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ORIGINAL PAPER

Molecular mapping of leaf rust resistance gene *LrNJ97* in Chinese wheat line Neijiang 977671

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Abstract Neijiang 977671 and 19 near-isogenic lines with known leaf rust resistance genes were inoculated with 12 pathotypes of *Puccinia triticina* for postulation of leaf rust resistance genes effective at the seedling stage. The reaction pattern of Neijiang 977671 differed from those of the lines with known leaf rust resistance genes used in the test, indicating that Neijiang 977671 may carry a new leaf rust resistance gene(s). With the objective of identifying and mapping the new gene for resistance to leaf rust, F_1 and F_2 plants, and $F_{2:3}$ families, from Neijiang 977671 × Zhengzhou 5389 (susceptible) were inoculated with Chinese *P. triticina* pathotype FHNQ in the greenhouse. Results from the F_2 and $F_{2:3}$ populations indicated that a single dominant gene, temporarily designated *LrNJ97*, conferred resistance. In order to identify other possible genes in Neijiang 977671 other eight *P. triticina* pathotypes avirulent on Neijiang 977671 were used to inoculate 25 $F_{2:3}$ families. The results showed that at least three leaf rust resistance genes were deduced in Neijiang 977671. Bulked segregant analysis was performed on equal amounts of genomic DNA from 20 resistant and 20 susceptible F_2 plants. SSR markers polymorphic between the resistant and susceptible bulks were used to analyze the $F_{2:3}$ families. *LrNJ97* was linked to five SSR loci on chromosome 2BL. The two closest flanking SSR loci were *Xwmc317* and *Xbarc159* at genetic distances of 4.2 and 2.2 cM, respectively. At present two designated genes (*Lr50* and *Lr58*) are located on chromosome 2BL. In the seedling tests, the reaction pattern of *LrNJ97* was different from that of *Lr50*. *Lr50* and *Lr58* were derived from *T. armeniacum* and *Ae. triuncialis*, respectively, whereas according to the pedigree of Neijiang 977671 *LrNJ97* is from common wheat. Although seeds of lines with *Lr58* were not available, it was concluded that *LrNJ97* is likely to be a new leaf rust resistance gene.

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Introduction

Leaf rust, caused by *P. triticina*, is one of the most widespread diseases of common wheat (*Triticum aestivum* L.) worldwide (Bolton et al. 2008). In China, destructive epidemics of leaf rust occurred in 1969, 1973, 1975 and 1979 (Dong 2001). During the last few years, leaf rust has become increasingly important in the major wheat production regions, particularly in Northern China and the southern part of the Yellow and Huai Valleys (Zhao et al. 2008). With increasing temperatures and rainfall in recent years, epidemics have occurred in major wheat production regions. In 2012 yield losses were recorded in some regions of Gansu, Sichuan, Shaanxi, Henan and Anhui

(unpublished data). Resistant wheat cultivars are an efficient, economic and environmentally safe means to reduce losses caused by leaf rust.

Currently 68 leaf rust resistance genes are formally catalogued in wheat (McIntosh et al. 2011; Herrera-Foessel et al. 2012). Most of the genes are race-specific, and may be ineffective or lose effectiveness when virulent races emerge or increase. It is, therefore, very important to identify and use new leaf rust resistance genes in breeding programs.

There are many ways to study leaf rust resistance genes. Gene postulation based on comparative responses relative to lines with known genes can be used to identify the leaf rust resistance genes in a large number of lines within a relatively short time period. Li et al. (2010) tested seedlings of 102 Chinese wheat cultivars and advanced lines with 24 *P. triticina* pathotypes, and postulated the presence of *Lr1*, *Lr26*, *LrZH84* and 11 other leaf rust resistance genes in one or more lines. With the recent progress of molecular biology, markers are widely used to map resistance genes, and marker-assisted selection has been applied in breeding for resistance. Molecular markers, including simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), resistance gene analog polymorphism (RGAP), and now single nucleotide polymorphism (SNP), are useful tools for gene mapping in wheat. In total, the locations of 40 leaf rust resistance genes, including *Lr1*, *Lr3a*, *Lr9*, *Lr10*, *Lr13*, *Lr14a*, *Lr17a*, *Lr19*, *Lr20*, *Lr21*, *Lr24*, *Lr25*, *Lr27*, *Lr28*, *Lr29*, *Lr31*, *Lr32*, *Lr34*, *Lr35*, *Lr37*, *Lr38*, *Lr39*, *Lr40*, *Lr41*, *Lr44*, *Lr45*, *Lr46*, *Lr47*, *Lr48*, *Lr49*, *Lr50*, *Lr51*, *Lr52*, *Lr57*, *Lr58*, *Lr60*, *Lr61*, *Lr64*, *Lr67*, and *Lr68*, have been confirmed and/or mapped to wheat chromosomes with molecular markers (Sacco et al. 1988; Yang and Liu 2004; Khan et al. 2005; Helguera et al. 2005; Hiebert et al. 2005; McIntosh et al. 2009, 2011; Herrera-Foessel et al. 2012). SSR markers have been employed much more frequently than other markers due to their many advantages over other methods except SNP (Röder et al. 1998). Previously, we mapped resistance gene *LrZH84* in Chinese wheat line Zhou 8425B, closely linked to SSR loci *Xgwm582-1B* and *Xbarc8-1B*, with genetic distances of 3.9 cM and 5.2 cM, respectively (Zhao et al. 2008). In another Chinese wheat line, Bimai 16, leaf rust resistance gene *LrBi16* was shown to be closely linked to SSR loci *Xcfa2257* and *Xgwm344* on chromosome 7BL using seven SSR markers (Zhang et al. 2011).

Neijiang 977671, which was developed in 1997 by the Neijiang Agricultural Science Institute, Neijiang, Sichuan province, showed high resistance to most Chinese *P. triticina* pathotypes in seedling test. Although Neijiang 977671 has lost resistance to a few Chinese virulent pathotypes, it can be used in the deployment of resistances based on gene combinations to control leaf rust in China. In this study, F_1 and F_2 plants and $F_{2:3}$ families from a cross between the resistant line Neijiang 977671 and susceptible line Zhengzhou 5389 were used for molecular mapping of leaf rust resistance genes in Neijiang 977671.

Materials and methods

Plant materials and *P. triticina* isolates

The resistant parent Neijiang 977671, susceptible parent Zhengzhou 5389, F_1 and F_2 plants and $F_{2:3}$ families were included in the genetic analysis. Neijiang 977671 originated from the cross Neimai 6/90T388//Mianyang 26/Chuan SW 89-5165. Nineteen 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 12 *P. triticina* pathotypes used in multi-pathotype comparisons (Table 1) and genetic analysis are maintained at the Biological Control Center for Plant Diseases and Plant Pests of Hebei, Hebei Agricultural University. The 12 pathotypes were designated on the coding system of Long and Kolmer (1989) with addition of a fourth letter for the reactions of a fourth set of differentials (Table 1, http://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Cerealrusts/pt_nomen.pdf).

Evaluation of seedling responses in the greenhouse

Neijiang 977671, Zhengzhou 5389, and 19 near-isogenic Thatcher lines were inoculated with 12 *P. triticina* pathotypes (Table 1) for comparing the leaf rust reaction arrays. Neijiang 977671, Zhengzhou 5389, 15 F_1 plants, 139 F_2 plants and 185 $F_{2:3}$ families with 30 seedlings each were inoculated with Chinese *P. triticina* pathotype FHNQ (virulent on Zhengzhou 5389 and avirulent on Neijiang 977671). In order to identify other possible genes in Neijiang 977671 other eight *P. triticina* pathotypes avirulent on Neijiang 977671 were used to inoculate 25 of 185 $F_{2:3}$ families with 30 seedlings each. The 185 F_2 plants that generated the $F_{2:3}$ families had been inoculated with pathotype FHNQ at the adult plant stage in the field.

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 °C with 70 % RH. Infection types were scored 14 days after inoculation according to the Stakman scale modified by Roelfs et al. (1992).

Table 1 Seedling infection types and adult plant resistance produced by 21 lines when tested with Chinese *Puccinia triticina* pathotypes

Tester set[a]	Line	*Lr* gene	Pathotypes and infection types											APR to THTT[b]
			FHNQ	THST	FHSQ	FHSS	THTT	PHTQ	PHFT	FHPT	THKM	PHRS	PHLS	PHMQ	
1	RL 6003	*Lr1*	;	4	;1	;1	4	3	3	;	3	4	3	3	60S
	RL 6016	*Lr2a*	1	4	;	;1	4	;1	3	2	3	2	3	3c	30S
	RL 6047	*Lr2c*	3	4	4	4	4	3	3	3	3	4	3	3	80S
	RL 6002	*Lr3*	3	4	4	4	4	4	3	3	3	4	3	3	50S
2	RL 6010	*Lr9*	0	0	0	;	;	0	0	0	0	0	0	0	1R
	RL 6005	*Lr16*	4	4	4	4	4	4	4	3	4	4	4	3	40MS
	RL 6064	*Lr24*	;	;	;	;	;	;	;	;	;	;	;	;	0R
	RL 6078	*Lr26*	3	3	4	4	4	4	4	3	3	4	4	4	30S
3	RL 6007	*Lr3 ka*	3	3	3	4	3	3	3c	3	;	3	3	3	40S
	RL 6053	*Lr11*	3c	3	3	4	4	3	3c	3c	3	3	3c	3c	80S
	RL 6008	*Lr17a*	3	3	4	4	4	3	3	3	3	2	2	2	15MS
	RL 6049	*Lr30*	;	X	2	X	3	3	3	3	3	3	2	3	30S
4	RL 6051	*LrB*	4	3	4	4	4	4	4	4	3	4	3	4	60S
	RL 6004	*Lr10*	4	3	4	4	4	3	4	4	2	3	3	3	50S
	RL 6013	*Lr14a*	2	3	X	3	3	X	3	3	X	3	3	X	40S
	RL 6009	*Lr18*	2	3	2	2	3	12	3	3	3	2	2	1	1MR
	RL 6006	*Lr14b*	4	4	4	4	4	4	4	4	4	X	3	3	5S
	RL 6147	*Lr44*	3	;	3	4	1	3c	3	3	;1	2	1	1	10MR
	TcLr50	*Lr50*	4	4	4	4	4	4	4	4	3	4	3	3	60S
	Neijiang 977671		;	3	1+	;	4	;1	;1	;1	3	X	;1	;	80S
	Zhengzhou 5389		4	4	4	4	4	4	4	4	4	4	4	4	80S

[a] The coding system of Long and Kolmer (1989)

[b] Reactions and disease severity tested in the field

Evaluation of leaf rust reactions in the field

Neijiang 977671, Zhengzhou 5389, 19 near-isogenic lines (Table 1) and 185 F_2 plants were grown at Baoding, Hebei province, in the 2010–2011 cropping season. Neijiang 977671, Zhengzhou 5389 and 19 near-isogenic lines were inoculated with pathotype THTT, while two parents and their 185 F_2 plants were inoculated with FHNQ. The F_2 population was planted as single plant hills spaced 15 cm apart. Spreader rows of Zhengzhou 5389 were planted perpendicular and adjacent to the rows of tested plants. Leaf rust epidemics were initiated by spraying an aqueous suspension of urediniospores of *P. triticina*, to which a few drops of Tween 20 (0.03 %) had been added, onto the spreader rows in mid-April.

Percentage leaf rust severities (Peterson et al. 1948) and host responses to infection (Roelfs et al. 1992) were recorded. Scoring took place when leaves of the susceptible line Zhengzhou 5389 were fully rusted in late May. The F_2 plants were harvested individually for progeny testing with pathotype FHNQ as mentioned above.

DNA extraction and bulk preparation

Genomic DNA was extracted from F_2 plants or $F_{2:3}$ families (30 plants bulked for each line) using the CTAB protocol (Sharp et al. 1988). DNA was quantified with a UV spectrophotometer, and diluted to a final concentration of 30 ng/μl prior to use.

Bulked segregant analysis (Michelmore et al. 1991) was performed to identify molecular markers putatively linked to leaf rust resistance in Neijiang 977671. Genomic DNA from 20 resistant and 20 susceptible F_2 plants inoculated with FHNQ at the adult stage was bulked in equal amounts to form resistant and susceptible bulks. DNA samples of the two parents and bulks were screened for polymorphisms of SSR markers.

SSR analyses

The 980 wheat SSR markers used in this study included 185 GWM (Gatersleben wheat microsatellite) primer sequences described by Röder et al. (1998), 316 WMC primers developed by the Wheat Microsatellite Consortium

(http://wheat.pw.usda.gov/ggpages/SSR/WMC), 357 BARC primers developed by Cregan and associates (USDA-ARS Beltsville) and 122 CFA primers developed by Sourdille et al. (2001). All sequences are available at the GrainGenes site (http://www.graingenes.org).

SSR markers showing polymorphism between resistant and susceptible bulks were used to genotype individual $F_{2:3}$ families. Microsatellite analysis followed the procedure described by Bryan et al. (1997) with minor modifications. PCR was performed in volumes of 20 µl containing 1.0 U *Taq* DNA polymerase (Zexing Biotechnology Co. Ltd, Beijing, China), 1× PCR buffer (50 mM KCl, 10 mM Tris–HCl, 1.5 mM $MgCl_2$, pH 8.3), 200 µM of each dNTP, 6 pmol of each primer and 60 ng of template DNA. The PCR conditions were a denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 51–68 °C (depending on the primer pair) for 1 min, 72 °C for 1 min and a final extension at 72 °C for 10 min. Five microliters of PCR product was mixed with 5 µl of formamide loading buffer (98 % formamide, 10 mM EDTA, 0.25 % bromophenol blue, 0.25 % xylene cynol, pH 8.0), and heated at 94 °C for 5 min and cooled on ice. The mixture was then loaded on 6 % denaturing polyacrylamide gels, run at 90 W for approximately 1 h, and visualized by silver staining (Bassam et al. 1991).

Linkage analysis and genetic mapping

Chi-square (χ^2) tests were used to evaluate the goodness of fit of observed and expected segregation ratios. Linkage analysis was performed using the software MapManager QTXb20 (Manly et al. 2001) and recombination values were converted to centiMorgens using the Kosambi mapping function (Kosambi 1944).

Results

Reactions of Neijiang 977671 and 19 NILs with known leaf rust resistance genes

In seedling tests with 12 *P. triticina* pathotypes (Table 1), Neijiang 977671 was resistant to FHNQ, whereas lines possessing 12 known leaf rust resistance genes (*Lr2c*, *Lr3*, *Lr16*, *Lr26*, *Lr3ka*, *Lr11*, *Lr17a*, *LrB*, *Lr10*, *Lr14b*, *Lr44*, *Lr50*) were susceptible, indicating *LrNJ97* was different from these 12 genes. The X response produced by Neijiang 977671 against pathotype PHRS could be due to *Lr14b* because RL 6006 (*Lr14b*) produced a similar response to pathotype PHRS. At the adult stage RL 6006 (*Lr14b*) showed slow rusting to pathotype THTT, but Neijiang 977671 was highly susceptible (80S) (Table 1), indicating that the resistance to PHRS in Neijiang 977671 was not due to *Lr14b* and therefore it must carry a different gene. Of three pathotypes (THST, THTT, and THKM) virulent on Neijiang 977671, at least one produced a low IT on lines with four genes (*Lr9*, *Lr24*, *Lr30*, and *Lr14a*), indicating that resistance gene *LrNJ97* in Neijiang 977671 was also different from these four genes. Three genes (*Lr1*, *Lr2a* and *Lr18*) gave low reactions to all the pathotypes avirulent to Neijiang 977671, but the locations of the three genes were different from that of *LrNJ97* (chromosome 2BL), therefore *LrNJ97* was different from those genes. The results indicated that *LrNJ97* was different from 19 known *Lr* genes.

Inheritance of leaf rust resistance in Neijiang 977671

In the seeding test with race FHNQ, Neijiang 977671 was resistant (infection type, IT; to 1), whereas Zhengzhou 5389 was susceptible (IT 4). F_1 plants were resistant (IT;1) and the F_2 population segregated 104 plants with IT; to 2 (resistant) and 35 plants with IT 3 to 4 (susceptible), indicative of a single dominant gene for resistance ($\chi^2_{3:1} = 0.0024$, $P_{1\text{d.f.}} > 0.95$). In the field test, Neijiang 977671 was resistant (IT;1) and Chinese Spring was susceptible (IT 4). The F_2 population segregated 145 plants IT; to 2 and 40 plants with IT 3 to 4, also indicating single locus segregation ($\chi^2_{3:1} = 1.13$, $P_{1\text{d.f.}} > 0.25$). When 185 $F_{2:3}$ families from these F_2 plants were tested as seedlings, 42 lines were homozygous resistant, 103 segregated and 40 were homozygous susceptible, fitting a single locus segregation ratio ($\chi^2_{1:2:1} = 2.43$, $P_{2\text{d.f.}} > 0.25$). The 40 homozygous susceptible lines were the progenies of plants scored susceptible in the field. Results from the F_2 and F_3 populations indicated that a single dominant gene, tentatively designated *LrNJ97,* conferred resistance to leaf rust pathotype FHNQ in Neijiang 977671.

When 25 $F_{2:3}$ families with 30 seedlings each were inoculated with nine *P. triticina* pathotypes avirulent on Neijiang 977671, the response patterns of 25 $F_{2:3}$ families were same for five pathotypes FHNQ, PHTQ, PHFT, PHLS and PHMQ, showing leaf rust resistance in Neijiang 977671 against these five pathotypes was provided by *LrNJ97*. The response patterns of 25 $F_{2:3}$ families were same for three pathotypes FHSQ, FHSS, and FHPT, but they were different from those produced by FHNQ. To further clarify the relationship between the leaf rust resistance genes identified with pathotypes FHNQ and FHSQ, 115 $F_{2:3}$ families with 30 seedlings each were inoculated with FHNQ and FHSQ. According to the results, the segregation to FHNQ and FHSQ is not independent and the gene *LrNJ97* for resistance to FHNQ is also effective against FHSQ (Table 2). The segregation to FHSQ is probably 7:8:1 ($\chi^2_{7:8:1} = 0.26, P_{2\text{d.f.}} > 0.75$) for

Table 2 Seedling reaction produced by 115 $F_{2:3}$ families from Neijiang 977671 × Zhengzhou 5389 when tested with *P. triticina* pathotypes FHNQ and FHSQ

Reaction to FHNQ	Reaction to FHSQ			Total
	HR	Seg	HS	
HR	25	–	–	25
Seg	18	44	–	62
HS	9	13	6	28
Total	52	57	6	115

Table 3 Seedling reaction produced by 25 $F_{2:3}$ families from Neijiang 977671 × Zhengzhou 5389 when tested with *P. triticina* pathotypes FHNQ and PHRS

Reaction to FHNQ	Reaction to PHRS			Total
	MR	MR:S	HS	
HR	3	2	–	5
Seg	1	6	6	13
HS	–	5	2	7
Total	4	13	8	25

HR homozygous resistant, *Seg* segregating, *HS* homozygous susceptible, *MR* moderately resistant infection type X, *MR:S* infection types X and 4

two independent genes (Table 2). When 28 homozygous susceptible lines without *LrNJ97* were tested with FHSQ, 13 lines were segregating (the ratios fit 3:1, pooled segregation: 257 resistant plants and 92 susceptible plants, $\chi^2_{3:1} = 0.34$, $P_{1d.f.} > 0.25$) and 9 lines were homozygous resistant, showing that resistance in these lines was conferred by a second gene. In another test 215 plants from one of 13 segregating $F_{2:3}$ families without *LrNJ97* were tested with pathotype FHSQ, and the plants of the line segregated 166 plants with IT;1 (resistant) and 49 plants with IT 3 to 4 (susceptible), indicative of another unknown dominant gene for resistance to FHSQ ($\chi^2_{3:1} = 0.56$, $P_{1d.f.} > 0.25$). Neijiang 977671 produced X response against PHRS and the response pattern of 25 $F_{2:3}$ families to PHRS was different from those produced by other pathotypes (Table 3), indicating other unknown gene was present in Neijiang 977671 and conferred X response to PHRS. These results suggested that at least three leaf rust resistance genes were present in Neijiang 977671. Molecular mapping of the second gene is underway.

Linkage analysis and genetic map

Of the 980 SSR markers, five (*wmc500*, *gwm547*, *wmc317*, *barc159*, and *wmc356*) on chromosome 2BL showed polymorphisms between the resistant and susceptible bulks as well as the parents. This indicated that *LrNJ97* was located on chromosome 2BL. The five polymorphic markers were then screened on DNA bulks from each of the 185 $F_{2:3}$ families previously tested with leaf rust. Resistance gene *LrNJ97* was linked to the five SSR loci with genetic distances ranging from 2.2 cM to 25.4 cM (Fig. 2). The two closest flanking SSR loci were *Xwmc317-2BL* and *Xbarc159-2BL* with genetic distances of 4.2 and 2.2 cM (Figs. 1, 2; Table 4), respectively.

Origin of *LrNJ97*

Neijiang 977671 was derived from the cross Neimai 6/90T388//Chuan SW 89-5165/Mianyang 26. Seedling tests with leaf rust pathotype FHNQ indicated that Neijiang 977671 and Chuan SW 89-5165 were resistant (IT;1), whereas Neimai 6 and Mianyang 26 were susceptible (IT 4). Seeds of 90T388 were not available. It seems likely that *LrNJ97* originated from Chuan SW 89-5165. SSR analyses with *Xbarc159-2B* and *Xwmc317-2B* closely linked to *LrNJ97* showed that Chuan SW 89-5165 possessed the same alleles as Neijiang 977671, further confirming that *LrNJ97* came from Chuan SW 89-5165. All parents of Chuan SW 89-5165 are common wheat; therefore *LrNJ97* originates from common wheat.

Discussion

Comparison of *LrNJ97* with leaf rust resistance genes located on chromosome 2BL

Formally named leaf rust resistance genes *Lr50* and *Lr58* are located on chromosome 2BL (Brown-Guerdira et al.

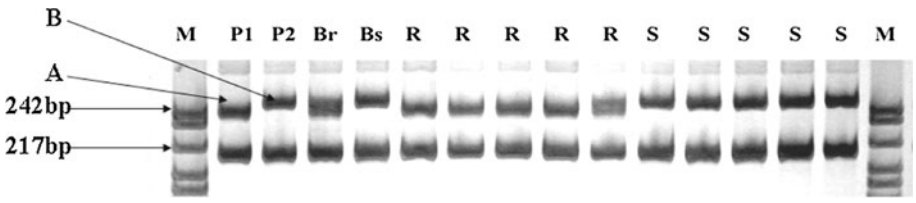

Fig. 1 Electrophoresis of PCR products amplified with SSR marker *barc159-2BL* on polyacrylamide gels. The *A* allele is present in the resistant parent Neijiang 977671 (P1); the *B* allele is present in Zhengzhou 5389 (P2); *Br* resistant bulk, *Bs* susceptible bulk, *R* resistant F_2 plants, *S* susceptible F_2 plants

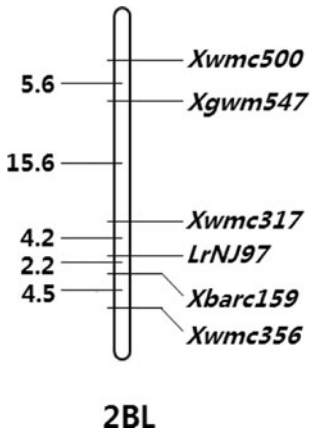

Fig. 2 Linkage map of the *LrNJ97* region constructed with five SSR markers on chromosome 2BL

Table 4 F_2 phenotypes and genotypes inferred from reactions of $F_{2:3}$ families inoculated with Chinese *P. triticina* pathotype FHNQ and the corresponding alleles at *Xbarc159* and *Xwmc317*

Locus name	F_2 phenotype	F_2 genotype	Allele		
			A	H	B
Xbarc159	Resistant (145)	RR (42)	40	2	0
	Susceptible (40)	Rr (103)	3	100	0
		rr (40)	0	3	37
Xwmc317	Resistant (145)	RR (42)	42	0	0
	Susceptible (40)	Rr (103)	7	92	4
		rr (40)	0	4	36

RR homozygous resistant, *Rr* segregating, *rr* homozygous susceptible, *A* homozygous for the Neijiang 977671 allele, *B* homozygous for the Zhengzhou 5389 allele, *H* heterozygous

2003; Kuraparthy et al. 2007). *Lr50* is present in wheat line KS96WGRC36, a *T. timopheevii* subsp. *armeniacum* derivative and is linked to SSR markers *Xgwm382* and *Xgdm87* with genetic distances of 6.7 and 9.4 cM, respectively (Brown-Guedira et al. 2003). In the study *LrNJ97* was closely linked to SSR loci *Xwmc317* and *Xbarc159* with genetic distances of 4.2 and 2.2 cM, respectively. In the map of Somers et al. (2004), the distances of the markers from the end of the short arm were listed as *Xwmc317* 106 cM, *Xbarc159* 109 cM, *Xgwm382* 114 cM and *Xwmc356* 117 cM; this places the *Lr50* locus in the same vicinity or distal to *LrNJ97*. In seedling tests, the *Lr50* line was susceptible to all 12 pathotypes, whereas Neijiang 977671 was resistant to most of them, indicating that *LrNJ97* was different from *Lr50*. *Lr58* was mapped on the terminal region of chromosome 2BL and closely linked to SSR marker *cfd50*, which was also close to *LrNJ97*, but *Lr58* was derived from *Ae. triuncialis* (Kuraparthy et al. 2007), according to the pedigree of Neijiang 977671, *LrNJ97* was derived from common wheat. Although seeds of lines with *Lr58* were not available, it was concluded that *LrNJ97* is likely to be a new leaf rust resistance gene.

Linkage map of leaf rust resistance gene *LrNJ97*

LrNJ97 was flanked by markers *barc159* and *wmc317*. The latter is proximal to the gene with a genetic distance of 4.2 cM whereas *barc159* is distal (2.2 cM) to it (Fig. 1). The distances of the markers from the end of the short arm were listed as *wmc500* 78 cM, *wmc317* 106 cM, *barc159* 109 cM, and *wmc356* 117 cM (Somers et al. 2004). The order and positions of these markers were consistent with the map of *LrNJ97*. The two closest flanking SSR makers can be used for marker assisted selection in breeding wheat cultivars with resistance to leaf rust, but for cloning the resistance gene, the two markers are too far away, and therefore it is necessary to look for more closely linked or even co-segregating markers for map-based cloning.

Use of *LrNJ97* in wheat breeding for durable resistance to leaf rust

Breeders and plant pathologist have focused on using wheat cultivars with durable resistance for controlling wheat leaf rust, and the deployment of resistances based on gene combinations (Watson and Singh 1952; Eriksen et al. 2004) or slow rusting types of adult plant resistance are considered to be useful ways to prolong leaf rust resistance. Currently, only a few leaf rust resistance genes are effective against prevalent Chinese *P. triticina* races (Li et al. 2010). Hence, it is very important to search for more resistance genes to cope with the dynamic and rapidly evolving pathogen population. There are abundant materials with leaf rust resistance in China, but limited information is available in respect of possible leaf rust resistance genes in Chinese wheat germplasm (Li et al. 2010). Therefore, identifying and tagging new leaf rust resistance genes in current Chinese wheat lines would be extremely useful for breeding new resistant cultivars and for gene deployment schemes (Li et al. 2010). Although *LrNJ97* as a new leaf rust resistance gene is effective against most of Chinese pathotypes, it is already ineffective against at least three pathotypes. The gene should be combined with other effective leaf rust resistance genes to achieve more widely effective resistance or perhaps could be used in a multi-line cultivar for durable control of leaf rust in China. In the field test Neijiang 977671 was highly susceptible (80S) to the *LrNJ97*-virulent pathotype THTT (Table 1) indicating an absence of effective minor or slow rusting resistance genes.

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