Identification of Leaf Rust Resistance Genes in Chinese Common Wheat Cultivars

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Abstract

Puccinia triticina Eriks. (*Pt*), the causal agent of wheat (*Triticum aestivum* L.) leaf rust, is the most widespread disease of common wheat worldwide. In the present study, 83 wheat cultivars from three provinces of China and 36 tester lines with known leaf rust resistance (*Lr*) genes were inoculated in the greenhouse with 18 *Pt* pathotypes to identify seedling effective *Lr* genes. Field tests were also performed to characterize slow leaf rusting responses at the adult plant growth stage in Baoding and Zhoukou in the 2014–15 and 2015–16 cropping seasons. Twelve *Lr* genes, viz. *Lr1*, *Lr26*, *Lr3ka*, *Lr11*, *Lr10*, *Lr2b*, *Lr13*, *Lr21*, *Lr34*,

Lr37, *Lr44*, and *Lr46* either singly or in combination were identified in 41 cultivars. Known *Lr* genes were not detected in the remaining 42 cultivars. The most commonly identified resistance genes were *Lr26* (20 cultivars), *Lr46* (18 cultivars), and *Lr1* (eight cultivars). Less frequently detected genes included *Lr13*, *Lr34*, and *Lr37* (each present in four cultivars), *Lr10* (three cultivars), and *Lr3ka* and *Lr44* (each in two cultivars). Evidence for the presence of genes *Lr11*, *Lr2b*, and *Lr21* (each in one cultivar) was also obtained. Seventeen cultivars were found to have slow rusting resistance in both field growing seasons.

Wheat leaf rust is one of the most important wheat diseases worldwide (Mebrate et al. 2008). It decreases grain quantity and quality, and can reduce production by 40% in susceptible cultivars (Khan et al. 2013). A severe leaf rust epidemic was reported in northwestern Mexico during 1976–77, which caused 70% estimated yield losses (Dubin and Torres 1981). Four significant leaf rust epidemics were documented in China, in 1969, 1973, 1975, and 1979 (Dong 2001). During the past decade, leaf rust has periodically caused serious yield losses in the major Chinese wheat production regions (Zhao et al. 2008). High yield losses were recorded in 2012 in five provinces of China, viz. Gansu, Sichuan, Shanxi, Henan, and Anhui (Li et al. 2014; Zhou et al. 2013).

Resistant wheat cultivars are the most economical, effective, and environmentally friendly method for controlling leaf rust (Pink 2002). Identification of *Lr* gene(s) present in wheat cultivars aids in appropriate cultivar deployment to help control the disease. Gene postulation via multipathotype testing and molecular marker genotyping are useful methods to understand the *Lr* genes present in wheat cultivars.

Gene postulation based on the gene-for-gene hypothesis (Flor 1956) has been used by researchers to determine the *Lr* genes present in wheat cultivars. For instance, Hysing et al. (2006) postulated nine *Lr* genes (*Lr1*, *Lr2a*, *Lr3*, *Lr10*, *Lr13*, *Lr14a*, *Lr17*, *Lr23*, and *Lr26*) from 84 wheat cultivars grown in Denmark, Finland, Norway, and Sweden using 12 pathotypes. McVey and Long (1993) identified 14 *Lr* genes, viz. *Lr1*, *Lr2a*, *Lr3*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a*, *Lr16*, *Lr17a*, *Lr18*, *Lr24*, *Lr26*, and *Lr30* from 86 hard red winter wheat breeding lines using 17 *Pt* pathotypes. D. Singh et al. (2001) found that *Lr1*, *Lr3a*, *Lr10*, *Lr13*, *Lr17b*, *Lr20*, *Lr26*, and *Lr37* were present in 70 U.K. wheat cultivars using six *Pt* pathotypes. *Lr1*, *Lr3a*, *Lr10*, *Lr13*, *Lr17b*, *Lr20*, *Lr26*, *Lr37*, *Lr3ka*, and *Lr14a* were identified by Pathan and Park (2006) in 105 European wheat cultivars using five *Pt* pathotypes. Eight *Lr* genes (*Lr1*, *Lr3*, *Lr10*, *Lr13*, *Lr16*, *Lr17*, *Lr23*, and *Lr26*) were postulated from 22 Iraqi bread wheat cultivars with 13 *Pt* pathotypes (Maaroof et al. 2005). R. P. Singh and S. Rajaram (1991) identified 14 *Lr* genes (*Lr1*, *Lr3*, *Lr3bg*, *Lr10*, *Lr13*,

Lr14a, *Lr16*, *Lr17a*, *Lr19*, *Lr23*, *Lr26*, *Lr27* + *Lr31*, and *Lr34*) in 76 wheat cultivars from Mexico using 17 *Pt* pathotypes. Mebrate et al. (2008) postulated 18 *Lr* genes (*Lr1*, *Lr2c*, *Lr3*, *Lr3ka*, *Lr9*, *Lr10*, *Lr14a*, *Lr14b*, *Lr13*, *Lr16*, *Lr18*, *Lr20*, *Lr21*, *Lr23*, *Lr27*+*Lr31*, *Lr30*, *Lr37*, and *Lr44*) in 36 wheat cultivars from Ethiopia and Germany using 31 *Pt* pathotypes. R. P. Singh et al. (1999) postulated nine *Lr* genes, namely *Lr1*, *Lr3*, *Lr3bg*, *Lr10*, *Lr13*, *Lr14a*, *Lr16*, *Lr23*, and *Lr26* from 163 Chinese spring and winter wheat cultivars using 14 *Pt* pathotypes. Li et al. (2010) identified 14 *Lr* genes, viz. *Lr1*, *Lr2a*, *Lr3bg*, *Lr3ka*, *Lr14a*, *Lr16*, *Lr17a*, *Lr18*, *Lr20*, *Lr23*, *Lr24*, *Lr26*, *Lr34*, and *LrZH84* in 102 Chinese winter wheat cultivars using 24 *Pt* pathotypes.

To identify *Lr* genes more accurately and efficiently, increasingly molecular markers linked to such genes are being developed. For example, genes like *Lr1* (Cloutier et al. 2007), *Lr9* (Gupta et al. 2005; Schachermayr et al. 1994), *Lr10* (Schachermayr et al. 1997), *Lr19* (Gupta et al. 2006b; Prins et al. 2001), *Lr20* (Neu et al. 2002), *Lr24* (Gupta et al. 2006a; Schachermayr et al. 1995), *Lr26* (Chai et al. 2006; de Froidmont 1998; Mago et al. 2002), *Lr34* (Dyck 1987), *Lr37* (Bariana and McIntosh 1993), and *Lr46* (R. P. Singh et al. 1998) can be identified by closely linked molecular markers.

To date, 76 *Lr* genes have been formally cataloged in the wheat genome (McIntosh et al. 2016). Most of these are seedling resistance genes or major genes that are presumed to interact with *Pt* on a gene-for-gene basis (McIntosh et al. 1995, 2003). However, host resistance conferred by major genes have a tendency to be easily overcome by new virulent rust pathotypes (McDonald and Linde 2002). Therefore, pyramiding several *Lr* genes into a single cultivar is a useful strategy as the combined effects of several genes give the cultivar a wider base of disease resistance, thereby extending the period of effectiveness (Roelfs et al. 1992). In recent years, only a few *Lr* genes, *Lr9*, *Lr19*, *Lr24*, and *Lr38*, have resistance against prevalent *Pt* pathotypes in China (Li et al. 2010; Yuan et al. 2007). Slow rusting genes or minor genes are more durable than the seedling resistance genes. The characteristics of minor genes include slow disease development in the field, longer latent period, low infection frequency, smaller pustule size, and less production of urediniospores (Caldwell 1968). Minor rust resistance genes in wheat are not only more durable but also have been found in some cases to have a pleiotropic effect on multiple diseases (Li et al. 2014; R. P. Singh 1992; Spielmeier et al. 2005). Of the 76 *Lr* genes, *Lr34* (Dyck 1987), *Lr46* (R. P. Singh et al. 1998), *Lr67* (Herrera-Foessel et al. 2011; Hiebert et al. 2010), and *Lr68* (Herrera-Foessel et al. 2012) are assumed to be slow rusting resistance genes.

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In China, there is little information on *Lr* genes present in wheat cultivars and this makes it difficult to manage resistant cultivars for controlling leaf rust. Therefore, identifying *Lr* genes in the currently released Chinese wheat cultivars is important for sustainable gene deployment and for breeding new resistant cultivars. This study was designed to identify the seedling and slow rusting resistance genes in 83 wheat cultivars from Jiangsu, Anhui, and Ningxia provinces in China.

Materials and Methods

Plant materials. Eighty-three wheat cultivars (Table 1) from Jiangsu, Anhui, and Ningxia provinces were selected to test their seedling responses to 18 *Pt* pathotypes. Thirty-six differential lines, mostly near isogenic lines (NILs) in the background of Thatcher with known *Lr* genes provided by USDA-ARS Cereal Disease Laboratory, University of Minnesota, St Paul, U.S.A., were also tested.

Table 1. The pedigree and origin of 83 wheat cultivars tested for leaf rust response from three provinces in China

Line	Genotype	Pedigree	Origin
1	An 1331	NA ^a	Anhui
2	Annong 0305	Aizao 781/Wansu 8802	Anhui
3	Fu 936	Wanmai 20/Jimai 5418/Neixiang 184	Anhui
4	Hua 2566	Een 1/hua 9528/Jiayin 175/Chuannong 6280	Jiangsu
5	Huaimai 18	Zhengzhou 891/Yan 1604	Jiangsu
6	Huaimai 19	NA	Jiangsu
7	Huaimai 21	Huaimai 17/Yumai 54	Jiangsu
8	Huaimai 25	NA	Jiangsu
9	Huaimai 30	Zhengmai 9023/Huai 86175	Jiangsu
10	Lian 0536	NA	Jiangsu
11	Lian 0809	NA	Jiangsu
12	Lian 9791-4	Jian 94/Lumai 21	Jiangsu
13	Lianmai 2	Jian 94(73)/Lumai 21	Jiangsu
14	Nannong 02y293	NA	Jiangsu
15	Nannong 0686	MV964 091×Ningmai 9	Jiangsu
16	Nannong 3519	NA	Jiangsu
17	Nannong 9918	Yangmai 158/92R137/Yangmai 158	Jiangsu
18	Ningchun 10	Yuzhonghong/Denong 3	Ningxia
19	Ningdong 1	Changwu 7125/Jinnong 3	Ningxia
20	Ningdong 10	NZT/302//ALD/4/NAD//TMP/CI12426/3/EMU/5/Beinong 2	Ningxia
21	Ningdong 11	RENAN//Beinong 2 /Beijing 841	Ningxia
22	Ningdong 13	Luo 9073	Ningxia
23	Ningdong 2	Xifeng 18/CA8055	Ningxia
24	Ningdong 3	Qingnong 3/Qingfeng 1	Ningxia
25	Ningmai 10	Sha Shanghai 7//PRL ^s /VEE#5	Jiangsu
26	Ningmai 11	Ningmai 8	Jiangsu
27	Ningmai 13	Ningmai 9	Jiangsu
28	Ningmai 14	Ningmai 9	Jiangsu
29	Ningmai 15	Ningmai 8	Jiangsu
30	Ningmai 16	Ningmai 8/Ningmai 9	Jiangsu
31	Ningmai 17	Ning9529*2/Yangmai5	Jiangsu
32	Ningmai 18	Ningmai 9*2//Ningmai 9/Yangmai 10	Jiangsu
33	Pengxuan 3	NA	Ningxia
34	Su 0663	NA	Jiangsu
35	Su 553	Yannong 19/Su 1264	Jiangsu
36	Sumai 3	Afu 98/Taiwan Xiaomai	Jiangsu
37	Sunong 6	NA	Anhui
38	Wan 23094	Ji 845034/Wan 825508/Wan 8656/Yang 8617	Anhui
39	Wanmai 19	Boai 7422/Yumai 2	Anhui
40	Wanmai 29	Yili xiaomai/Afuleer	Anhui
41	Wanmai 33	Zhong 8131-1/Annong 8326	Anhui
42	Wanmai 38	Yanzhong 114/85-15-9	Anhui

(continued in next column)

All 85 genotypes, including Zhengzhou 5389 (Chinese susceptible check) and the CIMMYT line SAAR (resistant check) for slow rusting resistance, were tested in the field in the 2014–15 and 2015–16 cropping seasons.

***Pt* pathotypes.** To identify *Lr* genes in the wheat cultivars, 18 *Pt* pathotypes were used to inoculate 36 differentials carrying the resistance genes *Lr1*, *2a*, *2c*, *3*, *9*, *16*, *24*, *26*, *3ka*, *11*, *17*, *30*, *B*, *10*, *14a*, *18*, *2b*, *3bg*, *13*, *14b*, *15*, *19*, *20*, *21*, *23*, *28*, *29*, *33*, *36*, *39*, *42*, *44*, *45*, *47*, *51*, and *53* (Table 2). The *Pt* pathotypes were sourced from the culture collection from the Department of Plant Pathology, Agricultural University of Hebei, China. Fresh inoculum was obtained by multiplying urediniospores on the susceptible cultivar, Zhengzhou 5389. These pathotypes were named following the *Prt* code system described by Long and Kolmer (1989), with the addition of a fourth letter for the reactions to the fourth quartet of differentials (https://www.ars.usda.gov/ARUserFiles/50620500/Cerealarusts/pt_nomen.pdf).

Table 1. (continued from preceding column)

Line	Genotype	Pedigree	Origin
43	Wanmai 46	Shan 7859/83(165)	Anhui
44	Wanmai 47	Wanxi 8906/Boshou 7422/Ningmai 3	Anhui
45	Wanmai 50	Zhengzhou 8329/Wanmai 19	Anhui
46	Wanmai 52	Zhengzhou 8329/Wanmai 1	Anhui
47	Wanmai 53	Yumai 29/Wanmai 19	Anhui
48	Wanmai 68	Wanmai 19/Zheng 79201	Anhui
49	Wansu 9908	Bo 74-22/Yumai 2	Anhui
50	Xu 9069	NA	Jiangsu
51	Xu 9128	NA	Ningxia
52	Xumai 24	7924-13-2-2/Aimengniu	Jiangsu
53	Xumai 25	7904-13-2-2/Bainong 792	Jiangsu
54	Xumai 26	Xiaoyan 6/Shan 7859	Jiangsu
55	Xumai 27	Zaofeng 1/Yan 1934	Jiangsu
56	Xumai 29	Zhengzhou 8329/Zhoumai 9	Jiangsu
57	Xumai 30	Zhou91098/Xuzhou 25	Jiangsu
58	Xumai 31	Yanfu 188/Xuzhou 26	Jiangsu
59	Xumai 4036	Yangfu188/xuzhou26	Jiangsu
60	Xuzhou 14	Zaoyangmai/Nangda 2419	Jiangsu
61	Xuzhou 23	Punong 3665/UP301//Zhengzhou 761	Jiangsu
62	Xuzhou 25	7904-13-2-2/Bainong 792	Jiangsu
63	Xuzhou 438	Land race	Jiangsu
64	Yangfumai 2	Yangmai 158/101-90	Jiangsu
65	Yangfumai 3	Yangmai 158/1-9012	Jiangsu
66	Yangfumai 4	Ningmai 8/Ningmai 9	Jiangsu
67	Yangmai 10	Yuma/8*Cc//Yangmai 5/3/4*Yang 85-85/4/2*Yangmai 158	Jiangsu
68	Yangmai 11	Yang 158/3Y.C/Jianer//Yang 85-85	Jiangsu
69	Yangmai 12	Yangmai 158/3/TP114/Yangmai 5//85-853	Jiangsu
70	Yangmai 13	Yang 88-84//Yangmai 8/Maristdove	Jiangsu
71	Yangmai 158	Yangmai 4/ST 1472/506	Jiangsu
72	Yangmai 16	Yang 91F138/Yang 90-30	Jiangsu
73	Yangmai 17	92F101/Chuanyu 21256	Jiangsu
74	Yangmai 19	Yangmai 96/4/Yang 158/3/4×Y.C/Yangmai 5//85-854	Jiangsu
75	Yangmai 4	Nanda 2419/Shenglimai/Axuan 2	Jiangsu
76	Yangmai 5	9-16-4/St1472/506	Jiangsu
77	Yangmai 9	Jian 2/Jian 3	Jiangsu
78	Zhen 02168	Sumai 6/97G59	Jiangsu
79	Zhen 05185	Sumai 6/97G59	Jiangsu
80	Zhen 06101	Yangmai15	Jiangsu
81	Zhenmai 168	Sumai 6/97G59	Jiangsu
82	Zhenmai 6	Yangmai 158/Zhenmai 1	Jiangsu
83	209-1-2-1	NA	Jiangsu

^a NA = Not available.

Seedling testing. A total of 83 cultivars, Zhengzhou 5389, and 36 differential lines were tested using 18 *Pt* pathotypes. Five to eight seeds of each were sown in a plastic growth chamber (3 × 2 cm). Inoculation of seedlings was carried out by the method provided by Li et al. (2010) and R. P. Singh et al. (1999). Infection types (ITs) were recorded 10 to 14 days after inoculation based on the 0 to 4 Stakman scale modified by Roelfs et al. (1992) where 0 = immune, ; = hypersensitive flecks with necrosis, 1 = small uredinia surrounded by necrosis, 2 = small to medium uredinia surrounded by necrosis, X = random distribution of variable-sized uredinia, 3 = moderate size uredinia that may be associated with chlorosis, 4 = large uredinia without chlorosis, + = uredinia somewhat larger than normal, - = uredinia somewhat smaller than normal, and C = more chlorosis than normal. The gene postulation was conducted using the method reported by Dubin et al. (1989).

Field testing. All 85 genotypes, including Zhengzhou 5389 and SAAR, were evaluated for leaf rust reaction in the field nurseries at Baoding in Hebei Province and Zhoukou in Henan Province in the 2014–15 and 2015–16 wheat cropping seasons. Field tests were conducted in randomized complete blocks with two replicates at each location. Seeds (50 to 100 in number) of each cultivar were sown in single rows (1.5-m length and 25 cm between rows). The susceptible genotype Zhengzhou 5389 was sown adjacent and perpendicular to the test rows in order to aid the spread of spores within the trials. Epidemics were initiated by spraying aqueous suspensions containing equal amounts of mixed *Pt* pathotypes THTT, THTS, and FHHT to which a few drops of Tween 20 (0.03%) were added, onto the spreader rows at tillering stage. Three *Pt* pathotypes, THTT, THTS,

and FHHT, were virulent pathotypes to most of the 83 tested cultivars at the seedling stage in the greenhouse (Table 3). Four weeks after inoculation, leaf rust severity was scored once a week using the modified scale by Peterson et al. (1948). The ITs to the mixed pathotype were scored on a 0 to 4 scale as reported by Roelfs et al. (1992). The final disease severity (FDS, %) data, when the susceptible check (Zhengzhou 5389) displayed maximum disease severity, was used for statistical data analysis.

Statistical analysis. Analysis of variance (ANOVA) was performed using the generalized linear model procedure (PROC GLMMIX) of Statistical Analysis System (version 9.1; SAS Institute Inc., Cary, NC). Cultivars, environments (the combination of location and year), and their interaction were treated as fixed effects, and replications nested in environments were as random. Fisher's least significant difference (LSD) test at *P* = 0.05 was used to separate the treatment means (Sokal and Rohlf 1989) of FDS data. Genotypes with high ITs to mixed *Pt* pathotypes at the seedling stage and low FDS values (nonsignificantly higher FDS value than the slow rusting check, SAAR) in the field were considered to have slow rusting resistance.

DNA extraction and molecular marker detection. Genomic DNA of the cultivars was extracted according to the modified CTAB method (Gill et al. 1991; Ren et al. 2015). Twelve STS and SCAR markers for 10 known *Lr* genes, viz. *Lr1*, *Lr9*, *Lr10*, *Lr19*, *Lr20*, *Lr24*, *Lr26*, *Lr34*, *Lr37*, and *Lr46*, were utilized to test all cultivars. The sequences and annealing temperatures of all primers are provided in Table 4. PCR was performed following the protocol of Helguera et al. (2003). The PCR product was separated on a 1.5%

Table 2. Infection types^a of 36 tester lines inoculated with 18 *Puccinia triticina* Eriks (*Pt*) pathotypes

No.	Leaf rust resistance (<i>Lr</i>) genes	Infection types to <i>Pt</i> pathotypes																	
		FH	TH	TH	FH	PH	PH	FH	PH	KH	PH	TH	PH	FH	FH	FH	TG	FH	FG
		PR	JS	TS	JQ1	SL	JT	JQ2	SS	SS	TT1	TT	TT2	TR	HT1	HT2	GT	TT	MT
1	RL6003 (<i>Lr1</i>)	;1	4	4	1	4	3	1	4	;1	4	4	4	1	1	1	4	1	1
2	RL6016 (<i>Lr2a</i>)	2	3c	3+	1	;	2	2	1	4	1	4	1	;	1	1	4	;	1
3	RL6047 (<i>Lr2C</i>)	4	4	4	3	3+	4	4	4	4	4	4	4	3	4	4	3	3	4
4	RL6002 (<i>Lr3</i>)	4	3	4	4	3+	4	4	4	4	4	4	4	4	4	4	3	4	4
5	RL6010 (<i>Lr9</i>)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	RL6005 (<i>Lr16</i>)	3	4	3+	4	3+	4	4	3	4	3	4	3	3	3	3	3	3	3
7	RL6064 (<i>Lr24</i>)	;	;	;	;	;	;	;1	;	;	;	;	;	;	;	;	;	;	0
8	RL6078 (<i>Lr26</i>)	3	3+	4	4	4	3	3+	3	3	3	3	3	3	3	3	2	3	2
9	RL6007 (<i>Lr3ka</i>)	3	1	3+	2	3+	2	12	3	3	3	3	3	3	2	2	1	3	3
10	RL605 (<i>Lr11</i>)	2	3+	4	4	4	4	3	3	3	4	4	3	4	3	3	3	3	2
11	RL6008 (<i>Lr17</i>)	3	4	4	3	4	3	3-	3	3	3	4	3	3	2	1	2	3	2
12	RL6049 (<i>Lr30</i>)	4	1	3+	1	2+	2	12	2	2	3	4	4	4	3	4	1	3	4
13	RL6051 (<i>LrB</i>)	4	4	4	4	3+	4	4	4	4	4	4	3	4	4	3	4	3	4
14	RL6004 (<i>Lr10</i>)	4	3+	4	3	2	4	4	4	4	4	3	4	3	3	3	4	4	4
15	RL6013 (<i>Lr14a</i>)	X	3c	3+	2	2X	3	2X	3	3	4	3	4	X	3	3	3	3	3
16	RL6009 (<i>Lr18</i>)	3	2+	2	2	2+	3	12	2	2	4	4	4	3	4	4	3	4	3
17	RL6019 (<i>Lr2b</i>)	3	4	3+	2	2+	3	4	1	3	3	4	3	3	3	3	3	4	3
18	RL6042 (<i>Lr3bg</i>)	4	3	4	4	4	3	3+	4	3	4	4	3	3	3	3	3	4	4
19	RL4031 (<i>Lr13</i>)	3	3	3	3	3+	3	3+	4	3	3	4	2	3	2	3	3	2	2
20	RL6006 (<i>Lr14b</i>)	4	4	4	4	3	4	4	4	4	4	4	3	4	4	3	4	4	4
21	RL6052 (<i>Lr15</i>)	2	1	3	1	4	;4	1	4	1	1	4	1	3	1	1	1	2	12
22	RL6040 (<i>Lr19</i>)	0;	0	0	0	0	0	0	0	0	0;	0	;	0	;	0	0	0	0
23	RL6092 (<i>Lr20</i>)	;1	1	3	1	3+	1	;1	1	1	3	1	4	0	3	1	3	3	1
24	RL6043 (<i>Lr21</i>)	3	3	4	4	2	3	3+	4	3	3	3	3	3	4	3	2	3	2
25	RL6012 (<i>Lr23</i>)	3	3	4	3	32	3	12	3-	3	3	1	1	1	1	1	3	?	1
26	RL6079 (<i>Lr28</i>)	0	0	0	0	0	0	0;	0	0	;	0	;	0	;	0	0	;	;
27	RL6080 (<i>Lr29</i>)	1	12	12	2	12	1	2	1	2	1	2	;4	2	3	3	1	3	2
28	RL6057 (<i>Lr33</i>)	4	4	4	3+	3+	4	4	4	3+	4	4	4	3	3	3	3	3	3
29	E84018 (<i>Lr36</i>)	1	3c	2	1	2	12	1	12	12	3	1	2	1	1	1	3	1	1
30	KS86NGRC02 (<i>Lr39</i>)	2	12	0	12	3-	12	12	2+	1	4	4	3	3	2	2	4	1	1
31	KS91WGRC11 (<i>Lr42</i>)	2	;	12	;	4	4	1	1	3	1	1	2	2	2	3	1	3+	3
32	RL6147 (<i>Lr44</i>)	3+	3+	3	3	1	3	3	2+	3	4	4	4	4	4	3	3	4	4
33	RL6144 (<i>Lr45</i>)	4	4	4	4	4	3+	4	4	4	1	1	1	1	1	1	3	1	1
34	PAVON76 (<i>Lr47</i>)	0	0	0	0	0	0	0	0	0	;	0	;	;	;	;	;	0	;
35	C78.5 (<i>Lr51</i>)	;1	;	;	;	;	1	;1	;	;	1	1	;	1	1	;	1	1	;
36	98M71 (<i>Lr53</i>)	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).

agarose gel in 1× TAE buffer and stained with ethidium bromide, then photographed under UV light.

Results

Lr genes from seedling reactions and molecular marker detection.
Seedling genes were postulated in the 83 wheat cultivars based on the

comparison of ITs of the genotypes tested to ITs of 36 differentials with known *Lr* genes. Variations of ITs on the 36 differentials inoculated with 18 *Pt* pathotypes (Table 2) indicated the possibility of postulating 22 *Lr* genes, namely *Lr1*, *Lr2a*, *Lr2b*, *Lr3ka*, *Lr10*, *Lr11*, *Lr13*, *Lr14a*, *Lr15*, *Lr17*, *Lr26*, *Lr30*, *Lr18*, *Lr20*, *Lr21*, *Lr23*, *Lr29*, *Lr36*, *Lr39*, *Lr42*, *Lr44*, and *Lr45*. Seven genes (*Lr9*, *Lr24*, *Lr19*,

Table 3. Seedling infection types^a and presence or absence of leaf rust resistance (*Lr*) genes in 83 wheat cultivars collected from three provinces in China based on gene postulation using 18 *Puccinia triticina* Eriks. (*Pt*) pathotypes and molecular markers

Line no. ^b	<i>Lr</i> gene	Infection types to <i>Pt</i> pathotypes																	
		FH	TH	TH	FH	PH	PH	FH	PH	KH	PH	TH	PH	FH	FH	FH	TG	FH	FG
		PR	JS	TS	JQ1	SL	JT	JQ2	SS	SS	TT1	TT	TT2	TR	HT1	HT2	GT	TT	MT
1	-	4	4	4	4	4	4	4	4	4	4	3	4	3	4	4	3+	3+	3+
2	-	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3+	4	4	4
3	-	4	4	4	4	4	4	4	4	4	4	4	3	3	4	4	3	4	3
4	-	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3+	3+	4
5	<i>Lr26</i> ^c	4	4	4	4	4	4	4	4	4	3	4	4	3	3	3	2	3	2
6	<i>Lr1</i> ^c	1	4	4	1	4	4	0;	4	1	4	3	4	2	1	1	4	;	2+
7	<i>Lr1</i> ^c	1	1	1	1	3-	2	0;	4	1	0	1	0	2+	;	1	2	0	2
	<i>Lr26</i> ^c																		
	<i>Lr3ka</i> ^d																		
	<i>Lr13</i> ^{d+}																		
8	<i>Lr26</i> ^c	4	4	4	4	4	4	4	4	4	3	4	3+	3	3	3	2	3+	1
9	-	4	4	4	4	4	4	4	4	4	4	3	3+	4	4	4	3	3	3
10	<i>Lr1</i> ^c	1	4	3c	1	4	2	;	1	3c	1	4	4	3+	3	1	1	2	0
	<i>Lr26</i> ^c																		
11	<i>Lr26</i> ^c	4	4	4	4	4	4	4	4	4	3+	3	3+	3	3+	3	2	4	2
	<i>Lr34</i> ^e																		
12	<i>Lr26</i> ^c	4	4	4	4	4	4	4	4	4	3	4	3+	3	3	3	2	3+	2
13	<i>Lr26</i> ^c	3+	4	4	4	4	4	4	4	4	4	4	3	3	3	3	2	3+	1
14	-	4	4	4	4	4	4	4	4	4	4	3+	4	4	3+	3	4	3+	3
15	<i>Lr37</i> ^e	4	4	4	4	4	3+	4	4	4	3	4	4	4	4	4	4	3+	3+
	<i>Lr46</i> ^e																		
16	<i>Lr1</i> ^c	12	1	2	1	3	12	12	3+	2	3+	3	2	1	1	1	2	1	1
	<i>Lr26</i> ^c																		
	<i>Lr3ka</i> ^d																		
	<i>Lr13</i> ^{d+}																		
17	-	4	4	4	4	4	4	4	4	4	4	4	4	3	4	4	3	3	3
18	<i>Lr46</i> ^e	4	4	4	4	4	4	4	4	4	3+	3+	3	4	3+	3	3	3	3
19	<i>Lr46</i> ^e	3+	4	4	4	4	4	4	4	4	4	3+	3	3	3	3	3	3+	3
20	<i>Lr46</i> ^e	3+	4	3+	4	4	4	4	4	4	3+	4	4	3+	3+	4	3	3	3
21	-	4	4	4	4	4	4	4	4	4	4	3+	3	4	3	3	3+	3	3
22	-	3+	4	4	4	4	4	4	4	4	3	4	4	3	3+	3	3	3	3
23	-	3+	4	4	4	4	4	4	4	4	3+	3+	4	4	3+	3	3+	3	3
24	<i>Lr26</i> ^c	4	4	4	4	4	3+	4	4	4	3+	3+	3+	4	3+	3	2	3	1
	<i>Lr46</i> ^e																		
25	<i>Lr46</i> ^e	4	4	4	4	4	4	4	4	4	4	4	3	4	3+	3+	3+	3+	3
26	<i>Lr26</i> ^c	3+	3c	3+	4	4	4	4	4	4	3	3	3	3	3-	3	2	3	1
	<i>Lr46</i> ^e																		
27	<i>Lr46</i> ^e	4	4	4	4	4	4	4	4	4	4	4	3	4	4	4	4	3	3
28	-	4	4	4	4	4	4	4	4	4	4	4	3+	3	4	4	3+	4	3+
29	<i>Lr46</i> ^e	4	4	4	4	4	4	4	4	4	4	4	3	3+	4	3	3	3	3
30	<i>Lr1</i> ^c	1	4	4	1	4	4	;	4	1	4	4	4	2	1	1	4	1	2
31	<i>Lr46</i> ^e	4	4	4	4	4	4	3+	4	4	2+	4	3+	3	12	1	3+	4	3+
32	-	3+	4	4	4	4	4	4	4	4	3+	3+	4	3+	3	3	3	3+	3
33	<i>Lr34</i> ^e	4	4	4	4	4	4	4	3	4	3	3	3+	3	3	3	3	3	3
	<i>Lr46</i> ^e																		
34	-	3+	4	4	4	4	4	4	4	4	2	4	4	3+	4	4	4	3+	3
35	<i>Lr1</i> ^c	1	4	4	1	4	3	;	1	3	1	4	4	3c	2	1	1	4	0
	<i>Lr11</i> ^d																		
36	<i>Lr34</i> ^e	4	3+	4	4	4	4	3+	4	4	4	4	3	3+	3	3	3+	3	4
	<i>Lr46</i> ^e																		
37	<i>Lr1</i> ^c	1	4	3+	1	3	3+	0;	4	1	3+	3	3	1	2	1	2	1	2

(continued on next page)

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

^b Line numbers correspond to those in Table 1.

^c Postulation of *Lr* genes based on gene postulation and molecular marker.

^d Postulation of *Lr* genes based on gene postulation.

^e Detection of *Lr* genes based on molecular marker.

Lr28, *Lr47*, *Lr51*, and *Lr53*) had low ITs to all the 18 *Pt* pathotypes. High ITs were noted with most of the pathotypes for *Lr* genes, viz. *Lr2c*, *Lr3*, *Lr16*, *LrB*, *Lr3bg*, *Lr14b*, and *Lr33*, hence, it was impossible to postulate these genes.

The susceptible check Zhengzhou 5389 conferred a high (susceptible) IT 4 to all pathotypes. Based on the ITs of the 83 wheat cultivars inoculated with the 18 *Pt* pathotypes (Table 3), nine *Lr* genes, *Lr1*, *Lr26*, *Lr3ka*, *Lr11*, *Lr10*, *Lr2b*, *Lr13*, *Lr21*, and *Lr44* either singly or in

Table 3. (continued from preceding page)

Line no. ^b	<i>Lr</i> gene	Infection types to <i>Pt</i> pathotypes																	
		FH	TH	TH	FH	PH	PH	FH	PH	KH	PH	TH	PH	FH	FH	FH	TG	FH	FG
		PR	JS	TS	JQ1	SL	JT	JQ2	SS	SS	TT1	TT	TT2	TR	HT1	HT2	GT	TT	MT
	<i>Lr26</i> ^c																		
	<i>Lr37</i> ^e																		
38	-	3+	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3+	4
39	-	3+	4	4	4	4	4	4	4	3	4	3	3	3	3+	3	3+	4	3
40	-	3+	4	4	4	4	4	3+	4	4	3+	3	2+	3+	4	3	4	3+	2+
41	<i>Lr10</i> ^c	4	4	4	4	2	4	3	2+	4	4	2	2+3-	2	3	1	33c	3+	3
	<i>Lr44</i> ^d																		
42	-	4	4	4	4	4	4	4	4	4	3	3	3+	2+	3	3	4	3	2+
43	<i>Lr26</i> ^e	3+	4	4	4	4	4	4	4	3+	4	4	3+	3+	3+	4	2	3	1
44	<i>Lr26</i> ^c	3	4	4	1	1	2	1	12	1	3+	4	3	3	2+	3	1	1	1
	<i>Lr10</i> ^c																		
	<i>Lr2b</i> ^d																		
	<i>Lr21</i> ^d																		
	<i>Lr44</i> ^d																		
45	-	3+	4	4	4	4	4	4	4	4	4	3	3	3+	3+	4	3	3	3
46	-	3+	4	4	4	4	4	4	4	4	4	3+	3	3	4	3	3+	3	2+
47	-	4	4	4	4	4	4	4	4	4	4	3	4	4	3+	3	3+	3	4
48	-	3+	4	4	4	4	3+	4	4	4	4	3	3+	4	4	4	4	3	3
49	-	4	4	4	4	4	4	4	4	4	3+	4	4	3	3+	3	3+	3	3
50	<i>Lr26</i> ^c	4	4	4	4	4	4	4	4	4	3	4	3+	3	3	3	2	3+	2
51	<i>Lr46</i> ^e	4	4	4	4	4	4	3	4	4	3	3	4	3	3	3	3	3+	3+
52	-	4	4	4	4	4	4	4	4	4	3+	4	3	3	3+	3	3	3+	3
53	-	4	4	4	4	4	4	4	4	4	4	4	3	3	3	3	3	3	3
54	<i>Lr26</i> ^c	4	4	4	4	4	4	4	4	3	3	3+	3	3-	3	3	2	4	2
55	-	4	4	4	4	4	4	4	4	4	3	4	4	3+	3	3	3+	3+	3+
56	<i>Lr26</i> ^c	4	4	4	4	4	4	4	3+	4	4	3+	4	3	4	3	2	3+	2
57	-	2	4	4	4	4	4	4	4	4	4	4	3	3+	3+	4	3+	3	3
58	+	3c	4	12	12	3c	2+	12	2+	12	2	2+	2	3	2	1	3+	3c	2
59	<i>Lr10</i> ^c	1	4	12	1	2	2+	1	3	1	3+	4	2+	3	1	2	3+	3	3
	+																		
60	-	4	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	3+	3+
61	<i>Lr1</i> ^c	1	4	4	1	4	3+	1	4	0;	4	4	4	2	1	1	2	;	2
	<i>Lr26</i> ^c																		
	<i>Lr37</i> ^e																		
62	-	3+	4	4	4	4	4	4	4	3+	4	3	3	3	3	3	3+	3	3
63	<i>Lr34</i> ^e	4	4	4	4	3+	4	4	4	4	4	4	4	3+	4	3	3+	3	4
	<i>Lr46</i> ^e																		
64	<i>Lr46</i> ^e	4	4	4	4	4	4	3+	4	4	2+	3+	2	12	1	1	4	2	1
	<i>Lr13</i> ^d																		
65	<i>Lr26</i> ^c	3+	4	4	4	4	4	4	4	4	3	3+	2	2	2	4	2	2+	1
	<i>Lr13</i> ^d																		
66	-	4	4	4	4	4	4	4	4	4	4	4	4	3	3+	3	3	4	3
67	-	4	4	4	4	4	4	4	4	4	3c	4	3	3	3	4	3	3+	3
68	<i>Lr46</i> ^c	4	4	4	4	4	4	4	4	4	4	3+	3	3c	3+	4	3	3	3
69	-	4	4	4	4	4	3+	4	4	4	4	3	3	3	4	2	3	4	3
70	-	4	4	4	4	4	4	4	4	4	4	4	2+	2+	4	2	3+	3	3
71	<i>Lr26</i> ^c	4	4	4	4	4	4	3+	4	4	3	3+	2+	3	3	3	2	3+	2
72	-	4	4	4	4	4	4	4	4	4	4	3+	3+	4	4	4	3	3	3
73	-	4	4	4	4	4	4	4	4	4	4	4	3-	4	4	3	3+	3	3
74	-	4	4	4	4	4	4	4	4	4	4	4	3+	4	4	4	3	4	3+
75	-	3+	4	4	4	3+	4	4	3	4	4	3	3	2+3-	3+	3	3+	3	3
76	-	4	4	4	4	4	4	4	4	4	3+	3	3	3	4	3	3+	3	3+
77	-	4	4	4	4	4	4	4	4	4	4	4	3+	4	4	3	4	3+	3+
78	-	4	4	4	4	4	4	4	4	4	4	4	3+	3	4	3	4	4	3+
79	-	4	4	4	4	4	4	4	4	4	4	4	4	3	4	3	3	3+	4
80	-	4	4	4	4	4	4	4	4	4	4	4	3+	4	4	4	4	3+	3
81	<i>Lr37</i> ^e	4	4	4	4	4	4	4	4	4	4	4	4	4	3+	4	3	3	4
	<i>Lr46</i> ^e																		
82	-	4	4	4	4	4	4	4	4	4	4	3+	4	3+	3+	4	3	3	3+
83	<i>Lr26</i> ^c	4	4	4	4	4	4	4	4	4	4	3	3	3	3	3	2	3	2

combination, were identified in 26 cultivars, while the remaining 57 genotypes likely contained unknown *Lr* gene(s) or lacked detectable *Lr* genes.

Lr26 was present in 20 cultivars. Thirteen cultivars contained only *Lr26* because they were resistant with two *Lr26* avirulent pathotypes (TGGT and FGMT) and susceptible with the other 16 pathotypes. Three cultivars, Lian 0536, Sunong 6, and Xuzhou 23, contained *Lr26* combined with *Lr1* because they showed resistance to *Lr26* and *Lr1* avirulent pathotypes. Based on their resistant responses to other pathotypes, four genotypes, Huaimai 21, Nanong 3519, Wanmai 47, and Yangfumai 3, had *Lr26* and other *Lr* genes in combination. All genotypes postulated with *Lr26* were confirmed by molecular marker for *Lr26* (Table 3). Eight cultivars were found to have *Lr1* because they were resistant to eight *Lr1* avirulent pathotypes (FHPR, FHJQ1, FHJQ2, KHSS, FHTR, FHHT1, FHHT2, FHHT, and FGMT) (Tables 2 and 3). All cultivars postulated to contain *Lr1* were also confirmed by the molecular marker for *Lr1*.

Four cultivars, viz. Huaimai 21, Nannong 3519, Yangfumai 2, and Yangfumai 3, contained *Lr13* because they had intermediate reactions to four *Lr13* avirulent pathotypes (PHTT2, FHHT1, FHHT, and FGMT). *Lr13* and *Lr26* were present in Yangfumai 3. Huaimai 21 and Nannong 3519 were found to contain *Lr3ka*, *Lr1*, *Lr26*, and *Lr13* genes in combination because they were resistant to all avirulent pathotypes to *Lr3ka*, *Lr1*, *Lr26*, and *Lr13*.

All the avirulent pathotypes to *Lr17* were also avirulent to *Lr1*. Therefore, *Lr17* could not be postulated in the cultivars with *Lr1*.

Lr10 was probably present in Wanmai 33, Wanmai 47, and Xumai 4036 because they gave intermediate ITs to *Lr10* avirulent pathotype PHSL. *Lr10* in these cultivars was also confirmed by molecular marker for *Lr10*.

Wanmai 47 contained *Lr2b*, *Lr21*, *Lr10*, and *Lr26* as it displayed low or intermediate ITs with all avirulent pathotypes to *Lr2b*, *Lr21*, *Lr10*, and *Lr26*. *Lr11* and *Lr1* were found in Su 553 as it showed low or intermediate ITs to all the *Lr11* and *Lr1* avirulent pathotypes.

Xumai 31 and Xumai 4036 were resistant to most of *Pt* pathotypes, and their resistance could not be attributed to any known *Lr* gene. Therefore, these two cultivars appear to contain unknown *Lr* genes.

Fifty-six cultivars showed high ITs to all tested *Pt* pathotypes, as did differential lines carrying the resistance genes *Lr2c*, *Lr3*, *Lr16*,

LrB, *Lr3bg*, *Lr14b*, and *Lr33*. Therefore, it was impossible to postulate *Lr* genes in these cultivars.

Twelve STS and SCAR molecular markers closely linked to *Lr1*, *Lr9*, *Lr10*, *Lr19*, *Lr20*, *Lr24*, *Lr26*, *Lr34*, *Lr37*, and *Lr46* genes were used to genotype the 83 wheat cultivars (Table 4). The presence of *Lr1*, *Lr26*, and *Lr10* were confirmed by gene postulation and molecular marker analysis. All cultivars lacked *Lr9*, *Lr19*, *Lr20*, and *Lr24* based on the seedling reaction and molecular marker genotyping. It was not possible to postulate *Lr34*, *Lr37*, and *Lr46* in the seedling tests because all are APR genes. Therefore, the molecular markers for these three APR genes were used to test all the cultivars. The results revealed that *Lr46* was detected in 18 cultivars followed by *Lr34* and *Lr37*, each present in four cultivars.

The combined results of gene postulation and molecular marker genotyping indicated that 12 *Lr* genes, namely *Lr1*, *Lr26*, *Lr3ka*, *Lr11*, *Lr10*, *Lr2b*, *Lr13*, *Lr21*, *Lr34*, *Lr37*, *Lr44*, and *Lr46* either singly or in combination were identified in 41 cultivars, whereas any known *Lr* genes could not be found in the remaining 42 genotypes.

Slow rusting resistance. The analysis of variance showed significant differences between cultivars and environments for FDS in the field (at $P = 0.05$) (Table 5). This indicated that slow rusting resistance was influenced by cultivar and environment. There were not significant differences between replication, cultivar-environment, and cultivar-replication interactions.

In both cropping seasons, there was good development of leaf rust disease because Zhengzhou 5389 (susceptible check) scored FDS value of 85 and 95% in the 2014–15 and 2015–16 cropping seasons, respectively (Table 6). On the other hand, SAAR (slow rusting check) scored FDS value of 1 and 3% in both cropping seasons, respectively. Seventeen cultivars were susceptible to the mixed pathotypes at the seedling stage but showed low FDS value at the field across all the environments (Table 6).

Discussion

Postulation of seedling effective *Lr* genes was performed based on the gene-for-gene specificity concept described by Flor (1956). This enables one to quickly identify the genes existing in wheat cultivars with diverse genetic backgrounds (Mebrate et al. 2008).

In the present study, *Lr26* was the most commonly identified *Lr* gene among the 83 tested cultivars. The presence of *Lr26* may relate

Table 4. PCR primers and their conditions for detecting leaf rust resistance (*Lr*) genes as well as specific fragment sizes

<i>Lr</i> gene	Marker type	Primer	Sequence of primer (5'→3')	Annealing temp. (°C)	Size (bp)	Reference
<i>Lr1</i>	STS	WR003F WR003R	GGGACAGAGACCTTGGTGGG GAC GATGATGATTGCTGCTGG	65	760	Cloutier et al. 2007
<i>Lr9</i>	STS	J13/1 J13/2	TCCTTTATTTCCGCACGCCGG CCACACTACCCCAAAGAGAG	66	1100	Schachermayr et al. 1994
<i>Lr10</i>	STS	Lrk10D1 Lrk10D2	GAAGCCCTTCGTCTCATCTG TTGATTTCATTGCAGATGAGATCACG	58	282	Schachermayr et al. 1997
<i>Lr19</i>	SCAR	SCS265 F SCS265 R	GGCGGATAAGCAGAGCAGAG GGCGGATAAGTGGGTTATGG	65	512	Gupta et al. 2005
<i>Lr19</i>	SCAR	SCS253 F SCS253 R	GCTGGTTCACAAAGCAAA GGCTGGTTCCTTAGATAGGTG	60	736	Gupta et al. 2005
<i>Lr20</i>	STS	STS638-L STS638-R	ACAGCGATGAAGCAATGAAA GTCCAGTTGGTTGATGGAAT	60	542	Neu et al. 2002
<i>Lr24</i>	STS	Lr24 J 9/1 Lr24 J 9/2	TCTAGTCTGTACATGGGGGC TGGCACATGAATCCATACG	57	310	Schachermayr et al. 1995
<i>Lr26</i>	STS	Glu-B3F Glu-B3R	GGTACCAACAACAACAACCC GTTGCTGCTGAGGTGGTTC	65	210	de Froidmont 1998
<i>Lr26</i>	STS	ω-secalin F ω-secalin R	ACC TTCCTCATCTTTGTCCT CCGATGCCTATACCACTACT	65	1076	Chai et al. 2006
<i>Lr34</i>	STS	csLV34 F csLV34 R	GTTGGTTAAGACTGGTGATGG TGCTTGCTATTGCTGAATAGT	58	150	Dyck 1987
<i>Lr37</i>	STS	VENTRIUP LN2	AGGGGCTACTGACCAAGGCT TGCAGTACAGCAGTATGTACACAAAA	65	259	Bariana and McIntosh 1993
<i>Lr46</i>	STS	csLV46G22F csLV46G22R	AGG GAAAAGACATCTTTTTTTC CGACCGACTTCGGGTTC	58	335	R. P. Singh et al. 1998

more to its connotation with widespread adaptability and higher yield rather than the resistance it confers to avirulent *Pt* pathotypes (Pathan and Park 2006; Zeller and Hsam 1983). This gene was overcome by *Pt* in many wheat producing areas of the world including North America (Kolmer 1996), Europe (Bartos et al. 1984), South America (Huerta-Espino 1992), the Indian subcontinent (Nayar et al. 1991), South Africa (Pretorius et al. 1990), and China (Li et al. 2010; R. P. Singh et al. 1999). Li et al. (2010) and He et al. (2001) reported that in the early 1970s, the translocation of 1BL/1RS with *Yr9* and *Lr26* was introduced to Chinese wheat breeding programs through the introduction of Lovrin 10, Lovrin 13, Predgornaia 2, Kavkaz, and Neuzuch wheat germplasm. Li et al. (2010) identified 44 cultivars containing *Lr26* among 102 wheat lines, including Huaimai 18 which was confirmed by this research. According to pedigree analysis, *Lr26* in Huaimai 18 and Xumai 29 might be derived from Zhengzhou 891 and Zhoumai 9, respectively (Table 1), because Zhengzhou 891 and Zhoumai 9 carried *Lr26* (R. P. Singh et al. 1999). *Lr26* in Yangfuma 3 might be derived from Yangmai 158 (Table 1), because Yangmai 158 carried *Lr26* and it was one of the parents of Yangfuma 3 (He et al. 2001). In validating the present study, Ren et al. (2015) also reported that Lianmai 2 contained *Lr26*.

R. P. Singh et al. (1999) reported *Lr1* was present in 13 cultivars among a collection of 163 Chinese wheat cultivars inoculated with 14 *Pt* pathotypes. Li et al. (2010) found that seven cultivars carried

Lr1 from 102 tested lines with 24 pathotypes. Virulence for *Lr1* is common in China (Li et al. 2010; R. P. Singh et al. 1999), Iran (Boroujeni et al. 2011), and Australia (D. Singh et al. 2001). In the present study, *Lr1* in combination with other resistance genes was identified in seven cultivars (Table 3). *Lr1* in Xuzhou 23 might be derived from UP301, based on its pedigree analysis (R. P. Singh and A. K. Gupta 1991). *Lr1* in Su 553 and Huaimai 21 might be derived from Yannong 19 (Li et al. 2010) and Yumai 54 (R. P. Singh et al. 1999), respectively (Table 1).

The seedling resistance in 56 cultivars could not be determined because all of them showed high ITs with most of the pathotypes. It may be better to use different pathotypes with diverse pathogenicity or additional differential lines with other resistance genes to postulate the genes in these cultivars.

Although the seedling *Lr* genes were postulated on the basis of gene-for-gene strategy, there was observable limitation to this approach. It was not possible to identify all the *Lr* genes in the cultivars due to limitation of virulence in *Pt* pathotypes. Detecting *Lr* genes using molecular markers was more efficient and economic than gene postulation. In the present study, the identification of *Lr1*, *Lr26*, *Lr10*, *Lr34*, *Lr37*, and *Lr46* using molecular markers was vigorous and stable. Therefore, the molecular markers for above listed *Lr* genes may be very useful in MAS for developing wheat cultivars for resistance to leaf rust in China.

All 17 cultivars identified to carry slow rusting resistance were also tested with 18 *Pt* pathotypes at seedling stage (Tables 3 and 6). Nine cultivars, viz. Huaimai 21, Lian 0536, Nannong 3519, Sunong 6, Wanmai 46, Wanmai 47, Xu 9069, Xumai 26, and Xumai 29 contained *Lr26*. Huaimai 21, Lian 0536, Nannong 3519, Sunong 6, and Wanmai 47 had other known *Lr* genes. Cultivar Wanmai 33 contained *Lr10* and *Lr44* in combination. Three cultivars, Pengxuan 3, Sumai 3, and Xuzhou 438, contained *Lr34* and *Lr46* in combination. The mixed pathotype was virulent to all these genotypes at the seedling stage and the slow rusting resistances in these cultivars might be from *Lr34*, *Lr46*, and other APR genes. Unknown APR genes might be present in four cultivars (Nannong 02y293, Wanmai 53, Xumai 30, and Yangmai 12) that were found to have slow rusting resistance at both field trials.

Table 5. Analysis of variance of final disease severity (FDS, %) in 85 wheat genotypes, including slow rusting cultivar SAAR and susceptible Zhengzhou 5389 checks, tested in the 2014–15 and 2015–16 growing seasons

Parameter	Source of variation	df	MS	F value	P
FDS	Cultivar	84	13350.08	48.22 ^a	<0.0001
	Environment	1	7160.22	25.86 ^a	<0.0001
	Replication	1	936.70	3.38	0.0660
	Cultivar*Environment	84	247.39	0.89	0.7445
	Cultivar*Replication	84	133.09	0.48	1.0000
	Error	2870	276.88		

^a Significant at the 0.05 level of probability.

Table 6. Seedling infection types (ITs)^a with mixed *Puccinia triticina* Eriks (*Pt*) pathotype in the seedling test and final disease severity (FDS, %) in field experiments in Baoding and Zhoukou in 2014–15 and 2015–16 growing seasons for wheat cultivars with slow rusting resistance to leaf rust

Line no. ^b	Cultivar	APR ^c genes	Seedling ITs to mixed pathotype	2014–15 Baoding FDS (%)	2015–16 Baoding FDS (%)	2014–15 Zhoukou FDS (%)	2015–16 Zhoukou FDS (%)
7	Huaimai 21	-	3	5	3	1	10
10	Lian 0536	-	3	12.5	1	12.5	3
14	Nannong 02y293	-	3	10	12.5	7.5	12.5
16	Nannong 3519	-	3	7.5	10	1	3
33	Pengxuan 3	<i>Lr34</i> , <i>Lr46</i>	3+	10	10	5	12.5
36	Sumai 3	<i>Lr34</i> , <i>Lr46</i>	4	3	15	3	10
37	Sunong 6	<i>Lr37</i>	3	5	5	7.5	12.5
41	Wanmai 33	-	4	10	5	12.5	7.5
43	Wanmai 46	-	4	15	5	10	3
44	Wanmai 47	-	4	10	12.5	5	15
47	Wanmai 53	-	3	7.5	15	12.5	15
50	Xu 9069	-	4	5	15	12.5	10
54	Xumai 26	-	3+	15	10	7.5	15.5
56	Xumai 29	-	3+	10.5	15	15	10
58	Xumai 31	-	4	7.5	7.5	8	3
63	Xuzhou 438	<i>Lr34</i> , <i>Lr46</i>	4	7.5	12.5	5	15
69	Yangmai 12	-	3	5	5	15	12.5
	Saar ^d	-	4	1	3	1	3
	Zhengzhou 5389 ^e	-	4	80	95	85	95
	LSD	(P = 0.05)			12.35		

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

^b Line numbers correspond to those in Table 1.

^c Adult plant resistance genes based on molecular marker.

^d Slow rusting check.

^e Susceptible check.

The results of this study are useful to incorporate the resistance genes from the source identified here into the Chinese facultative wheat genotypes to improve the genetic diversity of cultivars and reduce future leaf rust damage in China.

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