Identification of known leaf rust resistance genes in bread wheat cultivars from China

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Abstract: Leaf rust caused by *Puccinia triticina* Eriks. (*Pt*) is one of the most devastating fungal pathogens affecting wheat (*Triticum aestivum* L.) production worldwide. Deployment of resistant cultivars is the most environmentally friendly approach to control the disease. In this study, thirty-seven wheat lines from the Hubei and Shaanxi provinces in China were evaluated for seedling resistance in the greenhouse using eighteen *Pt* races. These lines were also tested for slow rusting resistance in the field in the 2014 to 2018 growing seasons. Eleven molecular markers closely associated with known *Lr* genes were used as part of the postulation process. Seven known *Lr* genes, *1, 13, 18, 14a, 26, 34* and *46* either singly or in combination were postulated in twenty-five cultivars. *Lr1* and *Lr26* were the most commonly identified genes detected in thirteen and ten cultivars, respectively. *Lr13* and *Lr46* were each found in four and five cultivars. *Lr34* was present in three cultivars. *Lr18* and *Lr14a* were identified in cultivar Xi'nong 538. Six cultivars displayed slow rusting resistance in the field tests. The resistant cultivars identified in the present study can be used as resistance parents in crosses aimed at pyramiding and the deployment of leaf rust resistance genes in China.

Keywords: adult plant resistance; gene postulation; molecular markers; *Triticum aestivum*

Bread wheat provides about 20% of the calories consumed by humankind (Fu et al. 2009). Wheat leaf rust caused by *Puccinia triticina* Eriks. (*Pt*), is one of the most important wheat diseases in many regions worldwide. This disease occurs in almost all wheat-growing areas, including North America, Europe, Asia, Australia, etc. (Dehne & Oerke 1998), and causes severe yield losses ranging from 30 to 50% (McIntosh et al. 1995). A particularly severe leaf rust epidemic in north-western Mexico caused an estimated yield loss of up to 70% during the 1970s (Dubin & Torres 1981). Widespread damaging leaf rust epidemics in China

were recorded in 1969, 1973, 1975 and 1979 (Dong 2001; Li et al. 2014). In 2012 and 2015, leaf rust caused destructive yield losses in the major wheat production regions of China, especially in North China (Zhou et al. 2013; Peng et al. 2016). Deployment of resistant cultivars is an economical and environmentally friendly way to control wheat leaf rust. To ensure the continuing effectiveness, it is important to identify and utilise new sources of leaf rust resistance (*Lr*) genes in wheat breeding programmes.

The principle of gene postulation is based on the gene-for-gene concept proposed by Flor (1955) to

identify resistance genes to the disease possibly present in the tested cultivars. A specific resistance gene in a host cultivar can be postulated from the response array produced by a series pathogen races with known avirulence/virulence characteristics. In addition, many resistance genes can be postulated using molecular markers although not all markers are fully diagnostic of the gene to which they are linked. *Lr* genes with reliable markers include *1*, *10*, *9*, *19*, *20*, *24*, *34*, and *46*. Numerous researchers have used these multi-race tests to postulate the *Lr* genes in different sets of wheat cultivars. For example, Yuan et al. (1992) identified the *Lr* genes *1*, *3*, *3bg*, *9*, *10*, *13*, *16*, *23*, *26,* and *34* in forty-seven wheat cultivars using seventeen *Pt* races. Gebrewahid et al. (2017) identified twelve *Lr* genes, *1*, *26*, *3ka*, *11*, *10*, *2b*, *13*, *21*, *34*, *37*, *44*, and *46* in eighty-three Chinese common wheat cultivars using eighteen *Pt* races. Wu et al. (2019) postulated six *Lr* genes (*1*, *26*, *33*, *34*, *45* and *46*) in forty-four wheat accessions using twenty *Pt* races.

Currently, there is limited information regarding leaf rust resistance genes in the leading contemporary cultivars grown in some regions of China. In this study, thirty-seven wheat cultivars grown in the Hubei and Shaanxi provinces were subjected to multi-race seedling tests, field tests and molecular marker detection. The objective of this study was to identify *Lr* genes in thirty-seven wheat cultivars from China.

MATERIAL AND METHODS

Plant materials and *Pt* races. Thirty-seven wheat cultivars from the Shaanxi and Hubei were used in this study. The regions and pedigrees of the thirty-seven cultivars are listed in Table 1. All the cultivars were tested for seedling response using eighteen *Pt* races in the greenhouse (Tables 2 and 3), and for slow rusting to leaf rust in the field during the 2014 to 2018 growing seasons. The International Maize and Wheat Improvement Center (CIMMYT) line, Saar with typical slow leaf rusting (Lillemo et al. 2008; Zhuang et al. 2009) and the highly susceptible line, Zhengzhou 5389 (final disease severity (FDS) > 90%) were used as the slow rusting and susceptible controls, respectively. Thirty-six lines with known *Lr* genes were utilised as a reference base to compare the seedling infection types (ITs) produced by each test line (Table 2). All the *Pt* races were named following the three-letter coding system of Long and Kolmer

(1989), with the addition of a fourth letter for the fourth set of test differentials (http://www.ars.usda. gov/SP2 User Files/ad _hoc/36400500 Cereal rusts/ *pt* nomen.pdf). The seeds of all the thirty-seven test cultivars, thirty-six differential lines with known leaf rust resistance genes, susceptible line Zhengzhou 5389, and CIMMYT line Saar were provided by the Wheat Rust Laboratory of Hebei Agricultural University.

Seedling test. All the cultivars were planted in a greenhouse and inoculated with eighteen *Pt* races for the gene postulation (Tables 2 and 3). The gene postulation was conducted following the method described by Singh et al. (1999) with minor modifications. The seedling inoculations were performed by brushing urediniospores from sporulating susceptible seedlings onto the test seedlings when the first leaves were fully expanded. The inoculated seedlings were placed in plastic-covered cages and incubated at 18 °C and 100% relative humidity (RH) for 24 h before being transferred to a growth chamber maintained with 12 h light/12 h darkness at 18 °C to 20 °C with 70% RH. The ITs were scored 10 to 14 days postinoculation according to the 0 to 4 infection type scale as modified by Roelfs et al. (1992).

Adult plant tests. All the thirty-seven cultivars, along with the susceptible control, Zhengzhou 5389 and the slow rusting check, Saar were planted in a randomised complete block design with two replicates at Zhoukou, in Henan Province and Baoding, in Hebei Province during the 2014 to 2018 cropping seasons. Approximately fifty seeds of each line were sown in 1.5 m single-row plots with 0.3-m spacing. Spreader rows of Zhengzhou 5389 were planted perpendicular and adjacent to the test rows. The field inoculation was conducted using a mixture of an equal amount of urediniospores from the FHRT, THTT and THJT *Pt* races suspended in 0.03% Tween 20 onto the spreader rows at the tillering stage. The disease severities as a percentage of the leaf area covered with uredinia were scored three times at about the 1-week interval with the first scoring 4 weeks after inoculation (Feekes growth stage 10) in each environment according to the modified Cobb scale (Peterson et al. 1948). The FDS was collected when the susceptible control, Zhengzhou 5389 was fully infected. The FDS data evaluation to leaf rust response was conducted following the methods described by Li et al. (2010).

Statistical analysis. The analysis of variance (ANOVA) and for determining the least significance

differences (LSDs) for comparing the FDS values among the cultivars were performed using the IBM SPSS Statistics 19.0 software. Cultivars susceptible to the mixed *Pt* races at the seedling stage and having FDS values significantly lower than the slow rusting control, Saar in the field trials were considered to be slow rusting cultivars.

Molecular makers testing. The genomic DNA was extracted following the cetyltrimethyl-ammonium bromide (CTAB) method (Sharp et al. 1988). Eleven molecular markers reported as diagnostic or closely linked to nine *Lr* genes *1, 9, 10, 19, 20, 24, 26, 34*

and *46* were used to test all the cultivars (Table 3). The polymerase chain reaction (PCR) assay was conducted following the protocol of Helguera et al. (2000), in a 20 μL reaction volume containing: 10 μL of 2 × TaqPCR Master Mix (Tiangen Biochemical Incorporation, Beijing), 6 μL of ddH$_2$O, 2 μL (4 mol per μL) of the primer, and 2 μL 4 ng/μL of the template DNA. All PCR amplification conditions are listed in Table 4. The amplified products were detected by 1.5% agarose gel electrophoresis or 12% non-denaturing polyacrylamide gel electrophoresis in the case of *Lr46*.

Table 1. The region and pedigree of 37 Chinese wheat cultivars tested for the leaf rust response

Line No.	Genotype	Region	Pedigree	Line No.	Genotype	Origin	Pedigree
1	E'mai 580	Hubei	(Taigu sterile lines)/ (wheat line 957565)	20	Shaanmai 139	Shaanxi	Xiaoyan 22 × 94156/ N9134 F1
2	E'mai 17	Hubei	E'mai 12 variation plant	21	Xi'nong 538	Shaanxi	Lankao 90(6)52-30/ Xiaoyan 6/Huaihe 9412
3	E'mai 12	Hubei	750025-12/E'mai 6	22	Xiaoyan 166	Shaanxi	87135-1-3-2-1-2/88111
4	E'mai 18	Hubei	SKUA/865146//E'mai 11	23	Shaannong 138	Shaanxi	Xinmai 9/HangShaan 354
5	Xi'nong 291	Shaanxi	(Xiaoyan 5) 4DN/ hexaploid triticale WOH45	24	Xiaoyan 54	Shaanxi	Xiaoyan 6
6	Xiaoyan 22	Shaanxi	(Xiaoyan 6/775-1)/ Xiaoyan 107	25	Shaan 253	Shaanxi	Shaan 229/Shaan 213
7	Shaan 150	Shaanxi	(4/6811(2)F7/8435-1-1-8) F1/Xiaoyan 6	26	Xi'nong 6028	Shaanxi	Jingyan 60/Zhongnong 28
8	Gaoyou 503	Shaanxi	78506/84s504	27	Shaanmai 159	Shaanxi	Xiaoyan 597/89605
9	Xi'nong 88	Shaanxi	*Aegilops variabilis* cytoplasm	28	Xiaoyan 216	Shaanxi	Lankao 906/ Xiaoyan 22
10	Xi'an 93991	Shaanxi	NA	29	Xi'nong 2611	Shaanxi	Shaan229/ [84(14)43/83(2)3-3]/ (Xinong65 × Xiaoyan)
11	E'mai16	Shaanxi	The variation of 7023 plant	30	Shaan 558	Shaanxi	Xiaoyan 22/v9511
12	Xi'nong 2000	Shaanxi	Xi' nong 2611/386/ Xiaoyan22/Shaan 354	31	Xi'nong 9871	Shaanxi	Xinong 2208/Xiaoyan 22
13	Xiaoyan 4	Shaanxi	Fengchang 1/Xiaoyan 759	32	Xi'nong 889	Shaanxi	E Han-4/(Xiaoyan 6/ Xiaoyan 83352)
14	Shaan 627	Shaanxi	NA	33	Xiaoyan 228	Shaanxi	NA
15	Xi'nong 223	Shaanxi	Xi'nong 389 variation plant	34	Xiaoyan 319	Shaanxi	NA
16	Xi'nong 126	Shaanxi	NA	35	Shaan 160	Shaanxi	Shaan 213/winter wheat lines 167-6-4
17	Shaannong 981	Shaanxi	NA	36	Shaanmai 175	Shaanxi	NA
18	Xi'nong 3517	Shaanxi	Xi'nong 1376/Xi'nong 88	37	Shaan 512	Shaanxi	Shaanmai 150(A2)
19	Shaanken 6	Shaanxi	Lankao 906/Xiaoyan 22				

NA – not available

Table 2. Infection types produced by 36 reference lines with the single leaf rust resistance genes inoculated with 18 *Puccinia triticina* (*Pt*) races

No.	*Lr* gene(s)	PH GQ	TH JT	PH JT	KH JS	PH JS	THTT I	KH HT	FH RT	FH JQ	PHTT I	THTT II	PHTT II	FH TR	FHHT I	FHHT II	TG GT	FH TT	FG MT
											Infection types[a] to *Pt* races								
1	*TcLr1*	4	3+	3+	;1	4	4	1	1	1	4	4	4	1	1	1	4	1	1
2	*TcLr2a*	1	3+	2+	3	1	4	4	1	1	1	4	1	0, ;	1	1	4	;	1
3	*TcLr2C*	4	4	4	3+	4	4	3+	4	4	4	4	4	3+	4	4	3+	3+	4
4	*TcLr3*	4	4	4	3+	4	4	4	4	4	4	4	4	4	4	4	3	4	4
5	*TcLr9*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	*TcLr16*	4	4	4	4	4	4	4	4	4	3+	4	3	3	3	3	3	3+	3+
7	*TcLr24*	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	0	0
8	*TcLr26*	3+	3+	4	4	4	4	4	4	4	3	3	3	3	3	3+	2	3+	2
9	*TcLr3ka*	1	1	1	2	;, 1	3	1	4	1	3	3	3c	3+	2	2	1	3	3
10	*TcLr11*	4	4	3+	3	3	4	3	4	4	4	4	3c	4	3+	3	3+	3+	2+
11	*TcLr17*	2	3c	2+	1	3	4	1	1	1	3+	4	3	3	2+	1	2+	3	2
12	*TcLr30*	1	1	2+	1	1	3+	3c	3	1	3+	4	4	4	3+	4	1	3	4
13	*TcLrB*	4	3+	4	4	4	4	3+	4	4	4	4	3	4	4	3+	4	3+	4
14	*TcLr10*	3+	4	4	4	4	4	3+	4	4	4	3c	4	3	3	3	4	4	4
15	*TcLr14a*	X	4	3+	4	3+	4	4	3+	X	4	3+	4	X	3+	3	3+	3+	3+
16	*TcLr18*	1	3+	3	1	2	3	3+	3	2	4	4	4	3	4	4	3+	4	3+
17	*TcLr2b*	2	4	3+	4	3+	3+	3+	2+	3	3+	4	3	3+	3+	3	3+	4	3+
18	*TcLr3bg*	4	4	3+	4	4	4	3+	3+	3+	4	4	3+	3	3+	3	3+	4	4
19	*TcLr13*	3	3	3	3	4	3	3+	3	3	3	4	2	3	2	3	3+	2	2
20	*TcLr14b*	4	4	4	4	4	4	3+	4	4	4	4	3	4	4	3	4	4	4
21	*TcLr15*	4	1	1	1	3+	4	1	1	1	1	4	1	3	1	1	1	2	1, 2
22	*TcLr19*	0	;	0	0	0	0	0	;	0	0, ;	0	;	0	;	0	0	0	0
23	*TcLr21*	2	4	3	3	4	3	3	;, 1	4	3+	3	3	3	4	3	2	3+	2+
24	*TcLr20*	1	1, 2	1	1, 2	1	1	1, 2	;	1	1	1, 2	3	0	3+	1	3+	2+	1
25	*TcLr23*	1, 2	1	3	1	1	3	3	2+	3	3	1	1	1	1	1	3+	1	1
26	*TcLr28*	0	0	0	0	0	0	0	0	0	;	0	;	0	0, ;	0	0	;	;
27	*TcLr29*	1	1	1	2	3	1	2	2	2	1	2	;	2	3	3	1	3	2
28	*TcLr33*	3+	3+	3+	3+	3+	4	3+	4	3+	4	4	4	3	3+	3+	3+	3+	3
29	*TcLr36*	1	1	1	1	1	1	1	1	1	3	1	2	1	1	1	3	1	1
30	*TcLr39*	3	2+	2+	2	3+	3	1	2	1	4	4	3	3	2	2	4	1	1
31	*TcLr42*	3	3	3	2	1	2	3	3+	3	1	1	2	2	2	3	1	3+	3
32	*TcLr44*	4	4	4	3	4	4	4	4	4	4	4	4	4	4	4	3+	3	4
33	*TcLr45*	1	1	1	;, 1	1	1	1	3	;, 1	1	1	1	1	1	1	3+	1	1
34	*TcLr47*	0	0	0	0	0	0	0	0	0	;	0	;	;	;	;	;	0	;
35	*TcLr51*	;	;	;	0	;	;	;	;	;	1	1	;	1	1	;	1	1	;
36	*TcLr53*	0	0	0	0	0	0	0	0	0	;	0	;	;	;	;	;	;	0, ;

[a]According to the 0–4 Stakman scale modified by Roelfs et al. (1992); 0 – no flecks or uredinia, ; – hypersensitive flecks, 1 – small uredinia with necrosis, 2 – small uredinia with chlorosis, 3 – moderate size uredinia, 4 – large uredinia, + indicates slightly larger uredinia, C – more chlorosis than normal for the infection type, X – random distribution of variable-sized uredinia

Table 3. Seedling infection types and the presence or absence of the *Lr* genes in 37 Chinese wheat cultivars based on the gene postulation using 18 *Puccinia triticina* (*Pt*) races and molecular markers

No.[b]	*Lr* gene based on gene postulation	*Lr* gene based on gene marker detection	*Lr* gene(s)	PH GQ	TH JT	PH JT	KH JS	PH JS	THTT I	KH HT	FH RT	FH JQ	PHTT I	THTT II	PHTT II	FH TR	FHHT I	FHHT II	TG GT	FH TT	FG MT
1	+	none	unknown APR genes	4	4	4	4	4	4	4	4	4	3+	4	3+	4	3	4	3	4	3+
2	*Lr26, Lr13*, +	*Lr26*	*Lr26*c, *Lr13*d, +	4	3	3+	3	4	4	4	3	4	2	2	2	1	2	1	2+	2	1
3	+	none	unknown seedling resistance gene	4	4	4	4	4	4	4	4	4	3+	4	2+	2+	3	3+	4	3+	2+
4	*Lr1, Lr26*, +	*Lr1, Lr26*	*Lr1*c, *Lr26*c, +	4	4	4	2	4	4	2+	2	2+	2	4	3	2+	2	2+	2+	2+	2+
5	+	none	unknown APR genes	4	3	3	3+	4	3+	4	3	4	4	4	3	3+	3	3	3+	3+	3
6	*Lr1, Lr26*, +	*Lr1, Lr26*	*Lr1*c, *Lr26*c, +	4	4	4	2	4	4	2	2+	2	3	4	3	2	2+	2+	2+	2	2
7	*Lr1*, +	*Lr1*	*Lr1*c, +	3+	4	4	2+	4	3	2+	2+	2	4	3	4	2	2	2+	4	2+	2+
8	*Lr1, Lr13*, +	*Lr1*	*Lr1*c, *Lr13*d, *Lr46*e, +	3+	4	4	2	4	4	2	2+	2	4	3	2+	2+	2	2	4	2	2+
9	+	*Lr46*	*Lr46*e, +	3+	4	4	4	4	4	4	4	4	4	4	3+	4	4	4	4	4	3+
10	*Lr1, Lr13*, +	*Lr1*	*Lr1*c, *Lr13*d, +	4	4	4	2	4	4	2c	2+	2	3	4	2	2+	2	2	3+	2+	2
11	+	none	unknown APR genes	4	4	4	3+	4	4	4	4	4	4	4	4	3+	3	4	3	4	3+
12	+	none	unknown APR genes	4	3+	3+	3+	4	4	4	4	4	4	4	3+	3	4	3	3+	3	3+
13	*Lr1*, +	*Lr1*	*Lr1*c, +	4	4	4	2+	4	3+	2+	2	2	3	4	3	2c	2+	2	3+	2+	1
14	+	none	unknown seedling resistance gene	3	1	1	1, 2	1, 2	1	1	4	1	4	2	2	3	2	1	3	3	1
15	+	none	unknown APR genes	4	4	4	3	3+	4	4	4	4	4	3	3+	3	4	4	4	4	4
16	+	none	unknown APR genes	4	4	4	3+	4	4	4	4	4	3	4	3+	3	4	4	4	3+	4
17	*Lr1*, +	*Lr1*	*Lr1*c, +	3	4	2+	1	4	3	2+	1	1	3	3	3	2+	2+	2	3+	2	1
18	+	*Lr46*	*Lr46*e, +	4	4	4	4	4	3	3+	4	4	4	3+	3+	3	3+	3	4	3+	3+
19	*Lr26*, +	*Lr26*	*Lr26*c, +	4	3	4	4	4	3	3+	4	4	3	4	4	3	3	3	2+	3+	2+
20	+	none	unknown seedling resistance gene	4	4	4	4	4	4	3+	4	4	4	4	4	4	3	4	4	2	3
21	*Lr13, Lr18, Lr14a*, +	none	*Lr13*d, *Lr18*d, *Lr14a*d, +	1	;1	1	1	1, 2	4	1	4	;	3+	2	2+	1	2	1	3c	2	2+
22	*Lr26*, +	*Lr26*	*Lr26*c, +	3	3	3+	3	4	3	4	4	4	3	3	3	1	3	3	2+	3	2+
23	*Lr26*, +	*Lr26*	*Lr26*c, +	3+	3	3	3+	4	3	4	4	4	3	4	3	3	3	3	2+	3	2+
24	*Lr1*, +	*Lr1*	*Lr1*c, +	3+	4	3	2+	4	4	2	2	2+	3+	4	3+	2+	2	2	3+	2+	2
25	*Lr1*, +	*Lr1, Lr46*	*Lr1*c, *Lr46*e, +	4	4	3+	2	4	4	2	1	2+	died	4	3+	2+	2+	2	3+	2	2+
26	+	*Lr34, Lr46*	*Lr34*e, *Lr46*e	4	4	4	4	4	4	4	4	4	4	3+	3	3+	4	4	3+	3+	3+
27	*Lr26*, +	*Lr26, Lr34*	*Lr26*c, *Lr34*e, +	3+	4	3	3	4	4	3+	3+	3+	4	4	3	3	4	3+	2+	2+	2+
28	*Lr1*, +	*Lr1*	*Lr1*c, +	4	3	3	2+	3	3	2+	2	2	3+	4	3	2+	2	2	3	2	2
29	*Lr26*, +	*Lr26, Lr34*	*Lr26*c, *Lr34*e, +	3	4	3	3+	3+	3	4	4	4	3	1	2+	2+	1	2+	2+	3	2
30	*Lr26*, +	*Lr26*	*Lr26*c, +	4	4	3	4	3	4	4	4	4	3+	4	3+	3	3	3+	2+	3+	2+

Table 3 to be continued

No.[b]	Lr gene based on gene postulation	Lr gene based on gene marker detection	Lr gene(s)	Infection types[a] to Pt races																	
				PH GQ	TH JT	PH JT	KH JS	PH JS	THTT I	KH HT	FH RT	FH JQ	PHTT I	THTT II	PHTT II	FH TR	FHHT I	FHHT II	TG GT	FH TT	FG MT
31	Lr1, +	Lr1	Lr1[c], +	4	3+	4	2	4	4	2+	2	1	2+	4	3+	2+	2	2	3+	2+	2
32	+	none	unknown APR gene	4	4	4	4	4	4	4	4	1	4	4	4	3	4	4	3+	3+	3+
33	Lr26, +	Lr26	Lr26[c], +	4	4	4	3+	4	4	4	4	4	3	3	4	3	3	3+	2+	3	2
34	Lr1, +	Lr1	Lr1[c], +	4	4	2	2+	4	1	2	2	2	3c	4	4	2+	2+	2+	3+	2	2
35	Lr1, +	Lr1	Lr1[c], +	4	4	3+	2	4	4	2+	2	2	3+	4	4	2c	2+	2+	3	2+	2+
36	+	none	unknown APR genes	4	4	4	4	4	4	4	4	4	4	4	4	3	3	3	3	3	3
37	+	none	unknown APR genes	4	4	4	4	4	4	4	4	4	4	4	4	3	4	3+	3+	4	3
38	–	none	CK	4	4	4	4	4	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); 0 – no flecks or uredinia, ; – hypersensitive flecks, 1 – small uredinia with necrosis, 2 – small uredinia with chlorosis, 3 – moderate size uredinia, 4 – large uredinia, + indicates slightly larger uredinia, C – more chlorosis than normal for the infection type, X – random distribution of variable-sized uredinia; [b]line numbers corresponding to those in Table 1; [c]postulation of the Lr genes based on the gene postulation and molecular marker; [d]postulation of the Lr genes based on the gene postulation; [e]detection of the Lr genes based on the molecular marker and adult plant resistance

RESULTS

***Lr* genes postulated and molecular marker detection.** The infection types of thirty-six differentials (Table 2), thirty-seven wheat cultivars and the susceptible control, Zhengzhou 5389 were evaluated with eighteen *Pt* races at the seedling stage in a greenhouse (Table 3). Zhengzhou 5389 showed IT 4 with all eighteen *Pt* races. The differential lines, containing *Lr9*, *Lr19*, *Lr24*, *Lr28*, *Lr47*, *Lr51*, and *Lr53* conferred a resistance response to all the *Pt* races, however, the differential lines carrying the *Lr* genes *2c*, *3*, *16*, *B*, *3bg*, *14b*, *33*, and *44* were susceptible to all the *Pt* races, hence, none of these genes could be postulated based the present response arrays. Twenty-one differential lines, *Lr1*, *Lr2a*, *Lr26*, *Lr3ka*, *Lr11*, *Lr17*, *Lr30*, *Lr10*, *Lr14a*, *Lr18*, *Lr2b*, *Lr13*, *Lr15*, *Lr20*, *Lr21*, *Lr23*, *Lr29*, *Lr36*, *Lr39*, *Lr42* and *Lr45* showed differential responses, and one or more of those genes could be postulated. The susceptible check, Zhengzhou 5389 was highly susceptible to all the *Pt* races (Table 3). Based on the IT arrays, *Lr* genes *1*, *13*, *14a*, *18* and *26*, either singly or in combination were postulated in twenty-two cultivars (Table 3). Fifteen cultivars were found with unknown resistance gene(s).

Lr26 was present in ten cultivars (Table 3). Five cultivars (Shaanken 6, Xiaoyan 166, Shaannong 138, Shaan 558 and Xiaoyan 228) contained only *Lr26* because they were only resistant to two *Lr26* avirulent races (TGGT and FGMT) and susceptible with the other sixteen *Pt* races. Two cultivars (E'mai 18 and Xiaoyan 22) contained *Lr26* combined with *Lr1* because they showed resistance to the *Lr26* and *Lr1* avirulent *Pt* races. Based on their resistant responses to other races, three cultivars (E'mai 17, Shaanmai 159 and Xi'nong 2611) had *Lr26* and other *Lr* genes in combination. Thirteen cultivars had *Lr1* based on the IT reactions (Table 3). Eight cultivars (Shaan 150, Xiaoyan 4, Shaannong 981, Xiaoyan 54, Xiaoyan 216, Xi'nong 9871, Xiaoyan 319 and Shaan 160) carried *Lr1* alone because, like the *Lr1* control, they were resistant to the same nine avirulent races (KHJS, KHHT, FHRT, FHJQ, FHTR, FHHTI, FHHTII, FHTT, and FGMT). Five lines contained *Lr1* combined with other *Lr* genes (*13*, *26* or *46*). All the cultivars with *Lr26* and *Lr1* were confirmed by the respective molecular markers for *Lr26* and *Lr1* (Table 3). Four cultivars contained *Lr13* because they had intermediate reactions to four *Lr13*-avirulent races (PHTTII, FHHTI, FHTT, and FGMT) (Table 3). Two cultivars, Gaoyou

Table 4. Primer sequences and PCR amplification programmes for the different primer combinations

Lr gene	Primer Name	Sequence (5′–3′)	Cycle condition	Genetic distance (cM)	Size (bp)	Reference
Lr1	WR003F WR003R	GGGACAGAGACCTTGGTGGA GACGATGATGATTTGCTGCTGG	94 °C 5 min; 35 cycles (94 °C 1 min; 55 °C 1 min; 72 °C 1 min); 72 °C 10 min; 10 °C forever	co-segregated	745	Qiu et al. (2007)
Lr9	J13/1 J13/2	TCCTTTTATTCCGCACGCCGG CCACACTACCCAAAGAGACG	94 °C 6 min; 35 cycles (94 °C 1 min; 68.5 °C 1 min; 72 °C 2 min); 72 °C 10 min; 10 °C forever	co-segregated	1 100	Schachermayr et al. (1994)
Lr10	Fl.2245 Lr10-6/r2	GTGTAATGCATGCAGGTTCC AGGTGTGAGTGAGTTATGTT	94 °C 3 min; 35 cycles (94 °C 45 s; 60 °C 45 s; 72 °C 30 s); 72 °C 3 min; 10 °C forever	co-segregated	282	Schachermayr et al. (1997)
Lr19	SCS265-F SCS265-R	GGCGGATAAGCAGAGCAGAG GGCGGATAAGTGGGTTATGG	94 °C 5 min; 35 cycles (94 °C 1 min; 65 °C 1 min; 72 °C 2 min); 72 °C 10 min; 10 °C forever	co-segregated	525	Gupta et al. (2006)
Lr19	SCS253-F SCS253-R	GCTGGTTCCACAAAGCAAA GGCTGGTTCCTTAGATAGGTG	94 °C 5 min; 35 cycles (94 °C 1 min; 60 °C 1 min; 72 °C 2 min); 72 °C 10 min; 10 °C forever	co-segregated	736	Gupta et al. (2006)
Lr20	STS638-L STS638-R	ACAGCGATGAAGCAATGAAA GTCCAGTTGGTTGATGGAAT	94 °C 5 min; 35 cycles (94 °C 1 min; 60 °C 1 min; 72 °C 2 min); 72 °C 10 min; 10 °C forever	complete linkage	500	Neu et al. (2002)
Lr24	J09/1 J09/2	TCTAGTCTGTACATGGGGGC TGGCACATGAACTCCATACG	94 °C 5 min; 35 cycles (94 °C 1 min; 60 °C 1 min; 72 °C 2 min); 72 °C 10 min; 10 °C forever	complete linkage	315	Schachermayr et al. (1995)
Lr26	ω-secalinF ω-secalinR	ACCTTCCTCATCTTTGTCCT CCGATGCCTATACCACTACT	94 °C 5 min; 35 cycles (94 °C 1 min; 65 °C 1 min; 72 °C 2 min); 72 °C 10 min; 10 °C forever	co-segregated	1 100	Chai et al. (2006)
Lr26	O11B5 O11B3	GGTACCAACAACAACAACCC GTTGCTGCTGAGGTTGGTTC	94 °C 5 min; 35 cycles (94 °C 1 min; 65 °C 1 min; 72 °C 2 min); 72 °C 10 min; 10 °C forever	co-segregated	636	Froidmont (1998)
Lr34	csLv34F csLv34R	GTTGGTTAAGACTGGTGATGG TGCTTGCTATTGCTGAATAGT	94 °C 5 min; 35 cycles (94 °C 1 min; 55 °C 1 min; 72 °C 2 min); 72 °C 10 min; 10 °C forever	0.4	150	Lagudah et al. (2006)
Lr46	csLV46G22-F csLV46G22-R	TCGACTTTGGAATGGAGTTGC GGCGAAGATGCCATCATCCACCAG	94 °C 5 min; 35 cycles (94 °C 1 min; 60 °C 1 min; 72 °C 2 min); 72 °C 10 min; 10 °C forever	complete linkage	520	Suenaga et al. (2001)

Table 5. Analysis of variance of the final leaf rust severities for 37 test lines and Saar and Zhengzhou 5389 controls tested over four growing seasons

Source	*df*	MS	*F*	*P* value
Cultivars	38	4 710.609	3 740.173**	< 0.001
Years	3	8 496.879	6 746.431**	< 0.001
Places	1	30 240.692	2 4010.787**	< 0.001
Replications	1	4.858	3.857	0.051
Cultivars × replications	38	1.357	1.077	0.359
Cultivars × environments	230	554.123	439.967 **	< 0.001
Error	234	1.259		

R^2 = 0.999; **significance at level 0.01 probability; *df* – degree of freedom; MS – mean square

503 and Xi'an 93991, contained *Lr13* combined with *Lr1*. *Lr13* and *Lr26* were present in E'mai 17. Xi'nong 538 contained *Lr13*, *Lr18* and *Lr14a* in combination because they were resistant to races that were avirulent to the respective single gene controls. E'mai 12, Shaanmai 139 and Shaan 627 were resistant to some races and their resistance could not be attributed to any known *Lr* gene. E'mai 580, Xi'nong 291, Xi'nong 88, E'mai16, Xi'nong 2000, Xi'nong 223, Xi'nong 126, Xi'nong 3517, Xi'nong 6028, Xi'nong 889, Shaanmai 175 and Shaan 512 were susceptible to all the races (Table 3).

Nine molecular markers, eight STS and one SCAR closely linked to *Lr1*, *Lr9*, *Lr10*, *Lr19*, *Lr20*, *Lr24*, *Lr26*, *Lr34* and *Lr46* were used to genotype all the thirty-seven cultivars (Table 4). No line carried *Lr9*, *Lr10*, *Lr19*, *Lr20*, or *Lr24* based on the molecular marker

detection and gene postulation. The molecular marker tests predicted that *Lr46* was present in five cultivars Gaoyou 503, Xi'nong 88, Xi'nong 3517, Shaan 253 and Xi'nong 6028, And *Lr34* was detected in three cultivars (Xi'nong 6028, Shaanmai 159, and Xi'nong 2611) (Table 3). The cultivars containing *Lr34* and *Lr46* also showed symptoms of leaf tip necrosis (LTN) at the adult plant stage.

The combined results of the gene postulation and molecular marker detection indicated that seven *Lr* genes, *1*, *26*, *13*, *14a*, *18*, *34*, and *46*, either singly or in combination were found in twenty-five lines, but twelve cultivars possibly contained unknown *Lr* gene(s) or lacked detectable *Lr* genes.

Adult plant resistance in field tests. The analysis of variance results of the FDS data (at *P* = 0.05) of the tested cultivar showed significant differences among

Table 6. Infection types (IT) in the seedling stage test with *Puccinia triticina* (*Pt*) races mixed *Pt* races and the mean final disease severity (FDS) in the field experiments with the same race in the 2014–2015, 2015–2016, 2016–2017 and 2017–2018 growing seasons for 37 wheat genotypes with the slow rusting resistance to leaf rust

No.[a]	Cultivar (line)	Seedling ITs to race mixture[a]	FSD (%)						
			2014–2015		2015–2016		2016–2017		2017–2018 Henan
			Baoding	Henan	Baoding	Henan	Baoding	Henan	
2	E'mai 17	4	2.5	15	2.5	20	5	15	5
3	E'mai 12	4	2.5	17.5	2.5	20	1	15	35
16	Xi'nong 126	4	12.5	25	12.5	15	5	1	15
17	Shaannong 981	4	10	35	10	7.5	5	5	15
27	Shaanmai 1591	4	20	7.5	20	15	1	20	10
36	Shaanmai 175	4	10	15	10	10	7.5	2.5	8.75
	Saar[b]	4	2.5	1	5	1.75	5	1	1
	Zhengzhou5389[c]	4	100	100	100	100	100	100	100
LSD	(*P* = 0.05)					22.17			

[a]Line numbers corresponding to those in Table 1; [b]slow rusting check; [c]susceptible check; *LSD* – least significance difference

the cultivar-environment interaction, cultivars, and environments, but there were not significant differences between the cultivar-replication interaction (Table 5). In all the cropping seasons, the mean value of the FDS of the susceptible check Zhengzhou 5389 and the slow rusting check Saar was 100% and 2.5%, respectively, indicating an adequate level of disease. Six cultivars exhibiting high ITs to the *Pt* races mixture in the seedling tests showed consistent slow leaf rusting resistance in the field (Table 6). Among those cultivars, Shaanmai 159 carried *Lr34* and none of them carried *Lr46*.

DISCUSSION

The postulation of resistance genes based on the response to multiple races in seedling tests is a quick and traditional method of gene identification across diverse genetic backgrounds (Mebrate et al. 2008); and can be significantly strengthened by molecular marker genotyping and pedigree analysis. *Lr26* was derived from rye (*Secale cereal* L.) and located on the 1BR/1RS chromosome of wheat. *Lr26* is widely present in Chinese wheat lines in a high frequency through the introduction of wheat germplasm such as Lovrin 13, Lovrin 10, Predgornaia 2, Kavkaz, and Neuzucht (Zhuang 2003). According to Zhou et al. (2004), the frequency of the 1BR/1RS translocation line in the northern winter wheat area was 59%, and its frequency in the Huanghuai winter wheat area was 42%. In the present study, ten cultivars contained *Lr26* (Table 3). According to the pedigree analysis, *Lr26* in Xiaoyan 22 might be derived from Fengchan 3. Because Fengchan 3 carried *Lr26* (Li & Yan 1985; Zheng 2019).

The *Lr1* gene is widely distributed in various regions of the world such as Australia (McIntosh 1992), America (Kolmer et al. 2009) and Europe (Urbanovich et al. 2006). In China, *Lr1* was reported in more than 500 wheat varieties (Liu et al. 2014). Singh et al. (2000) also reported a high frequency of *Lr1* in Chinese cultivars and lines. In the present study, thirteen cultivars contained *Lr1*. The distribution frequencies of *Lr1* in Chinese cultivars are from four founder wheat parents, viz. the Nanda 2419, Funo, Yanda 1817 and Bima 4 derivatives (Liu et al. 2014). *Lr1* in Xiaoyan 216, Xi'nong 9871 and Xiaoyan 22 might be derived from Xiaoyan 22. Ren et al. (2012) reported that Xiaoyan 54, Xi'nong 9871, and Xiaoyan 22 contained *Lr1*. *Lr34* and *Lr46* are currently important leaf rust resistance genes in

China and are the most widely assessed slow rusting leaf rust resistance genes (Yuan & Chen 2011). *Lr34* and *Lr46* might not be highly effective when they are found alone, but they contribute higher levels of resistance when used in combination with other resistance genes (Sui et al. 2016).

In the present study, six cultivars were identified to carry slow rusting leaf rust resistance genes (Tables 3 and 6). E'mai 17, E'mai 12, Xi'nong 126, Shaannong 981, Shaanmai 1591 and Shaanmai 175 had other known *Lr* genes or unknown *Lr* genes. *Lr34*, *Lr46*, *Lr67* and *Lr68* are APR *Lr* genes, the molecular markers co-segregated with or closely linked to these were used to test these cultivars (Wu et al. 2019). E'mai 17 carried *Lr26* and *Lr13*, and this cultivar was found to have slow rusting based on the FDS. *Lr26* had lost resistance, although originally *Lr13* was described as an APR gene, which can be detected at the seedling stage especially at high temperatures (Pretorius et al. 1984). If *Lr26* and *Lr13* are found in combination, the resistance is significant.

The identified seedling or slow rusting *Lr* genes in the present tested wheat cultivars may facilitate the breeding process of Chinese wheat cultivars and might contribute to reducing the leaf rust damage in China.

CONCLUSION

In the study, seven *Lr* genes, *1*, *13*, *18*, *14a*, *26*, *34* and *46* either singly or in combination were identified in twenty-five lines. *Lr9*, *Lr10*, *Lr19*, *Lr20* and *Lr24* were not identified in all the tested cultivars. The known *Lr* genes, *9*, *19*, *24*, *28*, *47*, *51* and *53* were effective at all the plant growing stages. Five and three cultivars possessed *Lr46* and *Lr34*, respectively. Six cultivars showed slow leaf rusting resistance in the field. The results of this study are useful to incorporate the resistance genes from the sources identified here into the Chinese facultative wheat genotypes to improve the genetic diversity of the cultivars.

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