance locus on chromosome 7BL. It was closely linked to the SSR markers *Xgwm344* and *Xcfa2040* with genetic distances of 2.8 cM and 1.4 cM, respectively. Compared with other chromosome 7BL-located leaf rust resistance genes (*Lr14a*, *Lr14b*, *Lr68*), we demonstrate, that *Lrshi* is a novel wheat leaf rust resistance gene.

Keywords: gene mapping; molecular marker; SSR; wheat leaf rust

Leaf rust, caused by *Puccinia triticina* (*Pt*), is one of the most important and widespread diseases in wheat (*Triticum aestivum* L.). It occurs in a wide range of climates wherever wheat is grown and causes yield losses up to 65% under favourable conditions (Saari & Prescott 1985). With increased planting density and changed management of wheat production in China, leaf rust has expanded its infection area from the southwest and northeast regions of China to the major wheat planting region (Dong 2001). Utilization of resistant cultivars is still the most efficient, economical, and eco-friendly way to control leaf rust.

There are two types of leaf rust resistance genes with different phenotypic features: qualitative resistance conferred by single resistance gene (also defined as major, seedling, and race specific resistance) and quantitative resistance mediated by multiple genes or quantitative trait loci (QTL) (minor, adult, non-race specific and slow-rusting resistance). To date, more than 100 leaf rust resistance genes have been discovered in wheat, with 72 formally designated ones (McIntosh *et al*. 2013). Most of these genes are qualitative and interact with rust following the gene-for-gene theory (Flor 1956). However, these qualitative genes are rapidly losing resistance due to newly emerged leaf rust virulent races. Currently, only a few genes are still effective against prevalent *Pt* races in China (Yuan *et al*. 2007; Li *et al.* 2010). Hence, the identification of novel leaf rust resistance genes will greatly facilitate the genetic improvement of wheat (Chen *et al*. 1998).

Molecular markers, including RFLP, RAPD, SSR, AFLP and EST, have been widely used in mapping of leaf rust resistance genes with segregation populations. To date, 46 leaf rust genes have been mapped in wheat by various molecular markers (Xing *et al.* 2014). SSR markers, with their advantages of higher

Lingzhi Shi and Zaifeng Li contributed equally to this work.

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polymorphism and known chromosome location, provide a powerful tool for pyramiding of leaf rust resistance genes and marker-associated selection during breeding programs (Karakousis *et al*. 2003). 19HRWSN-129, developed at CIMMYT, Mexico, selected from the 19th High Rainfall Wheat Screening Nursery (Han *et al.* 2011), showed high levels of resistance to all pathotypes at the seedling stage. Here in this study, we have identified and mapped the resistance gene in this line as *Lrshi* on chromosome 7BL.

MATERIAL AND METHODS

Plant materials and *Puccinia triticina* races. The resistant parent lines 19HRWSN-129, the susceptible parent Zhengzhou5389 and $F_{2:3}$ families were included in the genetic analysis. Two near-isogenic lines *Lr14a*, *Lr14b* (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. Fifteen *Puccinia triticina* (*Pt*) races (PHTS, MHJS, FHDQ, FGBQ, FHBR, FHBQ, FGBR, THJL, FHDR, FGDQ, FHDS, THJP, TGTT, PHGN and THJC) were used for gene postulation (Table 1). Wheat planting and leaf rust inoculation were carried out following an instructed method at the Biological Control Center for Plant Diseases and Plant Pests of Hebei, Agricultural University of Hebei, China. All the *Pt* races were named using the Prt-code System (Long & Kolmer 1989).

Leaf rust evaluations in the greenhouse. Wheat seedlings of 19HRWSN-129, Zhengzhou5389 and 2 near-isogenic lines were phenotyped with fifteen collected *Pt* races. Wheat seedlings of 19HRWSN-129, Zhengzhou5389, and 144 of their segregating $F_{2:3}$ populations (20 seedlings each) were inoculated with *Pt* race PHGN (compatible on Zhengzhou5389 and incompatible on 19HRWSN-129). Seedlings were grown in a growth chamber. When the first leaves fully expanded, inoculations were performed by brushing urediniospores of corresponding *Pt* races. Once inoculated, seedlings were placed in a sealed box at 18°C and 100% relative humidity for 12 h in darkness. They were then transferred to a growth chamber at 12/12 h light/darkness, 18–22°C and 70% RH. Infection types (ITs) were scored at 14 days after inoculation (Roelfs *et al*. 1992). Plants with ITs of 0 to 2 were considered resistant, while those with ITs of 3 to 4 were considered susceptible (Dubin *et al*. 1989).

DNA extraction and BSA analysis. Genomic DNAs were extracted from the seedlings of 19HRWSN-129, Zhengzhou5389 and their segregating $F_{2:3}$ populations (10 seedlings each) by the cetyltrimethylammonium bromide (CTAB) method (Sharp *et al*. 1988). DNA concentrations were measured with a UV spectrophotometer, and diluted to final concentrations of 30 ng/µl. Bulked segregant analysis (BSA) (Michelmore *et al*. 1991) was used to identify molecular markers putatively linked to the leaf rust resistance loci in 19HRWSN-129.

Markers analysis. The initial screening was carried out using a total of 1023 wheat SSR markers derived from each of the chromosomes. SSR markers showing polymorphism between resistance and susceptible bulks were further used to genotype $F_{2:3}$ populations. Microsatellite analysis followed the procedure developed by Bryan *et al.* (1997) with minor modifications. Polymerase chain reactions (PCR) were performed in volumes of 10 µl with 1.0 U Taq of DNA polymerase (Ze xing Biotechnology Co. Ltd, Beijing, China); 1× PCR buffer (25 mM KCl, 5 mM Tris–HCl, 0.75 mM $MgCl_2$, pH 8.3); 100 µM each of dNTP, 3 pmol of each primer, and 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 µl of formamide loading buffer (98% formamide, 10 mM EDTA, 0.25% bromophenol blue, 0.25% xylene cyanol, pH 8.0). The mixture was then loaded on 10% non-denaturing polyacrylamide gel 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

Seedling resistance postulation. In seedling tests, 19HRWSN-129 and two near-isogenic lines with known resistance genes were inoculated with fifteen *Puccinia triticina* (*Pt*) races (Table 1). 19HRWSN-129 lines showed a strong resistance to all the tested *Pt* races at seedling stages. It was different from *Lr14a* and *Lr14b.*

Inheritance of leaf rust resistance in 19HRWSN-129. A $F_{2:3}$ population was generated by crossing the leaf rust resistant line 19HRWSN-129 with

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Table 1. Seedling infection types of 2 check wheat lines with known leaf rust resistance and the two cultivars to 15 pathotypes of *Puccinia triticina*

Line	Gene	Pathotype														
		PH	MH	FH	FG	FH	FH	FG	TH	FH	FG	FH	TH	TG	PH	TH
		JS	JS	DQ	BQ	BR	BQ	BR	JL	DR	DQ	DS	JP	TT	GN	JC
RL6013	*Lr14a*	4	4	X	X	X	X	X	X	X	2	4	4	4	3+	X
RL6006	*Lr14b*	4	4	4	4	4	4	4	4	4	4	4	X	4	X	4
19HRWSN-129	*Lrshi*	1+	1+	;	;	1	;	;	;	;	0	;	;	;, 1	1	;
Zhengzhou 5389	+	4	4	4	4	3+	4	4	4	4	4	4	4	4	4	4

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

Table 2. Segregation of seedling reactions to the pathotype PHGN in 19HRWSN-129, Zhengzhou5389 and their $F_{2:3}$ progenies

Material	Total	Infection types		Chi-square tests
		resistant	susceptible	
19HRWSN-129	20	20		
Zhengzhou5389	20		20	
$F_{2:3}$	144	105	39	$\chi^2_{1:2:1} = 2.375$

the susceptible line Zhengzhou5389. *Pt* race PHGN, which shows a typical incompatible response on 19HRWSN-129 and a compatible response on Zhengzhou5389, was used to phenotype the $F_{2:3}$ population at a seedling stage. Of all the 144 lines in the $F_{2:3}$ population, 28 lines were homozygous resistant, 77 were recombinant and 39 were homozygous susceptible, fitting at a ratio of 1:2:1 ($\chi^2_{1:2:1} = 2.375$, 2 df, $P > 0.05$, Table 2). These results indicate that the leaf rust resistance of the wheat line 19HRWSN-129 to *Pt* race PHGN is conferred by a single dominant gene, temporarily designated as *Lrshi*.

Linkage analysis and genetic mapping. A total of 1023 wheat SSR markers derived from each of the chromosomes were used for the initial screening. SSR markers *Xcfa2040* (Figure 1) and *Xgwm344* (Figure 2) from chromosome 7BL showed polymorphism between resistant and susceptible bulks as well as each parent line. Another five SSR markers (*Xbarc32*, *Xbarc182*, *Xgwm344*, *Xcfa2040* and *Xgwm146*) from

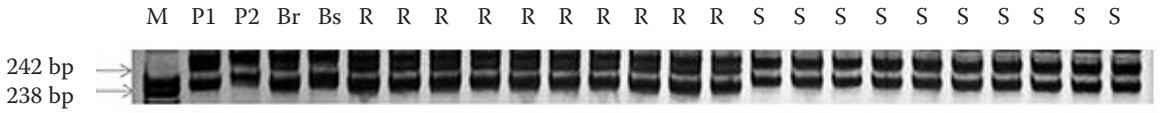

Figure 1. Specific PCR amplified fragments of the parents, resistant and susceptible bulks, and $F_{2:3}$ families with SSR marker Xcfa2040
M – PBR322 marker; P1 – resistant parent 19HRWSN-129; P2 – susceptible parent Zhengzhou5389; Br – resistant bulk; Bs – susceptible bulk; R – resistant plants in $F_{2:3}$ families; S – susceptible plants in $F_{2:3}$ families

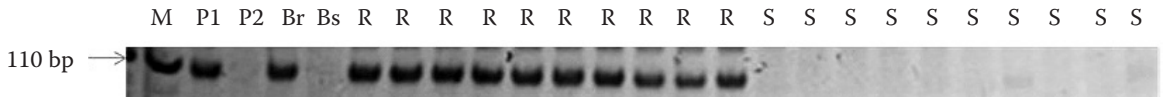

Figure 2. Specific PCR amplified fragments of the parents, resistant and susceptible bulks, and $F_{2:3}$ families with Xgwm344
M – PBR322 marker; P1 – resistant parent 19HRWSN-129; P2 – susceptible parent Zhengzhou5389; Br – resistant bulk; Bs – susceptible bulk; R – resistant plants in $F_{2:3}$ families; S – susceptible plants in $F_{2:3}$ families

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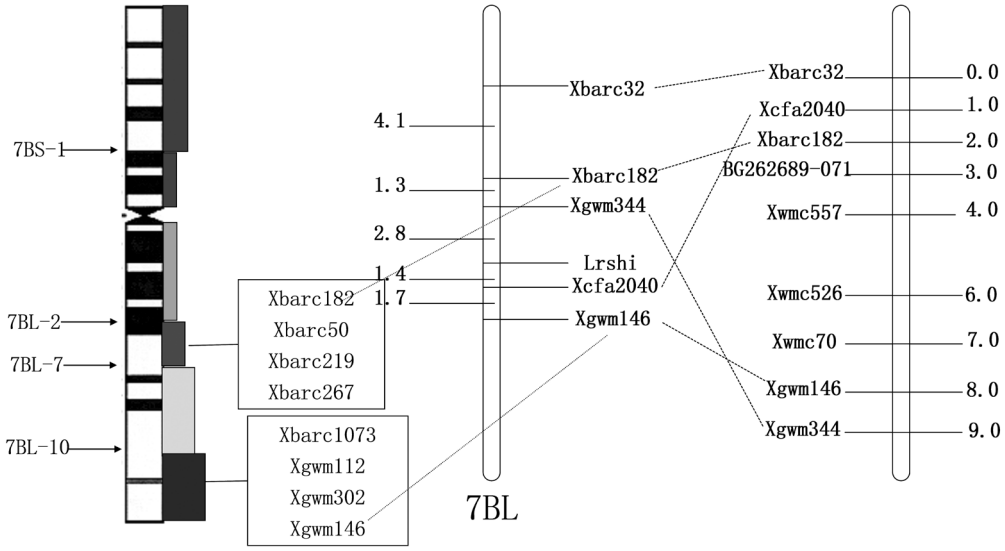

Figure 3. Linkage map of leaf rust resistance gene *Lrshi* and five markers based on $F_{2:3}$ population from the cross of 19HRWSN-129/Zhengzhou5389

Deletion bin map on chromosome 7BL (Sourdille *et al.* 2004) (left), linkage map of the leaf rust resistance region and five loci based on $F_{2:3}$ lines of 19HRWSN-129×5389; locus names and corresponding locations are indicated on the right, map distances are shown on the left in centiMorgans (centre) and compared with a previously published map (Somers *et al.* 2004) (right)

the nearby region of chromosome 7BL were used to generate the genetic map of *Lrshi* (Figure 3). The closest SSR markers *Xcfa2040* and *Xgwm344* are flanking *Lrshi* with genetic distances of 1.4 cM and 2.8 cM, respectively (Figure 3).

DISCUSSION

Seedlings of the wheat line 19HRWSN-129 from CIMMYT showed a very strong resistance to all the *Pt* races. Since the seedling resistance might be easily lost due to newly emerged leaf rust virulent races, e.g. *LrZH84* and *LrNJ97* (Zhao *et al.* 2008; Zhou *et al.* 2013), the wheat line 19HRWSN-129 seems to be a novel resource for wheat leaf rust resistance breeding. We have temporarily designated the single dominant gene that confers seedling resistance in the wheat line 19HRWSN-129 as *Lrshi*.

Comparison of *Lrshi* with *Lr14a*, *Lr14b*, *Lr68* located on chromosome 7BL. Two SSR markers, *cfa2040* and *gwn344*, closely linked to *Lrshi* were located on chromosome 7BL (Figure 3). At present, three leaf rust resistance genes, *Lr14a*, *Lr14b* and *Lr68*, are also located on chromosome 7BL (Law & Johnson 1967; Dyck & Samborski 1970; Herrera-Foessel *et al.* 2008, 2012). Seedling responses in the greenhouse showed that *Lrshi* was different from *Lr14a* and *Lr14b* (Table 1). *Lr68* is an adult plant resistance gene (Herrera-Foessel *et al.* 2012), but *Lrshi* is a seedling resistance gene, therefore it is different from *Lrshi*. *Lrshi* was closely linked to *Xcfa2040*-7B and *Xgwm344*-7B with genetic distances 1.4 and 2.8 cM. Therefore it can be concluded that *Lrshi* is different from *Lr14a*, *Lr14b*, *Lr68*, and is a new leaf rust resistance gene. At the same time, several *LR* resistance genes have been mapped in this region, suggesting that this region has a great potential for wheat leaf rust resistance breeding.

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Corresponding authors:

Xing Li, Ph.D., Agricultural University of Hebei, Biological Control Center of Plant Diseases and Plant Pests of Hebei, College of Plant Protection, Baoding, P.R. China; e-mail: lxkzh@163.com

Daqun Liu, Agricultural University of Hebei, Biological Control Center of Plant Diseases and Plant Pests of Hebei, College of Plant Protection, Baoding, P.R. China; e-mail: ldq@hebau.edu.cn