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Molecular mapping of leaf rust resistance gene in wheat line 19HRWSN-76

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Abstract

A highly leaf rust resistant genetic stock 19HRWSN-76 from CIMMYT was crossed to a susceptible variety Zhengzhou 5389 to generate F_1 and F_2 population to find out the mode of inheritance and map the resistance gene. The F_2 population was inoculated with uredosporic inoculum of physiological race FHJP of leaf rust. The 3:1 ratio of resistant and susceptible plants in F_2 suggested that the leaf rust resistance in 19HRWSN-76 is controlled by a single gene, tentatively named *Lr19HRWSN-76*. Relationship between the known leaf rust resistance gene *Lr24* and *Lr19HRWSN-76* located on chromosome 3D was established by SSR marker barc71 and STS 24-16.

Key words: Leaf rust resistance gene, molecular marker, genetic analysis, molecular mapping

Introduction

Wheat is a widely grown cereal crop around the world. In China, the acreage of wheat is only second to rice. Wheat leaf rust (*Puccinia triticina* Eriks.) is one of the major diseases inflicting 40% or more yield losses under severe conditions and thereby affecting the production (Knott 1989). The cultivation of resistant varieties of wheat is the most economic, safe and effective method to prevent leaf rust. More than 100 wheat leaf rust resistance genes have been discovered domestic and foreign, 76 have been officially named (Singh et al. 2013). The majority of wheat leaf rust resistance genes have been rendered ineffective by new virulences of leaf rust, only few genes such as *Lr*9, *Lr*19, *Lr*24 , *Lr*38 still possess resistance against Chinese leaf rust pathotypes. Therefore, the study of inheritance of resistance to leaf rust of wheat, and searching for new resistance genes constantly is important in controlling leaf rust incidence in wheat.

The use of molecular markers is a common research method to assist in biological studies, not limited by season, environment, high polymorphism, and is able to identify homozygous and heterozygous genotypes. Leaf rust resistance genes in EST, AFLP can be converted into stable and simple SCAR, STS markers (Sacco et al. 1988; Gupta et al. 2005). STS marking technology is simple, quantitative, easy to test and can be used for marker-assisted selection. Schachermayr et al. (1994) used STS markers of *Lr9*, *Lr19* and *Lr24* to test 55 common wheat varieties in Russia. Chen et al. (2008) detected 33 wheat leaf rust resistant varieties using *Lr19* STS molecular marker and provided reliable information for cultivating disease-resistant varieties. Simple sequence repeats (SSRs) is a series of dozens of nucleotide repeats by a few nucleotides (usually 1-6) as the repeat unit, can distinguish between homozygous and heterozygous genotype, with a low requirements for template DNA, simple operation, short cycle, widespread application in wheat, corn, barley, rice and other crops. At present, several wheat leaf rust resistance genes have been discovered and located using SSR markers (Suenaga et al. 2001; Zhang et al. 2005; Blaszczyk et al. 2004; Zhang et al. 2011; Kuraparthy et al. 2007).

Lr24 still possesses a high degree of resistance in many geographical areas of the world including China and India. Schachermayr et al. (1995) identified STS

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marker OPJ09 completely linked with *Lr24*, whereas Zhang et al. (2008) designed a STS marker using an EST-SSR marker co-segregating with *Lr24*, which can amplify a 180 bp fragment. The fragment has been cloned, without any overlap with OPJ09, a marker from the common wheat. SCAR marker S1302-609 developed and validated by Gupta et al. (2006) which amplifies a specific 609 bp band.

The wheat line 19HRWSN-76 derived from a cross THELIN/3/2*BABAX *Lr*42//BABAX is developed by CIMMYT having good comprehensive traits and resistance to leaf rust pathotypes. Han et al. (2011) determined that 19HRWSN-76 carried seedling and slow rusting resistance to leaf rust against Chinese pathotypes. The present study postulated and mapped the resistance gene carried by the line 19HRWSN-76.

Materials and methods

The resistant parent 19HRWSN-76, susceptible parent Zhengzhou 5389, F_1 (19HRWSN-76 x Zhengzhou 5389) and F_2 plants were included in the genetic analysis. Thirty-six near-isogenic lines with known leaf rust resistance genes were kindly provided by the USDA-ARS Cereal Disease Laboratory, University of Minnesota, St Paul, MN, USA. The 15 *P. triticina* pathotypes used in multi-pathotype comparisons (Table 1) and genetic analysis are maintained at the Biological Control Centre of Plant Diseases and Plant Pests of Hebei, Hebei Agricultural University, China.

Seedling resistance and genetic analysis

The parents 19HRWSN-76 and Zhengzhou 5389 and 36 near-isogenic lines in Thatcher background were inoculated with 15 leaf rust pathotypes (Table 1) for comparing the leaf rust reaction arrays. Both the 19HRWSN-76 and Zhengzhou 5389, 20 F_1 plants and 207 F_2 individuals were inoculated with Chinese *P. triticina* pathotype FHJP (virulent on Zhengzhou 5389 and avirulent on 19HRWSN-76). Seedlings were grown in a growth chamber. When the first leaf was fully expanded, inoculations were performed by brushing urediniospores from a sporulating susceptible genotype onto the seedlings to be tested. Inoculated seedlings were placed in plastic-covered cages and incubated at 18°C and 100 % relative humidity for 12 h. They were then transferred to a growth chamber maintained with 12 h light/12 h darkness at 18-22^{o}C with 70 % RH. Infection types were scored 14 days after inoculation according to the Stakman scale modified by Roelfs et al. (1992). Which is divided into 0,; , 1, 2, 3, 4, which level 0 ~ 2 is for disease resistance, 3 ~ 4 for disease susceptible. Gene postulation was done according to the method put forward by Dubin et al. (1989).

DNA extraction and PCR amplification

Genomic DNA was extracted from F_2 plants using the CTAB protocol (Sharp et al. 1988), and diluted to the required concentration with TE. Refered to bulk segregant analysis (BSA), genomic DNA from 10 resistant and 10 susceptible F_2 plants were mixed in equal amounts to form resistant and susceptible bulks (Michelmore et al. 1991). DNA samples of the two parents and bulks were screened for polymorphisms of markers.

The 626 pairs of wheat SSR markers in 21 wheat chromosomes and two molecular markers of co-separation with *Lr24* (*STS24-16*, *S1302-609*) were used in this study. The SSR markers showing polymorphism between resistant and susceptible bulks were used for linkage analysis in F_2 plants. The primer sequences described by Roder et al. (1998) are available at the GrainGenes site (http://www.graingenes.org). The primer sequences for *STS24-16* are *STS24-16*F: 5'-CTTCGGACAGGAGGGTATGA-3', *STS24-16*R: 5'-GGACAGCTGTAAACGGGTTC-3' (Zhang et al. 2008). And the primer sequences for *S1302-609* are *S1302-609*F: 5'-CGCAGGTTCCAAATACTTTTC-3', *S1302-609*R: 5'-CGCAGGTTCTACCTAATGCAA-3' (Gupta et al. 2006).

Polymerase chain reactions (PCR) were performed in volumes of 10 ul with 1.0 U Taq of DNA polymerase (Zexing Biotechnology Co. Ltd, Beijing, China); 1× PCR buffer (25 mM KCl, 5 mM Tris-HCl, 0.75 mM MgCl2, pH 8.3); 100 uM each of dNTP, 3 pmol of each primer, and 30 ng of template DNA. The PCR conditions were a denaturation at 94^{o}C for 5 min, followed by 35 cycles of 94^{o}C for 1 min, 55-65^{o}C (depending on the primer pair) for 1 min, 72^{o}C for 1 min and a final extension at 72^{o}C for 10 min. Based on differences in molecular weight of the amplified products were detected by polyacrylamide gel electrophoresis and analyzed by agarose gel electrophoresis.

Results

Gene postulation

In seedling stage, inoculations were done with 15 *P. triticina* pathotypes on 19HRWSN-76, Zhengzhou 5389, and 36 nearly isogenic lines with single known resistance gene (Table 1) to compare the infection

Table 1. Seedling infection types in 19HRWSN-76, Zhengzhou 5389 and 36 near-isogenic lines with known leaf rust resistance gene to 15 pathotypes of *Puccinia triticina*

Line	Gene	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
RL6003	*Lr1*	4	4	;	;	;	;	;	4	0	;	0	4	4	4	4
RL6016	*Lr2a*	;	;	1+	;	;	1	1	3	;	;	2	3	3	;	4
RL6047	*Lr2c*	4	1	4	4	4	4	4	4	4	4	4	4	4	4	4
RL6002	*Lr3*	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
RL6010	*Lr9*	;	;	;	;	0;	0	0	;	0	0	;	;	;	0	;
RL6005	*Lr16*	4	4	4	4	4	4	3+	3+	4	4	4	4	4	3	4
RL6064	*Lr24*	;1	;	;	;	;	;	;	;	;	;	;	;	;	;	;
RL6078	*Lr26*	4	4	4	1	4	4	;	4	4	1	4	4	2	4	4
RL6007	*Lr3ka*	X	X	;	;	;	;	1	1	;	;	;	1	4	;	X
RL6053	*Lr11*	4	4	1	;	;	1+	2	3+	1	1	2	4	3+	4	4
RL6008	*Lr17*	4	3+	3+	2	2	2	2+	4	3+	4	4	4	4	2+	4
RL6049	*Lr30*	3C	1	1	;	;	;	;	1	;	;	;	;	4	;	1
RL6051	*LrB*	3+	4	4	4	3+	4	4	3+	4	4	4	4	4	4	X
RL6004	*Lr10*	3	3	4	4	4	4	4	2	4	4	4	2+	4	1	X
RL6013	*Lr14a*	4	4	X	X	X	X	X	X	X	2	4	4	4	3+	X
RL6009	*Lr18*	1	1+	2	2	4	2	4	1+	4	2+	2	4	3+	3C	3
RL6019	*Lr2b*	1	0;	4	;	3	3+	2	4	3	3+	3+	2	4	3C	4
RL6042	*Lr3bg*	4	4	4	4	4	3+	4	4	4	4	4	4	4	4	4
RL4031	*Lr13*	3	4	4	3	3	4	4	3	3	2	3+	4	4	4	4
RL6006	*Lr14b*	4	4	4	4	4	4	4	4	4	4	4	X	4	X	4
RL6052	*Lr15*	1	;	;	;	;	;	;	4	1	;	;	4	3+	4	4
RL6040	*Lr19*	0	0	;	0	0	;	0	0	0	0	;	0	0	0	;
RL6092	*Lr20*	4	4	;	;	;	;	0	;	;	;	;	4	1	4	;
RL6043	*Lr21*	4	2	2	;	2+	3	2	;	1	;	1+	;	3	1	1
RL6012	*Lr23*	4	4	4	3+	3+	4	3+	1	4	4	4	4	4	3+	4
RL6079	*Lr28*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL6080	*Lr29*	0	0	0	0	;	0	0	;	;	0	3+	4	;	0	0
RL6057	*Lr33*	3	4	3+	3+	3+	4	2+	3C	3+	3	4	4	4	3+	3+
E84018	*Lr36*	4	2	1+	;	2	2	1	1	2	2+	3	2+	3+	2+	3+
KS86 NGRC02	*Lr39*	;	;1	;	;	;	;	;	;	;	;	;	;	;	;	;
KS91 WGRC11	*Lr42*	;	;	;	0	0	;	;	;	1	;	;	0	;	0	1
RL6147	*Lr44*	1	;	4	4	4	4	4	1	4	4	4	;	1+	;1	1
RL614	*Lr45*	4	4	4	4	4	4	4	;	4	4	4	4	;	;	;
PAVON76	*Lr47*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C78.5	*Lr51*	;	;	;	;	1	;	0	;	;	;	;	0	;	;	;
-98M71	*Lr53*	;	0	0	0	0	0	0	;	0	0	0	0	0	0	0
19HRWSN-76	*LrHRWSN-76*	1	:	0	:	:	:	:	0	:	:	0	:	0	:	:
Zhengzhou 5389	+	4	4	4	4	3+	4	4	4	4	4	4	4	4	4	4

C = More chlorosis than normal for infection type; + = Uredinia somewhat larger than normal for the infection type

types of 19HRWSN-76 and 36 nearly isogenic lines to *P. triticina* pathotypes. The results showed that the 19HRWSN-76 was immune or highly resistant to all pathotypes. In 36 near isogenic lines, RL6010(*Lr9*), RL6064(*Lr24*), RL6040(*Lr19*), RL6079(*Lr28*), KS86/NGRC02(*Lr39*), KS91/WGRC11(*Lr42*), PAVON76 (*Lr47*), C78.5(*Lr51*), 98M71(*Lr53*) were immune or highly resistant to the 15 *P. triticina* pathotypes. So that, if there is a pair of disease-resistance gene in 19HRWSN-76, it be among those listed above.

Genetic analysis

The sedling reactions against FHJP pathotype of resistant parent (19HRWSN-76), the susceptible parent (Zhengzhou 5389) and their F_1 are presented in Table 1. All 20 seedlings each of the resistant and susceptible parents showed resistant and susceptible reactions to pathotype FHJP, respectively. Out of the 207 F_2 seedlings, tested, 147 were resistant while 60 susceptible. Chi-square test was in accordance with the ratio of 3:1 ($\chi^2_{0.05} = 1.91 < \chi^2_{0.05} = 3.84$). Results from the F_2 populations indicated that a single dominant gene, tentatively designated *Lr19HRWSN-76*, conferred resistance to leaf rust pathotype FHJP in 19HRWSN-76.

The linkage analysis and genetic mapping

Of the 626 SSR markers, *Xbarc71* (Fig. 1) at the distal end of chromosome 3D showed polymorphism between the resistant and susceptible bulks as well as the parents. This indicated that *LrHRWSN-76* was located on chromosome 3D. There were two known leaf rust resistance genes (*Lr32, Lr24*) located on chromosome 3D. *Lr32* is located on the short arm of chromosome 3D and located far away from *Xbarc71*. *Lr24* was located on the long arm without any linked SSR markers reported so far. The STS marker *STS24-16* and SCAR marker *S1302-609* co-segregated with *Lr24*. In F_2 population, it was found that these two markers are linked with *Lr19HRWSN-76*. The three polymorphic markers *Xbarc71*, *STS24-16* and *S1302-609* were then screened on DNA of 207 F_2 plants previously tested with leaf rust. A newly identified resistance gene *Lr19HRWSN-76* was linked to these three markers with genetic distances ranging from 3.0 cM to 4.0 cM (Fig. 2). The closest marker was SSR marker *Xbarc71* with genetic distance of 3.0 cM.

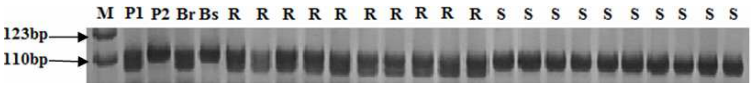

Fig. 1. Specific PCR amplified fragments of parents, resistant and susceptible bulks, and F_2 population with the SSR marker *Xbarc71.* M: PBR322 marker; P1: The resistant parent 19HRWSN-76; P2: The susceptible parent zhengzhou5389; Br: The resistant bulk; Bs: The susceptible bulk; R: The resistant plants in F_2 population; S: The susceptible plants in F_2 population

Discussion

Depending on the experimental conditions and goals, researchers have chosen various methods of developing wheat leaf rust resistance genes, including traditional hybridization, genetic postulation, and molecular markers. These methods can be used alone or in combination. The present method combined traditional hybridization, genetic postulation, and use of molecular markers methods to make up for the individual limitations of each method and produce more accurate and reliable results. The F_2 population derived from 19HRWSN-76 and Zhengzhou 5389 showed 3:1 ratio, proving that the seedling resistance is controlled by a dominant gene. In the field tests, F_2 population showed deviation from theoretical ratio of 3:1, proving that resistance at adult plant stage in 19HRWSN-76 may be controlled by a number of genes.

However, a planned study is needed to find out the number of genes governing adult plant resistance. The pedigree of 19HRWSN-76 indicate that it may carry another leaf rust resistance gene *Lr42*. However, the gene *Lr*42 is lacked on 1D chromosome and therefore the possibility of the presence of Lr42 is ruled out. Gene postulation showed that 19HRWSN-76 was resistant to all of the leaf rust pathotypes in seedling stage. Among the 36 near-isogenic lines, resistance to all the leaf rust pathotypes was observed on lines Tc*Lr9* Tc*Lr24*, Tc*Lr19*, Tc*L28*, Tc*Lr39*, Tc*L42*, Tc*Lr47*, Tc*Lr51*, Tc*Lr53*. Genetic analysis and chi-square test

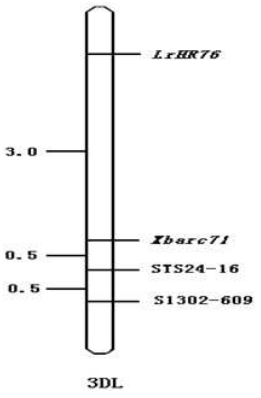

Fig. 2. Linkage map of leaf rust resistance gene *LrHRWSN*

indicated that there is a single dominant gene in 19HRWSN-76, which may be diverse than *Lr9, Lr24, Lr19, Lr28, Lr39, Lr42, Lr47, Lr51* and *Lr53*, as these genes are located on different chromosomes except the genes *Lr24* and *Lr32*. Linkage map contains three tags, all were located on 3DL chromosome, including a SSR and STS markers and a SCAR marker of co-separation with *Lr24*. This further proves that the resistance conferred by 19HRWSN-76 locatged on chromosome 3DL is different than displayed by *Lr9, Lr19, Lr28, Lr39, Lr42, Lr47, Lr51,* and *Lr53.*

Marker *STS24-16* showed co-separation with an alian gene *Lr24* derived from *Thinopyrum ponticum*. *STS24-16* and *S1302-609* are linked with *Lr19HRWSN-76*. Therefore, we speculate that the resistance in 19HRWSN-76 is different from *Lr24* and may be a new leaf rust resistance gene. However, to establish the relationship between 19HRWSN-76 and *Lr24*, a test of allelism will be required to perform.

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