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Identification of a Leaf Rust Resistance Gene in the Chinese Wheat Line LB0288

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Abstract

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Leaf rust (LR), caused by *Puccinia triticina* (*Pt*), is worldwide one of the most spread diseases in common wheat (*Triticum aestivum* L.). With the objective of identifying and mapping new genes for resistance to leaf rust, F_1 and F_2 plants from a cross between the resistant cultivar LB0288 and the susceptible cultivar Thatcher were inoculated with the Chinese *P. triticina* pathotype THTT in the greenhouse. A set of 1273 simple sequence repeat (SSR) primer pairs were used to test the parents and the resistant and susceptible bulks. The results indicated, that LB0288 carried a single dominant resistance gene *LrLB88*, closely linked to the CAPS marker of *Lr1* (WR003) and the SSR marker *Xbarc144*, with genetic distances of 0 cM and 5.3 cM, respectively. Based on the chromosomal location and seedling reactions, it can be concluded that *LrLB88* might be a new leaf rust resistance gene. These markers can be useful for marker assisted selection in breeding of leaf rust resistant wheat cultivars.

Keywords: leaf rust resistance gene; molecular mapping; wheat

Wheat leaf rust, caused by the fungus *Puccinia triticina*, is one of the most important foliar diseases in wheat (*Triticum aestivum* L.) worldwide. It occurs in a wide range of climates wherever wheat is grown and causes significant yield and economic losses, which can be up to 40% under conditions favourable for the disease (Knott 1989). In China leaf rust (LR) is traditionally important only in the southwest and northeast regions; but with increased planting densities and changing management practices it has become increasingly important in most of the major wheat producing areas. Destructive epidemics of LR occurred in 1969, 1973, 1975 and 1979 in China (Dong 2001) and the yield losses were incurred in some regions of Gansu, Sichuan, Shaanxi, Henan and Anhui provinces in 2012 (Zhou *et al.* 2013). Although fungicides can control the disease, the most effective, economic and environmentally safe way to control the disease is growing rust resistant cultivars.

More than 100 leaf rust resistance genes have been reported in wheat and its relatives, 72 of them permanently catalogued (McIntosh *et al.* 2013). Most are race-specific resistance genes that confer hypersensitive reactions. This kind of resistance often loses effectiveness after deployment in agriculture. Only a few designated leaf rust resistance genes, such as *Lr9*, *Lr19*, *Lr24* and *Lr38*, are effective against prevalent Chinese *P. triticina* races (Yuan *et al.* 2007). It is therefore important to identify new resistance genes to cope with dynamic and rapidly evolving pathogen populations.

There are several ways for studying wheat leaf rust resistance genetics. Genetic interactions between wheat and *P. triticina* involve gene-for-gene relationships (Samborski 1963). Gene postulations assume gene-for-gene specificities in hypothesizing probable resistance genes present in host genotypes (Person 1959). The presence of a specific resistance gene in a host line can be postulated from response arrays of pathogen cultures with known avirulence and virulence characteristics. Postulations of *Lr* genes in a series of wheat lines have been reported in many

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publications (Kolmer 2003; Oelke & Kolmer 2004; Wamishe & Milus 2004; Mebrate *et al.* 2008; Li *et al.* 2010b).

Molecular markers, including RAPD, RFLP, SSR, AFLP, and RGAP, are useful tools for gene mapping in wheat. In total, 46 leaf rust resistance genes have been mapped to wheat chromosomes with molecular markers (Xing *et al.* 2014). Closely linked SSR markers provide a useful tool for pyramiding leaf rust resistance genes and marker-assisted selection in breeding programs.

The cultivar LB0288 introduced from Sichuan province (pedigree: Mianyang 90-310/M180) exhibited high resistance to leaf rust and appeared to carry new leaf rust resistance genes based on its reaction pattern with these isolates (Li *et al.* 2010b), and it also had good agronomic characters. The leaf rust resistance gene has not been reported yet. The aim of this study was to map the leaf rust resistance gene in LB0288 using SSR markers.

MATERIAL AND METHODS

Plant material and *P. triticina* pathotypes. The resistant parent line LB0288 and susceptible parent line Thatcher, 20 F_1 and 120 F_2 plants, were used in genetic analysis. 35 lines with known *Lr* genes (Table 1) were used for gene postulation. A total of 13 *P. triticina* pathotypes used in multi-pathotype comparisons (Table 1) and genetic analysis are maintained at the Biological Control Centre for Plant Diseases and Plant Pests, Hebei Agricultural University, Baoding. These pathotypes were designated following the coding system of Long and Kolmer (1989), supplemented by addition of the fourth letter for reactions on the fourth quartet of differentials (http://www.ars.usda.gov/SP2 UserFiles/ad_hoc/36400500Cerealrusts/pt_nomen.pdf).

Leaf rust evaluations in the greenhouse. LB0288, Thatcher, and 35 lines with known *Lr* genes were inoculated with 13 *P. triticina* pathotypes (Table 1) for comparing the leaf rust reaction arrays. Seedlings of the parents and progeny generations were inoculated with *P. triticina* pathotype THTT.

Seedlings were grown in a growth chamber. Inoculations were performed when the first leaves were fully expanded by brushing urediniospores from fully infected susceptible genotype Zhengzhou 5389 onto the new seedlings. Inoculated seedlings were placed in plastic-covered cages and incubated at 18°C and 100% relative humidity for 12 h in darkness. They were then transferred to a growth chamber maintained with 12 h light/12 h darkness at 18–22°C and 70% RH. Infection types were scored 14 days after inoculation according to the Stakman scale as modified by Roelfs *et al.* (1992). Plants with IT 0 to 2 were considered to be resistant and those with IT 3 to 4 were susceptible. The resistance gene postulation was performed following the method of Dubin *et al.* (1989).

DNA extraction and BSA analysis. Total genomic DNA was extracted from the seedlings of F_2 plants by the cetyltrimethylammonium bromide (CTAB) method (Sharp *et al.* 1988). DNA concentrations were measured with a UV spectrophotometer, and diluted to final concentrations of 50 ng/µl.

Bulked segregation analysis (BSA) (Michelmore *et al.* 1991) was used to identify molecular markers putatively linked to the *Lr* gene in LB0288. Equal amounts of DNA were pooled separately from ten resistant DNA and ten susceptible F_2 plants to form resistant and susceptible bulks, respectively.

Marker analysis. A total of 1273 SSR markers covering all wheat chromosomes were used to screen the parents as well as resistant and susceptible bulks. All the SSR marker sets were publicly available in GrainGenes 2.0 (http://wheat.pw.usda.gov). The SSR markers used in the study included 458 BARC markers (Song *et al.* 2002), 420 WMC markers (Gupta *et al.* 2002), 185 GWM markers (Röder *et al.* 1998), 106 CFA and 104 CFD markers (Sourdille *et al.* 2004).

Markers showing polymorphism between resistant and susceptible bulks were further used to analyse all the F_2 plants for linkage analysis. SSR analysis followed the procedure developed by Bryan *et al.* (1997) with minor modifications. PCR were run in final volumes of 10 µl containing 0.5 U Taq DNA polymerase (Zexing Biotechnology Co. Ltd, Beijing, China), 1 µl of PCR buffer (50mM KCl, 10mM Tris-HCl, 1.5mM $MgCl_2$, pH 8.3), 100µM of each dNTP, 3 pmol of each primer and 50 ng of template DNA. Standard amplification conditions were 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 50, 55 or 60°C (as reported for the individual SSR by Röder *et al.* 1998), and 1 min at 72°C, followed by a final extension at 72°C for 10 min. Subsequently, 10 µl of PCR product was mixed with 2 µl of formamide loading buffer (98% formamide, 10mM EDTA, 0.25% bromophenol blue, and 0.25% xylene cynol, pH 8.0). PCR products were separated on 8% non-denaturing polyacrylamide gels and silver stained following Bassam *et al.* (1991). CAPS marker WR003 used in the study followed Qiu *et al.* (2007).

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Table 1. Seedling infection types produced on LB0288, Thatcher and 35 lines with known leaf rust resistant genes when inoculated with 13 Chinese *Puccinia triticina* pathotypes

Tester	*Lr* gene	Infection types to *P. triticina* pathotypes[a]												
		PHQT	FCQR	FCST	PCBT	FGSQ	PGTT	PCJT	FHHQ	FHTR	PHJT	THTT	FCTT	PCGR
RL6003	*Lr1*	3	;0	0;	3	;	3	3	0;	0;	3	4	;	3
RL6016	*Lr2a*	1	1	;	;1	1+	1	;2	0;	0;2	;	4	;	;
RL6019	*Lr2b*	3	4	3	4	3	3	3	3	3	3	4	4	3
RL6047	*Lr2c*	3	3	3	4	4	3	3	3	3	3	4	4	3
RL6002	*Lr3*	3	4	3	3	4	3	3	3	3	4	4	4	3
RL6042	*Lr3bg*	3	3	3	3	4	3	3	3	2	3	3	4	3
RL6007	*Lr3ka*	3	3	3	;1	4	3	0;	0;	3	;,2	3	3	;2
RL6010	*Lr9*	0	;	0	0	0	0	0	0	0	0	0	;	0
RL6004	*Lr10*	3	3	3	3	4	3	3	3	3	3	3	4	3
RL6053	*Lr11*	3	3	3	;12	3+	3	3	3	3	3	3	4	3
RL6013	*Lr14a*	3	3	3	4	2	3	3	2	2	3	3	4	2
RL6052	*Lr15*	0;	0	;	;1	1+	3	;	2	;2	;1	3	;1	3
RL6005	*Lr16*	3	1	1	1+	4	3	2	3	4	3	4	;2	2
RL6008	*Lr17a*	2	2	3	;	3+	3	3	1	3	3	3	3	2
RL6009	*Lr18*	3	3	3	3	1	3	3	2	3	3	3	4	3
RL6040	*Lr19*	0	;	;	0	;	1	0;	0;	0	0;	;	0;	;
RL6092	*Lr20*	3	3	;1	0;1	;	0;	3	3	3	3	3	3	3
RL6043	*Lr21*	0;	;1	3	;2	3	2	2	2	3	3	3	3	2
RL6012	*Lr23*	;	;	3	;1	4	4	4	;2	0;	;	3	–3	;1
RL6064	*Lr24*	0	0	;	;	;	;	;	;	0	;	;	;	;
RL6084	*Lr25*	3	4	3	4	4	3	3	3	3	3	3	4	3
RL6078	*Lr26*	3	3	3	3	0;	2+	3	3	3	-3	3	4	3
RL6079	*Lr28*	0	0;	;	0;	;	0	;	;	3	0	0	;	0
RL6080	*Lr29*	3	3	3	;2	4	3	1	3	3	;2	3	3	3
RL6049	*Lr30*	0	;	;	;1	1	3	0;2	3	3	;2	3	3	2
RL5497	*Lr32*	;	;	;	;1	4	1	;1	3	0	;2	3	4	2
RL6057	*Lr33*	3	3	3	3	4	3	4	3	3	3	3	3	3
E84018	*Lr36*	3	;1	;1	;	2+	;	2	2	0	;	3	;	;
RL6097	*Lr38*	0	0	;	0	;1	0	0	0	0	0;	;	;	;
KS86N-GRC02	*Lr39*	3	3	;	;2	1	2	;2	3	3	;2	;	1	2
KS91W-GRC11	*Lr42*	0;	0;	;	0;12	;	2	3	;	3	0	0;1	0;2	;
RL6147	*Lr44*	3C	2	3	3	3	2	1+	3	1	3	3	3	2
RL6144	*Lr45*	3	3	;	2;	1	1	0;	3	3	0	;	0;	3
TcLr50	*Lr50*	0;	0;	;	;	4	3	;	0;	0	;1	3	;	2
RL6051	*LrB*	4	3	3	3	4	3	3	3	3	3	3	4	3
LB0288	*LrLB88*	;	0	0;	0;1	;	0	0	0	0	0	0;	0;	1
Thatcher		4	4	4	4	4	4	4	4	4	4	4	4	4

[a]According to the 0–4 Stakman scale as modified by Roelfs *et al.* (1992)

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Table 2. Seedling reaction of F_1 and F_2 plants from the cross LB0288/Thatcher when inoculated with leaf rust pathotype THTT

Material	Total	Infection type					
		0	;	1	2	3	4
LB0288	15	5	10				
Thatcher	15						15
F_1 plants			20				
F_2 plants		8	65	8	4	15	20

F_2 plants – $\chi^2_{3:1}$ = 1.34, 1df, $P > 0.05$

Statistical and linkage analyses. Goodness of fit of observed and expected segregation ratios was evaluated by chi-squared (χ^2) tests. Linkage analysis was performed using MapManager QTXb20 (Manly *et al.* 2001) and recombination values were converted to centimorgans using the Kosambi mapping function (Kosambi 1944).

RESULTS

Reactions of LB0288 and 35 lines with known *Lr* genes in the greenhouse. In the seedling tests, LB0288, Thatcher and 35 lines with known *Lr* genes were inoculated with 13 Chinese *P. triticina* pathotypes (Table 1). Variation in ITs (infection types) conferred by 36 known leaf rust resistance genes in differential lines, inoculated with 13 pathotypes (Table 1), provided an ability to postulate 23 resistance genes (*Lr1*, *Lr2a*, *Lr3bg*, *Lr3ka*, *Lr11*, *Lr14a*, *Lr15*, *Lr16*, *Lr17a*, *Lr18*, *Lr20*, *Lr21*, *Lr23*, *Lr26*, *Lr29*, *Lr30*, *Lr32*, *Lr36*, *Lr39*, *Lr42*, *Lr44*, *Lr45* and *Lr50*). Resistance genes *Lr9*, *Lr19*, *Lr24*, *Lr28*, and *Lr38* conferred low ITs to all pathotypes. The postulation of genes *Lr2b*, *Lr2c*, *Lr3*, *Lr10*, *Lr25*, *Lr33*, and *LrB* was not possible because high ITs were recorded in most pathotypes. Wheat line LB0288 was highly resistant to all the 13 pathotypes. Five lines with known *Lr* genes, viz. *Lr9*, *Lr19*, *Lr24*, *Lr28* and *Lr38*, showed high resistance to all pathotypes. LB0288, Thatcher and most of the lines with known *Lr* genes showed susceptible to the *P. triticina* pathotype THTT, which indicated that (an) unknown leaf rust gene(s) conferred resistance to THTT. THTT was employed to inoculate the whole F_2 population.

Inheritance of leaf rust resistance in wheat line LB0288. In seedling tests with the pathotype THTT, LB0288 gave a resistant reaction with IT ;, Thatcher responded with IT 4, and F_1 plants were resistant with IT ;, indicating that resistance was dominant. The F_2 population segregated 85 plants with IT ; to 2 (resistant) and 35 plants with IT 3 to 4 (susceptible), indicative of a single dominant gene for resistance ($\chi^2_{3:1}$ = 1.34, 1df, $P > 0.05$, Table 2). Results from the F_2 populations indicated that a single dominant gene, tentatively designated *LrLB88*, conferred resistance to the *P. triticina* pathotype THTT in line LB0288.

SSR screen and linkage analysis and genetic map. Of all the markers tested, one SSR marker *Xbarc144*, one CAPS marker WR003 (Qiu *et al.* 2007) which were co-segregated with *Lr1* on 5DL, showed polymorphisms between the resistant and susceptible bulks as well as the parents, indicating that *LrLB88* was located on chromosome 5DL. The polymorphic markers were then assayed on the entire F_2 population. The resistance gene *LrLB88* was closely linked to one single SSR and one CAPS marker with

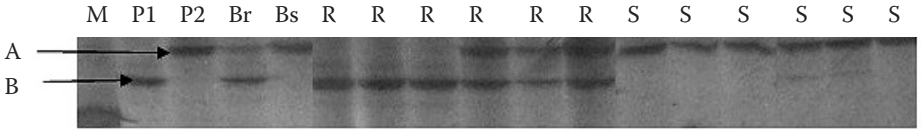

Figure 1. Electrophoresis of PCR products amplified with SSR marker Xbarc144 on polyacrylamide gels
M – PBR322/*Msp*I marker; A – allele in resistant parent LB0288 (P1); B – allele in Thatcher (P2); Br – resistant bulk; Bs – susceptible bulk; R – resistant F_2 plants; S – susceptible F_2 plants

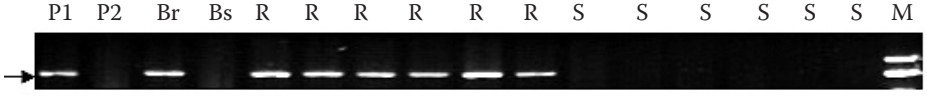

Figure 2. Electrophoresis of PCR products amplified with CAPS marker WR003 on agarose gel electrophoresis
M – DL2000 Marker; P1 – resistant parent LB0288; P2 – Thatcher; Br – resistant bulk; Bs – susceptible bulk; R – resistant F_2 plants; S – susceptible F_2 plants

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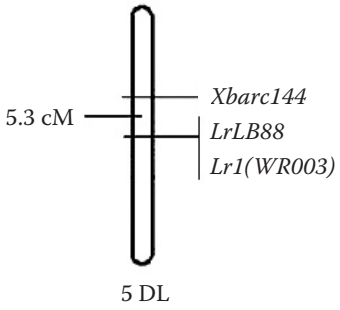

Figure 3. Linkage map of leaf rust resistance gene *LrLB88* and two markers based on F_2 population from the cross LB0288/Thatcher

genetic distances of 0 to 5.3 cM (Figures 1–3). The closest loci were WR003 with genetic distances of 0 cM. While RL6003 (*Lr1*) showed susceptible to the pathotype THTT, which indicated that *LrLB88* might be different from *Lr1*, and might be a new leaf rust resistance gene.

DISCUSSION

SSR markers. Compared with other markers, SSR markers are the most common due to advantages associated with co-dominance, accuracy, high repeatability, high levels of polymorphism, chromosome specificity, and ease of manipulation (Röder *et al.* 1998) and they have gained considerable importance in plant genetics and breeding, and have been the widely used molecular markers. They have been widely used in wheat for gene mapping. Currently, SSR markers have been successfully used in important wheat traits such as resistance to stripe rust and powdery mildew resistance gene mapping research (Jarve *et al.* 2000; Liu *et al.* 2002). In our laboratory, many leaf rust resistance genes such as *LrZH84* (Zhao *et al.* 2008), *LrG98* (Chen *et al.* 2010), *LrXi* (Li *et al.* 2010a), *LrBi16* (Zhang *et al.* 2011), *LrNJ97* (Zhou *et al.* 2013), and *LrFun* (Xing *et al.* 2014) were identified using SSR markers.

The presence of *Lr1* in Chinese wheat lines. Yuan *et al.* (2007) made a postulation of leaf rust resistance genes in 47 new wheat cultivars at the seedling stage. Results showed that *Lr1* was present in 11 wheat cultivars. Li *et al.* (2010b) inoculated 24 *P. triticina* pathotypes to postulate leaf rust resistance genes effective at the seedling stage of 102 Chinese winter wheat cultivars and advanced lines. Results showed that *Lr1* was identified in 6 cultivars. *Lr1* was commonly present in Chinese cultivars, while *Lr1* had lost resistance to most of the pathotypes in China. In the present test, *LrLB88* was mapped on 5DL near *Lr1*, but *LrLB88* showed resistance to the pathotype THTT while *Lr1* was susceptible, it can be indicated that *LrLB88* might be different from *Lr1*. On the other hand, wheat line LB0288 showed high resistance to all the pathotypes tested, which indicated that other leaf rust resistance genes might exist in LB0288. For future research, other pathotypes with high virulence should be employed to identify the other leaf rust gene. In another field test, LB0288 was susceptible to a mixture of pathotypes, including THTT, but at low disease severity, indicating that it might carry slow rusting resistance (unpublished data). LB0288 with slow rusting resistance to leaf rust could therefore be used in wheat breeding programs in China.

CONCLUSION

In the study, LB0288 carried a single dominant leaf rust resistance gene *LrLB88*, closely linked to the CAPS marker of *Lr1* (WR003) and SSR marker *Xbarc144*, with genetic distances of 0 cM and 5.3 cM. These markers should be useful for marker assisted selection in breeding leaf rust resistant wheat cultivars and will lay a foundation for improving leaf rust resistance in wheat breeding.

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