Identification of Wheat Leaf Rust Resistance Genes in Chinese Wheat Cultivars and the Improved Germplasms

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Abstract

Leaf rust is an important wheat disease that is a significant hindrance for wheat production in most areas of the world. Breeding resistant cultivars can effectively and economically control the disease. In the present study, a wheat collection consisting of 100 cultivars from China and 18 improved germplasms from global landrace donors together with 36 known single *Lr* gene lines were tested with 20 strains of *Puccinia triticina* Eriks. in the seedling stage to postulate the *Lr* gene in the cultivars and germplasms. In addition, 12 diagnostic molecular markers specific to 10 *Lr* genes were used to detect the presence of the *Lr* genes in the wheat collection. Resistance to leaf rust of these cultivars at the adult plant stage was tested in fields under natural infection during the 2016 to 2018 cropping seasons in Baoding, Hebei Province. The gene postulation combined with molecular marker detection showed that six *Lr* genes (*Lr1*,

Lr26, *Lr33*, *Lr34*, *Lr45*, and *Lr46*) were identified in 44 wheat accessions, including 37 cultivars and seven improved germplasms. Among the 44 wheat accessions postulated with *Lr* genes, *Lr1* was present in four accessions, *Lr26* in 12 accessions, *Lr33* in two accessions, *Lr34* in 14 accessions, *Lr45* in three accessions, and *Lr46* in 16 accessions. In the collection of 118 cultivars/germplasms, 34 wheat lines displayed adult-plant resistance carrying *Lr34*, *Lr46*, and/or underdetermined genes. Therefore, a high level of leaf rust resistance can be achieved through the combination of all-stage resistance and adult-plant resistance genes together in wheat cultivars.

Keywords: wheat leaf rust, gene postulation, slow-rusting resistance, molecular marker

Leaf rust of wheat, caused by *Puccinia triticina* Eriks., is one of the most important diseases in wheat worldwide. The leaf rust pathogen can infect wheat plants in most growth stages in the cropping season. It mainly reduces the photosynthetic efficiency of wheat, resulting in the reduction of plant organic matter synthesis. Leaf rust can decrease grain quantity and quality and reduce wheat production by 40% in susceptible cultivars (Khan et al. 2013; Li et al. 2010). A severe leaf rust epidemic was reported in northwestern Mexico during 1976–1977 and caused 70% yield losses (Dubin and Torres 1981). In China, leaf rust has been reported in the most winter-sown wheat production areas; for example, the most significant yield losses were documented in the provinces of Gansu, Sichuan, Shanxi, Henan, and Anhui in 2012 (Li et al. 2014; Zhou et al. 2013). Because wheat can be planted in different geographic and climatic conditions, the rust pathogens also have wider adaptation to survive in different environmental conditions. If the climate conditions are suitable for the rust pathogen and single resistant wheat varieties are planted in a large acreage, the resistance can be overcome by the emergence of the matching virulence in the rust population, leading to a disease pandemic. The use of rust resistant cultivars is the most economical

and efficient method to reduce the damage of the disease in a wide range of crops (Adhikari and Missaoui 2019; Hartman et al. 2005; Roelfs et al. 1992; Yu et al. 2018).

To date, more than 100 wheat leaf rust resistance (*Lr*) genes have been discovered, and 78 of them have been officially named (McIntosh et al. 2017). Most of them are effective through all wheat growth stages against leaf rust and can be easily identified in the seedling stage, known as all-stage resistance (ASR). This type of resistance is usually controlled by a single gene that starts expressing at the seedling stage (seedling resistance). However, some *Lr* genes often express at the adult plant stage. This type of resistance is known as adult-plant resistance (APR), further sorted as two types, race-specific and non-race-specific APR. Race-specific APR is characterized by a hypersensitive reaction around the rust pathogen infection sites such as *Lr12*, *Lr13*, *Lr22a* (Hiebert et al. 2007), *Lr22b*, *Lr35*, *Lr37* (Seah et al. 2001), *Lr48*, and *Lr49* (Bansal et al. 2008). Non-race-specific APR is controlled by non-hypersensitive genes such as *Lr34* (Dyck 1991), *Lr46* (Singh et al. 1998), *Lr67* (Herrera-Foessel et al. 2011), and *Lr68* (Herrera-Foessel et al. 2012). Wheat cultivars with a non-race-specific APR gene are characterized by slow disease progress with longer latent periods, low infection frequencies, smaller pustule size, and less spore production (Zhang et al. 2017). Although a single non-race-specific slow-rusting APR gene is susceptible or displays an intermediate reaction to leaf rust singly, a combination of four to five such genes can provide adequate resistance against the disease (Singh et al. 2000).

Rust resistance in wheat can be studied through genetic analysis, gene postulation, pedigree analysis, or marker-trait association analysis. Conventional genetic analysis includes making crosses, developing a segregated population, and evaluation of resistance in the population. It can provide essential information about the resistance in wheat, such as gene number, inheritance model, and the interaction of genes. Most of the officially named *Lr* genes were determined by this approach. This procedure is time-consuming and laborious. Morphological traits associated with resistance can be used as preliminary indicators to estimate the presence of the resistance gene. For example, wheat cultivars containing *Lr34*, *Lr46*, *Lr67*, and *Lr68* have leaf tip necrosis (Herrera-Foessel et al. 2011, 2012; Singh 1992; Singh et al. 1998), and cultivars carrying *Lr24* have red grains (McIntosh 1976).

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* The e-Xtra logo stands for “electronic extra” and indicates that there are supplementary materials published online.

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Table 1. The pedigree and origin of 118 wheat accessions

Line no.	Cultivars/germplasms	Pedigree	Origin
1	Jingzuo 208	Beijing 8*(Heine Hvede*Beijing 6)	Beijing, China
2	Jingzuo 210	Beijing 8*(Heine Hvede*Beijing 6)	Beijing, China
3	Zhongzuo 50009	(Jingzuo 348*Fengkang 4)*Nongda 139	Beijing, China
4	Yanda 1817	Selection from Pingyao White wheat	Beijing, China
5	Xinong 6028	Xibei 60*Zhongnong 28	Shaanxi, China
6	Nongda 139	(Nongda 183*Weier)*(30983*Yanda 1817)	Beijing, China
7	Dexuan 1	Bima 4*Soviet precocious 1	Shandong, China
8	Lovrin 13	Skorospelka3B*Heines VII	Beijing, China
9	Qingchun 1	Bima 4*Jubibeina 2	Shanxi, China
10	Beijing 8	Bima 4*Early Premium	Beijing, China
11	Beijing 14	Beijing 8*(Heine Hvede*Orofen)	Beijing, China
12	Beijing 16	Beijing 8*Etoile de Choisy	Beijing, China
13	54405	Bima 4*Soviet precocious 1	Shandong, China
14	Jingzuo 236	(Beijing 8*(Heine Hvede*Orofen))*Beijing 8	Beijing, China
15	Pulin 5	Zhengzhou 24*57Du-5	Shanxi, China
16	Luzhan 1	(Bima 4*Early Premium)*Orofen	Shandong, China
17	Taishan 1	(Bima 4*Soviet precocious 1)*Orofen	Shandong, China
18	Changwei 18	(Guandong'ai*Orofen)*54405(Bima 4*Soviet precocious 1)	Shandong, China
19	Jingzuo 278	Beijing 8*((Nongda 183*Early Premium)*Beijing 8)	Beijing, China
20	Youpi 1	NA ^a	Beijing, China
21	Youpi 2	NA	Beijing, China
22	Bima 2	Mazhamai*Biyumai	Shanxi, China
23	Kechun 14	Orofen*Beijing 8	Beijing, China
24	Linfen 10	((Bima 4*Early Premium)*6055mB)*((Bima 4*Early Premium)* Abbondanza)	Shanxi, China
25	Jingshuang 16	Lovrin 10*Youmanghong 7	Beijing, China
26	Fengkang 7	Youmanghong 7* Lovrin 10	Beijing, China
27	Fengkang 10	Youmanghong 7* Lovrin 10	Beijing, China
28	Fengkang 15	Youmanghong 7* Lovrin 10	Beijing, China
29	Jingnong 79-13	Youmanghong 7* Lovrin 10	Beijing, China
30	Jinghua 1	(Lovrin 18*5238-036)*(Beijing 8*(Heine Hvede*Orofen))	Beijing, China
31	Kedong 83	Beijing 8* Orofen	Beijing, China
32	Linfen 5064	(Linfen 5964*SaricF 74)*Linfen 5054	Shanxi, China
33	Beijing 8694	Fengkang 9*((Jingshuang 2*Lovrin 13)*Jingshuang 3)	Beijing, China
34	Jing 411	((Nongda 45*Beijing 8)*Lovrin 10)*((Nongda 45*Beijing 8)*Aiganza)	Beijing, China
35	Anhui 3	Nanda 2419*Shenglimai	Anhui, China
36	Ganmai 7	Nanda 2419*Abbondanza	Gansu, China
37	Jinghong 4	Nanda 2419* India 798(Nanda 2419*Mingni 50-25)	Beijing, China
38	Mianyang 62-31	Nanda 2419*Sichuan 51	Sichuan, China
39	Qunzhong 42	Nannong large black γ radial	Jiangsu, China
40	Shuwan 8	Hechang 5*Nanda 2419	Sichuan, China
41	Wangmai 17	Kunshanlaizihuang*Nanda 2419	Jiangsu, China
42	Weidong 4	Weidong 1 sib line	Gansu, China
43	Xiangnong 3	Nanda 2419*Funo	Qinghai, China
44	Xuyue 6	Early Premium*Nanda 2419	Henan, China
45	Jinmai 20	(Fengchan 2*Bima 4)*(Nanda 2419/3*Shanqianmai)	Shanxi, China
46	Jinmai 21	(Fengchan 2*Bima 4)*(Nanda 2419/4*Shanqianmai)	Shanxi, China
47	Jinmai 33	(Huadong 5*Nanda 2419)* Orofen	Shanxi, China
48	Jinghong 9	Jinghong 4*Moba 66	Beijing, China
49	Linmai 6	86Jian 22*84-346	Gansu, China
50	Ningmai 1	Chuanmai 4*Fumai 7	Fujian, China
51	Fufan 16	Jinmai 2148*B-5(white)	Fujian, China
52	Simai 2	(Orofen*Soviet precocious 3 σ)*(Neixiang 5*Jili)	Jiangsu, China
53	Yangmai 158	9-16*St1472/506	Jiangsu, China
54	Zheng 6 Fu	Zhengzhou 6 two times of continuous radiation	Henan, China
55	Zhengzhou 6	(Neixiang 5*Huixianhong)*Henan tritcale	Henan, China
56	Lumai 17	(Shanqianmai*Anhui 9* Red shell Orofen white)* Bainimo 62	Shandong, China
57	Mianyang 12	406C3-4-5*Fan 6	Sichuan, China
58	Ningmai 9	Yangmai 6*Xifeng	Jiangsu, China
59	E'mai 9	E'mai 6(Nanda 2419 γ radial) variation plant	Hubei, China
60	Wangmai 15	Selection from Nanda 2419	Jiangsu, China
61	Xinyang 1	Selection from Nanda 2419	Henan, China
62	Ningmai 3	St1472*506 γ radial	Jiangsu, China
63	Yangmai 10	(Yuma/8*Cc)*((Yangmai 5/3/4*Yang85-85/4/2*Yangmai 158)	Jiangsu, China
64	Ningmai 8	Yangmai 5*Yang 86-17	Jiangsu, China
65	Yangmai 11	((Yangmai 158/4*Yuma/8)*Cc)*(Yangmai 5/3*Yang 85-85)	Jiangsu, China

(Continued on next page)^a NA = Not available.

Table 1. (Continued from previous page)

Line no.	Cultivars/germplasms	Pedigree	Origin
66	Yangmai 13	Yang 88-84*(Yangmai 3*Marist Dove)	Jiangsu, China
67	Yangmai 17	Yang 92F101*Chunyu 21526	Jiangsu, China
68	Yang 85-85	Fu 428*9-6-1-2	Jiangsu, China
69	Jimai 26	(Aiganhan*Lovrin 10)*Jinfeng 1	Hebei, China
70	Zhengyin 4	Mara*San Pastore	Henan, China
71	Xiaoyan 4	Fengchan 1*Xiaoyan 96	Shaanxi, China
72	Xiaoyan 5	St2422/464*Xiaoyan 96	Shaanxi, China
73	Zhengmai 9023	(Xiaoyan 6*Xinong 65)*(83(2)3-3*84(14)43/3*Shan 213	Henan, China
74	Gaoyou 503	78506-2-4-6*845504	Beijing, China
75	Huabei 187	Shenglimai*Yanda 1817	Beijing, China
76	Nongda 311	Shenglimai*Yanda 1817	Beijing, China
77	Beijing 5	Selection from Huabei 187	Beijing, China
78	Beijing 6	Selection from Huabei 187	Beijing, China
79	Beijing 10	(Huabei 672*Xinshi 14)*(Soviet precocious 1*Huabei 672)	Beijing, China
80	Hanxuan 10	Nongda 16*Huabei 187	Shanxi, China
81	Taiyuan 566	Shenglimai*Yanda 1817	Shanxi, China
82	Taiyuan 116	(Shenglimai*Yanda 1817)*Mingxian 169	Shanxi, China
83	Taiyuan 567	Nongda 498* Early Premium	Shanxi, China
84	Hanxuan 1	Nongda 16*Huabei 187	Shanxi, China
85	Nanda 8	Funo*Jiangdongmen	Jiangsu, China
86	Zhengzhou 741	St2422/464*Zhengzhou 17	Henan, China
87	Bimai 6	Funo* Orofen	Guizhou, China
88	Linnong 11	Funo*Xinjiang Big seed	Gansu, China
89	Linnong 12	Funo*Xinjiang Big seed	Gansu, China
90	Linnong 13	NA	Gansu, China
91	Yunmai 27	Funo*rust resistance 1 (Orofen*Fuzhou white wheat)	Yunnan, China
92	Suzhou 7829	Funo*66-4768	Jiangsu, China
93	Pingliang 32	NA	Gansu, China
94	Shaan 6815-0-2-3	(Fengchan 3*Funu)*Xibulai 81	Shaanxi, China
95	Jingzhou HXB7561-16	Lovrin 13*(Funu*Taiwan wheat)	Hubei, China
96	Baiquan 565	(Huixianhong*Funu)*Orofen	Henan, China
97	Jingchun 70-5321	Funo*Orofen*Yindu 798	Beijing, China
98	Shaan 62(9)2-1	(Danmai 1*Funu)*58(18)2	Shaanxi, China
99	Xiannong 151	(Fengchan 3*Funu)*Jingmai 5	Shaanxi, China
100	Kezhen	(Songhuajiang 3*Kehua)*(Funu*3/AD20)	Heilongjiang, China
101	PI 660067	AvS*PI 480294	Ethiopia
102	PI 660068	AvS*PI 153779	Egypt
103	PI 660069	AvS*PI 159918	India
104	PI 660071	AvS*PI 178760	Iraq
105	PI 660072	AvS*PI 180957	India
106	PI 660073	AvS*PI 181410	Afghanistan
107	PI 660078	AvS*PI 182121	Pakistan
108	PI 660087	AvS*PI 189744	Pakistan
109	PI 660088	AvS*PI 189749	India
110	PI 660092	AvS*PI 192108	Mozambique
111	PI 660099	AvS*PI 205726	Peru
112	PI 660106	AvS*PI 268075	Hungary
113	PI 660110	AvS*PI 436382	Chile
114	PI 660111	AvS*PI 436384	Chile
115	PI 660113	AvS*PI 479242	Gansu, China
116	PI 660115	AvS*PI 479841	Ethiopia
117	PI 660123	AvS*PI 620708	U.S.A.
118	PI 480016	Landrace	U.S.A.

However, these morphological phenotypes are not stable, and their expressions are usually affected by environment conditions. DNA-based molecular markers are more stable and repeatable. Resistance gene linked markers have been extensively used in detecting resistance genes in wheat germplasms and in marker-assisted selection (MAS) for tracking resistance genes while breeding new wheat cultivars. Gene postulation is based on the gene-for-gene theory, which means a resistance phenotype is defined by the specific interaction between the resistance gene in the host and the corresponding determinant (avrulence) gene in the pathogen (Flor 1956). The responses of test lines are compared with the responses of an array of lines with known resistance genes when inoculated with sets of *P. triticulturae* pathotypes whose avirulence/virulence phenotypes are known (Dubin et al.

1989; Loegering et al. 1971). Although gene postulation has some drawbacks, such as difficulty for postulation of the slow-rusting gene and multiple genes or gene interaction in the cultivars, this approach can quickly estimate probable ASR genes in cultivars by appropriate experimental design. Kolmer (2003) used gene postulation to analyze soft red winter wheat in the U.S.A. and identified seven *Lr* genes in the test cultivars. When eliminating its drawbacks by combining it with pedigree analysis and molecular markers, gene postulation has been extensively used to understand leaf rust resistance in many wheat cultivars (Gao et al. 2019; Gebrewahid et al. 2017; Li et al. 2010; Randhawa et al. 2016; Yuan et al. 2007; Zhang et al. 2019).

China has a long history for wheat cultivation. Wheat landraces adapted to the local climatic conditions had been used by farmers for

centuries until the modern wheat breeding programs were established around the 1930s (He et al. 2001; Zhou et al. 2007). The approaches of wheat improvement in China include introduction and reselection of cultivars/germplasms from other countries, hybridization between local landraces and exotic elite cultivars/germplasms, and creation of new germplasms by introgression of alien species (He et al. 2001). The leaf rust resistance in many modern Chinese cultivars was derived from local landraces, alien species, or introduction of international germplasms, such as Mentana, Ardito, Orofen, and Lovrin 10 (He et al. 2001). However, the leaf rust resistance can be easily overcome by new virulent rust pathotypes. Only a few *Lr* genes, *Lr9*, *Lr19*, *Lr24*, *Lr28*, *Lr29*, *Lr47*, *Lr51*, and *Lr53*, have resistance against prevalent *P. tritricina* population in China (Li et al. 2010; Yuan et al. 2007).

The objective in this study was to identify the resistance in wheat cultivars and improved germplasms through gene postulation, molecular marker diagnostics, and pedigree analysis. Understanding the resistance in these wheat cultivars and identifying new resistance from germplasms will be valuable in wheat breeding for diversifying rust resistance in China.

Materials and Methods

Plant materials. One hundred wheat cultivars from 16 provinces in China and 18 improved germplasms from the National Small

Grain Collection (NSGC) of the U.S. Department of Agriculture (USDA) were to be postulated *Lr* genes (Table 1). A set of 36 wheat leaf rust differential lines differing for single *Lr* genes was used as reference (Table 2). A Chinese cultivar, Zhengzhou 5389, susceptible at both seedling and adult-plant stage to all *P. tritricina* pathotypes in China, was used as control. Cultivar Saar, containing *Lr34* and *Lr46*, was used as control for field adult-plant resistance test and *Lr34* and *Lr46* molecular marker test. The differential lines were kindly provided by USDA-ARS Cereal Disease Laboratory, University of Minnesota, St Paul, U.S.A., and seeds of 118 cultivars/germplasms and Zhengzhou 5389 were provided by the Wheat Rust Laboratory of Hebei Agricultural University, China.

***P. tritricina* races.** Twenty *P. tritricina* strains were used for characterization of the virulence/avirulence patterns in the wheat tester and the reference lines. These strains were collected from the main wheat production areas in China. The strains had various virulence/avirulence combinations. They were identified to 12 *P. tritricina* races, which were the historically predominant reported races in China. Races FHJQ had six strains, and FGJQ, FHSQ, and FHJS each had two strains. Spores of all *P. tritricina* strains were provided by the Wheat Rust Laboratory of Hebei Agricultural University, China. These races were named following the *P. tritricina* nomenclature code system described by Long and Kolmer (1989), with the

Table 2. Infection types^a of 36 *Lr*-gene differential lines inoculated with 20 *Puccinia tritricina* strains

Line no.	<i>Lr</i> genes	Infection types ^a to <i>P. tritricina</i> pathotypes						
		KHJS	THTS	PHSQ	FCJS	PHJS	PHTS	PHJQ
1	RL6003 (<i>Lr1</i>)	1	4	3	;;1	3+	4	4
2	RL6016 (<i>Lr2a</i>)	3	4	2	1	1	2	2
3	RL6047 (<i>Lr2c</i>)	4	4	4	3+	3+	3+	4
4	RL6002 (<i>Lr3</i>)	4	4	4	4	3+	4	4
5	RL6010 (<i>Lr9</i>)	0	0	0	0	0	0	0
6	RL6005 (<i>Lr16</i>)	3	3+	3+	4	3+	4	4
7	RL6064 (<i>Lr24</i>)	;	;	;;1	;	;	;	;
8	RL6078 (<i>Lr26</i>)	4	3-	3+	3+	3+	3+	3
9	RL6007 (<i>Lr3ka</i>)	2	4	3	2	2	4	2
10	RL605 (<i>Lr11</i>)	3+	3	3+	3	3	4	3+
11	RL6008 (<i>Lr17</i>)	4	3+	4	3+	3+	4	3
12	RL6049 (<i>Lr30</i>)	1	4	1	1	1	4	1
13	RL6051 (<i>LrB</i>)	4	3+	3+	3+	3+	4	3
14	RL6004 (<i>Lr10</i>)	3+	3+	3+	3+	3+	4	3+
15	RL6013 (<i>Lr14a</i>)	3	4	X	3	3+	4	2+
16	RL6009 (<i>Lr18</i>)	1	1	1	1	1	2	2
17	RL6019 (<i>Lr2b</i>)	3+	4	3	1	1	2+	2
18	RL6042 (<i>Lr3bg</i>)	3+	4	3+	3+	3+	3+	3
19	RL4031 (<i>Lr13</i>)	3+	4	3+	23	3+	3+	3+
20	RL6006 (<i>Lr14b</i>)	4	4	3+	3	3+	4	4
21	RL6052 (<i>Lr15</i>)	0	4	4	3	4	1	1
22	RL6040 (<i>Lr19</i>)	0	0	0	0	0	0	0
23	RL6092 (<i>Lr20</i>)	;	;	1	1	3+	1	1
24	RL6043 (<i>Lr21</i>)	2	3	2	2	2	3	2+
25	RL6012 (<i>Lr23</i>)	3	3+	3	3	3+	3+	3
26	RL6079 (<i>Lr28</i>)	0	0	0	;	0	0	0
27	RL6080 (<i>Lr29</i>)	;	;;1	;;1	;;1	1	;;1	1
28	RL6057 (<i>Lr33</i>)	3+	3	3	3,2+	2	3	3
29	E84018 (<i>Lr36</i>)	2	1	3	3	2+	3	3c
30	KS91 (<i>Lr39</i>)	3+	3	3+	3	3+	3+	3+
31	WGRC11 (<i>Lr42</i>)	3	2	3	3	3	2, 3	3c
32	RL6147 (<i>Lr44</i>)	3+	3	3	3	1	3+	3
33	RL6144 (<i>Lr45</i>)	4	3+	3	1	3+	3+	2,3+
34	C98.006 (<i>Lr47</i>)	0	0	0	0	0	0	0
35	C78.5 (<i>Lr51</i>)	0	;	0	;;1	;	0	;
36	98M71 (<i>Lr53</i>)	0	0	0	0	0	0	0
37	Zhengzhou 5389	4	4	4	3+	3+	4	4

(Continued on next page)

^a According to the 0 to 4 Stakman scale. 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, - indicates slightly smaller uredinia, C indicates more chlorosis than normal for the infection type, X indicates random distribution of variable-sized uredinia.

addition of a fourth letter for the reactions to the fourth quartet of differentials (https://www.ars.usda.gov/ARSEUserFiles/50620500/Cerealarusts/pt_nomen.pdf).

Seedling testing. The pathogenicity of all wheat accessions was tested in a controlled greenhouse condition in November to December in 2017. Five seeds of each known *Lr*-gene differential line, cultivar, improved germplasm, and Zhengzhou 5389 were sowed in plastic pots (3 × 2 cm) filled with soil mixture and placed in a growth chamber at 18 to 22°C with a diurnal cycle of 16 h light/8 h dark. The test plants were inoculated by each of 20 *P. tritricina* strains when the first leaf completely unfolded. Test plants were brushed against fully sporulating Zhengzhou 5389 plants (Wang et al. 2014). Inoculated plants were incubated in a dew chamber in the dark at 18°C and 100% relative humidity (RH) for 24 h. Then, the plants were transferred to a growth chamber at 18 to 22°C and 75% RH with a diurnal cycle of 16 h light/8 h dark. The infection types (ITs) were recorded 14 days after inoculation with a 0 to 4 scale (Roelfs et al. 1992). Plants with IT 0 to 2+ were considered as low IT (resistance), and 3 to 4 considered as high IT (susceptible). The gene postulation was conducted using the method proposed by Dubin et al. (1989).

Field testing. All 118 wheat accessions, susceptible check Zhengzhou 5389, and slow-rusting check Saar were evaluated for leaf rust reaction in the field nurseries at Baoding in Hebei Province

(115.47°E, 38.85°N) during the 2016 to 2018 cropping seasons. Field tests were designed with randomized complete blocks with three replicates at each location. Fifty seeds of each cultivar were sown in single rows (1.5 m length and 25 cm between rows). The susceptible cultivar Zhengzhou 5389 was planted one row following every 10 test accessions, and adjacent and perpendicular to the test blocks in order to spread spores within the trials. Epidemics were initiated by spraying aqueous spore suspensions, containing equal amounts of mixed spores of four *P. tritricina* races THTT, THTS, THTQ, and PHPS in Tween 20 (0.03%) solution, onto the spreader plants of Zhengzhou 5389 at the tillering stage in the spring. The final disease severity (FDS) was recorded according to the method proposed by Li et al. (2010).

Statistical analysis. Analysis of variance (ANOVA) was performed using the generalized linear model procedure (PROC MIX) of Statistical Analysis System (Version 9.1. SAS Institute Inc. Cary, NC, U.S.A.). 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 of FDS (Sokal and Rohlf 1989). Genotypes with high ITs to mixed *P. tritricina* races at the seedling stage and low FDS values (less than 60%) in the field were considered to have slow-rusting resistance.

Table 2. (Continued from previous page)

Infection types ^a to <i>P. tritricina</i> pathotypes												
THND	FHJQ-1	FHJQ-2	FHJQ-3	FHJQ-4	FHJQ-5	FHJQ-6	FGJQ-1	FGJQ-2	FHSQ-1	FHSQ-2	FHJS-1	FHJS-2
4	1	1	1	1	;;1	2	1	1	1	1	3	;
4	1	2	2	2	1+	2	2	1+	2+	2+	2	2+
4	4	4	4	3+	4	4	3+	4	3	3	4	3+
4	4	3+	4	3+	4	4	4	4	3+	3+	4	3+
0	;	0	0	0	0	0	0	0	0	0	0	;
3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3	4	3+
;	;;1	;	;	;	;	;	;	;	;	;	1	;
3+	3+	3	3+	3+	3+	3+	1	1	2+, 3c	3+	4	3
3+	2	2	2	2	2	2	2	2	3	3	2	2+
1	3+	3+	3+	3	3	3	3+	3+	3	3,3c	3+	3+
3+	3+	3+	3+	3	3+	3+	3+	3	3	3	3	3+
1	2	1+	;;1	2	1	1	2	1	2	1	2	2
3+	3+	3+	3+	3+	3+	3+	3+	3+	3	3+	3+	3+
2+	3+	3+	3+	3+	3+	3+	3+	3+	3	3+	3+	3
4	X	2	2	X	X	X	X	X	2	X	3	3c
1	1	1	1	1	1	1	1	1	1	1	1	1
4	3	3+	3	3	3	3	2	2	3	3+	3	3
4	3+	3+	3	3+	3+	4	3	3+	3	3+	3+	3
4	3	3	3	3	3+	3+	2+	2+	3+	3+	2+	2+
4	3+	3+	4	3	3+	4	3+	3+	3	3+	4	3
4	1	0	3	0	0	1	4	1	3+	;	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0
1	;	;	1	;	1	1	1	1	1	;	1	1
3	2	2	1	1	1	2+	1	2	2	2	2	3
4	3+	3+	3+	3	3+	3+	3	3	3+	3	3+	3
0	0	0	0	0	0	0	0	0	0	0	0	0
;	1	1	1	1	;	;;1	1+	1	1	1	1	;
3	3+	3+	3	3	3	3	3	3	3	3	3	3
1	3	3	3	3	3	3	2	3	2+	3c	3+	3
3	3	3+	3	3	3,3c	3	3+	3	3	3	3	3
2	3	3+	3+	3	3	3	3+	3	3	3	3	3
2	3	3+	3+	3	3	3	3	3	3	3	3	3
3+	4	3+	3+	4,2	3+	2,3+	;;3+	3+	1,3+	3+	3+	3+
0	0	0	0	0	0	0	0	0	0	0	0	0
;	;	1	;	0	;	;	;	;	;	;	;	;
0	0	0	0	0	0	0	0	0	0	0	0	0
4	4	4	4	3+	3+	4	3+	4	4	3	4	3+

Marker for leaf rust resistance gene. The plants were grown in a greenhouse and leaves of each plant were collected at the two-leaf stage. Genomic DNA was extracted from each wheat accession according to the modified CTAB method (Sharp et al. 1988) and the concentration and purity of DNA were measured by a spectrophotometer (NanoDrop ND-1000, Wilmington, DE, U.S.A.). Molecular markers linked to the 10 known *Lr* genes (*Lr1*, *Lr9*, *Lr10*, *Lr19*, *Lr20*, *Lr24*, *Lr26*, *Lr34*, *Lr37*, and *Lr46*) were used for detecting the presence of the genes in the test wheat accessions. The sequences, annealing temperatures, and *Lr* gene linked alleles of these markers are provided in Table 3. PCR was performed following the protocols of the original publications (Table 3). Genomic DNA from wheat lines carrying the known *Lr* genes was included as a reference in each marker test. The PCR products of STS markers were separated on 1.5% agarose gel and stained with ethidium bromide for 20 min. The PCR products of SSR markers were separated on 12% nondenaturing polyacrylamide gel and stained with silver nitrogen.

Results

***Lr* gene postulation.** Zhengzhou 5389 showed high ITs to each of 20 *P. tritricina* strains (IT 4). Nine *Lr* genes (*Lr9*, *Lr18*, *Lr19*, *Lr24*, *Lr28*, *Lr29*, *Lr47*, *Lr51*, and *Lr53*) showed low ITs to 20 *P. tritricina* strains, and nine *Lr* genes (*Lr2c*, *Lr3*, *Lr16*, *Lr17*, *LrB*, *Lr3bg*, *Lr14b*, *Lr23*, and *Lr39*) had high ITs to all *P. tritricina* strains. Thus, it is impossible to postulate the presence of these 18 *Lr* genes based on the test *P. tritricina* strains. The remaining 18 out of 36 *Lr* genes could be postulated based on their IT patterns on the 20 *P. tritricina* strains.

According to the IT patterns of the 118 wheat accessions produced by 20 *P. tritricina* strains (Table 4) and diagnostic alleles of the

molecular markers associated, four seedling resistance *Lr* genes, *Lr1*, *Lr26*, *Lr33*, and *Lr45*, were identified in 22 wheat accessions, including four improved germplasms and 18 cultivars. *Lr1* was present in four (3.4%) wheat accessions, *Lr26* in 12 (10.2%) accessions, *Lr33* in two (1.7%) accessions, and *Lr45* in three (2.5%) accessions. The reaction patterns of 57 accessions were different from the response of any known *Lr* gene or any gene combination, so these accessions contain unidentified *Lr* gene(s). On the other hand, 51 accessions displayed high ITs to all tested *P. tritricina* strains, indicating that the seedling *Lr* gene(s) could not be postulated.

Lr1 showed low ITs to 13 *P. tritricina* strains, KHJS, FCJS, FHJQ-1, FHJQ-2, FHJQ-3, FHJQ-4, FHJQ-5, FHJQ-6, FGJQ-1, FGJQ-2, FHSQ-1, FHSQ-2, and FHJS-2, and high ITs to the other seven strains. Four wheat accessions (Luzhan 1, Taishan 1, Linfei 10, PI 660011) showed similar avirulence/virulence patterns as the *Lr1* gene (Tables 2 and 4). The presence of the *Lr1* gene in these four wheat lines was confirmed by marker Lr1_WR003.

Lr26 showed low ITs to two strains, FGJQ-1 and FGJQ-2, and high ITs to the other 18 strains. Twelve cultivars were postulated carrying *Lr26* gene. Four cultivars (Fengkang 7, Jinghua 1, Beijing 8694, Jinmai 33) had similar avirulence/virulence patterns as *Lr26* and had the same alleles as the *Lr26* gene in marker loci Lr26- ω -secalin and Lr26_Glu-B3. Eight cultivars had additional resistance to the test *P. tritricina* strains. Except to strains FGJQ-1 and FGJQ-2, Lovrin 13 was resistant to strains KHJS, THTS, and PHJQ, Taishan 1 resistant to KHJS, PHSQ, FCJS, PHJS, FHJQ-1, FHJQ-2, FHJQ-3, FHJQ-4, FHJQ-5, FHJQ-6, FHSQ-1, FHSQ-2, FHJS-1, and FHJS-2, Fengkang 10 and Jimai 26 both resistant to THTS and FHJQ-5,

Table 3. PCR primers and their conditions for detecting leaf rust resistance (*Lr*) genes as well as specific fragment sizes

<i>Lr</i> gene	Primer		Reaction condition	Size (bp)	Genetic distance (cM)	Reference
	Name	Sequence (5'-3')				
<i>Lr1</i>	WR003F	GGGACAGAGACCTTGGTGGG	94°C 5 min; 35 cycles (94°C 1 min; 62°C 1 min; 72°C 1 min); 72°C 10 min; 10°C forever	760	Cosegregated	Qiu et al. (2007)
	WR003R	GACGATGATGATTTGCTGCTGG				
<i>Lr9</i>	J13/1	TCCTTTTATTCGACGCGCGG	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	1,100	Cosegregated	Schachermayr et al. (1994)
	J13/2	CCACACTACCCCAAAGAGACG				
<i>Lr10</i>	Lrk10D1	GAAGCCCTTCGTCTCATCTG	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	282	Cosegregated	Schachermayr et al. (1997)
	Lrk10D2	TTGATTCATTGCAGATGAGATCACG				
<i>Lr19</i>	SCS265-F	GGCGGATAAGCAGAGCAGAG	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	512	Cosegregated	Gupta et al. (2006)
	SCS265-R	GGCGGATAAGTGGGTTATGG				
<i>Lr19</i>	SCS253-F	GCTGGTTCCACAAAGCAAA	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	736	Cosegregated	Gupta et al. (2006)
	SCS253-R	GGCTGGTTCTTAGATAGGTG				
<i>Lr20</i>	STS638-L	ACAGCGATGAAGCAATGAAA	94°C 5 min; 35 cycles (94°C 1 min; 63°C 1 min; 72°C 2 min); 72°C 10 min; 10°C forever	542	Complete linkage	Neu et al. (2002)
	STS638-R	GTCCAGTTGGTTGATGGAAT				
<i>Lr24</i>	J09/1	TCTAGTCTGTACATGGGGGC	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	310	Complete linkage	Schachermayr et al. (1995)
	J09/2	TGGCACATGAAGTCCATACG				
<i>Lr26</i>	ω -secalinF	ACCTTCCTCATCTTTGTCCT	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	1,076	Cosegregated	Chai et al. (2006)
	ω -secalinR	CCGATGCCTATACCACTACT				
<i>Lr26</i>	Glu-B3F	GGTACCAACAACAACAACCC	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	636	Cosegregated	de Froidmont (1998)
	Glu-B3R	GTTGCTGCTGAGGTTGGTTC				
<i>Lr34</i>	csLv34F	GTTGGTTAAGACTGGTGATGG	94°C 5 min; 35 cycles (94°C 1 min; 57°C 1 min; 72°C 2 min); 72°C 10 min; 10°C forever	150	0.4	Lagudah et al. (2006)
	csLv34R	TGCTTGCTATTGCTGAATAGT				
<i>Lr37</i>	VENTRIUP	AGGGGCTACTGACCAAGGCT	94°C 5 min; 32 cycles (94°C 50 s; 65°C 50 s; 72°C 1 min); 72°C 10 min; 10°C forever	259	Cosegregated	Helguera et al. (2003)
	LN2	TGCAGCTACAGCAGTATGTACACAAAA				
<i>Lr46</i>	csLV46G22-F	TCGACTTTGGAATGGAGTTGC	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	520	Complete linkage	E. S. Lagudah, unpublished
	csLV46G22-R	GGCGAAGATGCCATCATCCACCAG				

Simai 2 resistant to FHSQ-1, FHSQ-2, and FHJS-1, Jingchun 70-5321 resistant to THTS and FHJQ-2, Jingnong 79-13 resistant to THTS, and Jingzhou HXB7561-16 resistant to FHJQ-3. The same alleles in two marker loci of *Lr26* were detected in these eight cultivars. The resistance to leaf rust in these eight cultivars was conferred by *Lr26* and additional unidentified *Lr* gene(s).

Lr33 showed low ITs (IT 2) to PHJS and high ITs in the other 19 strains (Table 2). Improved germplasms PI 660113 and PI 660067 were postulated carrying *Lr33*, for they had similar avirulence/virulence patterns on 20 *P. triticina* strains as *Lr33* (Table 4).

Lr45 had low IT (IT 1) to FCJS and high ITs on the other 19 strains (Table 2). Three wheat accessions were postulated carrying *Lr45*. Nanda 8, Yunmai 27, and PI 660073 had similar avirulence/virulence patterns against 20 *P. triticina* strains as the *Lr45* differential line.

Postulated adult-plant resistance genes by field test and molecular markers. The analysis of variance for FDS (at $P \leq 0.05$) of the tested wheat accessions showed the significant differences between genotype, environment, and genotype-environment interactions in the field trials (Table 5). This indicated that adult-plant resistance was influenced by genotype, environment and genotype-environment interactions. There were no significant differences among years and replications.

In both cropping seasons, the mean FDS value of susceptible check Zhengzhou 5389 was 80 to 100% while it was 1 to 3% for the slow rusting check Saar. A total of 34 accessions showed high ITs to at least one of the 20 *P. triticina* strains during the seedling stage in the greenhouse or mixed races in seedlings in fields but had FDS less than 60% in the adult-plant stage in fields in two years. These wheat accessions were considered as having adult-plant resistance (Table 6).

Markers specific to adult-plant resistance genes *Lr34* and *Lr46* were used to assess the 118 wheat accessions. Among the 34 adult-plant resistance accessions, 14 accessions had the *Lr34* associated allele (150 bp) of csLV34, and 16 had the *Lr46* associated allele (520 bp) of csLV46G22. The postulated *Lr34* carriers had mean FDS values of 11.9% in 2017, ranging from 1 to 35%, and a mean of 20.4% in 2018, ranging from 5 to 45%. The postulated *Lr46* carriers had mean FDS values of 7.9% in 2017, ranging from 1 to 15%, and a mean of 7.8% in 2018, ranging from 5 to 15%. Two cultivars, Shuwan 8 and Changwei 18, with *Lr34* and *Lr46* combination resistance, had mean FDS values of 1% and 15% in 2017 and both were 5% in 2018.

Molecular assessment of genes. Gene-specific markers of *Lr9*, *Lr10*, *Lr19*, *Lr20*, *Lr24*, and *Lr37* were used to detect if these genes were present in the 118 wheat accessions. Because *Lr9*, *Lr19*, and *Lr24* are resistant to all test *P. triticina* strains, the genes could not be postulated by phenotypes. The IT patterns of *Lr10* and *Lr20* on 20 strains were not found in 118 accessions; therefore, these two *Lr* genes also could not be postulated. *Lr37* has been used in many breeding programs; it is important to know if *Lr37* is present in this wheat collection. Marker Lr9_J13 produced a 1,100 bp allele in the *Lr19* differential line, marker Lr10_Lrk10D produced a 282 bp band in the *Lr10* differential line, marker Lr20_STS638 produced a 542 bp allele in the *Lr20* differential line, marker Lr24_J09 produced a 310 bp band in the *Lr24* differential line, and marker VENTRIUP/LN2 produced a 259 bp band in the *Lr37* differential line, but all these markers did not produce any target band in any of the 118 wheat accessions, indicating *Lr9*, *Lr10*, *Lr20*, *Lr24*, and *Lr37* are absent in these accessions. Marker Lr19_SCS265 having a 512 bp allele specifically associated with the *Lr19* gene and a null allele when *Lr19* was absent, and Lr19_SCS253 having a 736 bp allele when *Lr19* was absent and a null allele when *Lr19* was present were used to test the wheat accessions. Only a 736 bp allele was detected in all accessions and none of the accessions had a 512 bp allele, indicating that *Lr19* is not present in these accessions.

Discussion

The gene postulation approach can quickly identify the genes present in wheat cultivars with diverse genetic backgrounds and is

widely used for identification of leaf rust resistance genes among the wheat lines (Gebrewahid et al. 2017; Kolmer 2003; Kolmer et al. 2013; Randhawa et al. 2016; Singh et al. 1999; Yuan et al. 2007; Zhang et al. 2019). In the present study, we postulated four seedling resistance genes (*Lr1*, *Lr26*, *Lr33*, and *Lr45*) in 22 out of 118 wheat accessions. However, the genes present in other cultivars could not be postulated because of the nonmatching virulence patterns with any of the *Lr* reference lines. In these cases, more *P. triticina* races of diverse virulence phenotypes and/or reference lines with other *Lr* genes should be used to postulate the genes.

Molecular markers provided a more efficient and economical approach for gene detection. However, not all resistance genes have closely linked markers, and the accuracy of linked markers in predicting the presence of a target gene also depends on the linkage distance. In the study, closely linked markers for 10 genes were used to detect the presence of the genes. Results showed that these markers are stable and robust and can be well used for gene detection. Not all the genes contained good markers used in gene detection, however. In this case, it is necessary to develop more closely linked gene markers. In the study, two methods are used to verify each other, which shows the reliability of the results. The identification of *Lr1* and *Lr26* using molecular markers is consistent with the results obtained by gene postulation.

Lr1 was first reported in Malakoff (Mains et al. 1926) and designated as *Lr1* in 1946 (Ausemus et al. 1946). *Lr1* was widely present in bread wheats (Gebrewahid et al. 2017; Singh et al. 1999; Yuan et al. 2007). In the present study, *Lr1* was found in three cultivars, Luzhan 1, Taishan 1, and Linfen 10, and one improved germplasm. Analysis of their pedigree showed that all three cultivars have Bima 4 in their pedigrees (Table 1). The resistance of *Lr1* in these three cultivars is likely derived from Bima 4.

Lr26 was originated from rye (*Secale cereale* L.) chromosome arm 1RS and present in wheat carrying a translocation of 1BL/1RS. In addition to carrying resistance to leaf rust, this translocation also has resistance to stem rust (*Sr31*), stripe rust (*Yr9*), and powdery mildew (*Pm8*), and other good yield traits such as higher harvest index, spikes per square meter, thousand-kernel weight, and grain volume weight (Villareal et al. 2006). Cultivars Lovrin 10, Lovrin 13, Pedgornaia 2, Kavkaz, and Neuzucht carrying this translocation were introduced to breeding programs in China in the 1970s (He et al. 2001), leading to a high frequency of *Lr26* in Chinese wheat cultivars (Li et al. 2010; Zhou et al. 2004). In this study, *Lr26* was found in 12 wheat cultivars. The cultivars carrying *Lr26* resistance have either Lovrin 10, Lovrin 13, or Orofen in their pedigrees (Table 1). Lovrin 10 and Lovrin 13 carry *Lr26* (McIntosh et al. 1995; Zhuang 2003). The *Lr26* resistance in Beijing 8694 and Jingzhou HXB756-16 is likely derived from Lovrin 13, and the *Lr26* resistance in Fengkang 7, Fengkang 10, Jimai 26, and Jingnong 79-63 from Lovrin 10. Li et al. (2009) reported Orofen has *Lr26* resistance. The *Lr26* resistance in Taishan 1, Jinmai 33, Simai 2, Jinghua 1, and Jingchun 70-5321 is probably derived from Orofen.

Lr33 resistance was originally identified from a Chinese wheat germplasm PI 58548, and two other wheat germplasms, PI 268454 from Afghanistan and PI 268316 from Iran (Dyck 1977, 1987). *Lr33* resistance was also postulated in a global collection with 15 *P. triticina* strains (Chen and Wang 1997). This gene is rarely found in current Chinese wheat cultivars (Gebrewahid et al. 2017; Li et al. 2010; Singh et al. 1999; Yuan et al. 2007). In this study, *Lr33* was postulated in two germplasms, PI 660067 and PI 660113. The resistance donor of PI 660113 is a Chinese cultivar Tianxuan 15 (PI479242), and the donor of PI 660067 is an Ethiopian landrace PI 480294 (Wang et al. 2012). Since *Lr33* is not effective to the majority of the current prevalent *P. triticina* races in China, this gene has not been used in the current Chinese wheat breeding programs.

Lr45 resistance originated from rye and the *Lr45* gene was located on the translocation T2AS-3RS.2RL (McIntosh et al. 1995). *Lr45* has high level resistance in both seedling and adult plant stages to leaf rust, but the utilization in breeding programs is limited since it was associated with agronomic deficiencies and not normally inherited (McIntosh et al. 1995). Three wheat accessions in the present study

Table 4. Infection types^a of 118 wheat accessions produced with 20 *Puccinia triticina* strains in seedling stage

Line no. ^b	Resistance type ^c	<i>Lr</i> genes	Infection types ^a																			
			KH	TH	PH	FC	PH	PH	PH	TH	FHJ	FHJ	FHJ	FHJ	FHJ	FHJ	FGJ	FGJ	FHS	FHS	FHJ	FHJ
			JS	TS	SQ	JS	JS	TS	JQ	ND	Q-1	Q-2	Q-3	Q-4	Q-5	Q-6	Q-1	Q-2	Q-1	Q-2	S-1	S-2
1		N ^d	4	4	4	3+	3+	4	3+	4	4	3+	3+	3+	3	4	3+	3+	3+	3+	4	3+
2		+ ^e	3	3	2	1	3, 3c	3+	2	3	;	1	3	3	3	3	2	2	1	13	2	2
3	APR	<i>Lr34</i> ^f	3+	3+	4	3+	3	3	3	3+	3	3+	3	3	3	3	3	3	3	3	3+	3
4	APR	<i>Lr34</i> ^f	4	3+	3+	3	3+	3+	3+	3+	3+	3+	3+	3	3+	3	3+	3+	3+	3+	3+	3
5		N	3+	3+	4	3	3+	3+	3+	3	3+	3	3+	3	3	3+	3+	3+	3+	3+	4	3
6		N	3+	4	4	3	3+	3+	3	3+	4	3	3+	3+	3+	3	3+	3+	3+	3+	3+	3+
7	APR	N	4	3	4	3+	3+	4	3	3+	3+	3+	3+	3+	4	4	3	3	3+	4	3+	3+
8	ASR	<i>Lr26</i> ^g , +	2+	2+	3	3	3+	3+	1	3	3	3+	3	3	3	3+	1	1	3+	3+	4	3
9		N	3+	3	3	3+	3	3	3	3	3	3	3	3	3	3	3+	3	3	3+	3	3
10	APR	<i>Lr46</i> ^f	4	3+	4	3+	4	3+	3+	3+	4	4	3+	3+	3+	3+	3	3+	3+	3+	3+	3
11		+	3+	3+	3+	3+	3+	3+	3+	3+	3	3+	3+	3	1	4	3+	4	3	3+	4	3+
12		+	3+	3+	3+	3+	3+	4	3+	4	4	3+	3+	3+	1	3+	3+	4	3	3+	3+	3
13	APR	<i>Lr34</i> ^f , +	;	3+	3	2, 3	3	2, 3	3	3	3	2, 3	2, 3	2, 3	2, 3	2, 3	2, 3	3	1, 3	2+, 3+	3	3
14		N	3	4	4	3+	3+	3+	3+	3+	4	3	3+	3	3+	4	3	3+	3	3	3+	3
15	APR	<i>Lr34</i> ^f	3+	3+	3+	3+	2, 3+	3+	3+	3+	4	3+	4	3+	4	4	4	3	3	4	4	3+
16	ASRAPR	<i>Lr1</i> ^g , <i>Lr46</i> ^f , +	;	3	1	;	2	3	2	3	;	;	;	;	1	1	;	;	;	;	1	;
17	ASR	<i>Lr1</i> ^g , <i>Lr26</i> ^g , +	;	4	;	;	2	3+	3	3	;	;	;	;	1	1	;	;	1	;	1	;
18	APR	<i>Lr34</i> ^f , <i>Lr46</i> ^f , +	3+	3+	3	2, 3+	3	3	3+	3	1	2, 3+	3	2, 3	3	3	3	3	3+	3+	3+	3
19	APR	<i>Lr46</i> ^f	3+	4	4	3+	4	3+	3+	3+	4	3+	3+	3	3+	4	4	3+	3+	3	4	3
20		N	3+	3+	3	3	3+	3+	3+	3+	3	3+	3+	3+	3+	3+	3	3+	3	3+	3+	3
21		N	3+	4	3	3	3+	3+	3+	4	3	3	3	3	3	3+	3	3	3	3	3	3
22		N	3	3	3	3	2, 3+	3	3+	3+	3	3	3	2, 3+	3+	3	3+	3	3	3+	3	3
23	APR	<i>Lr34</i> ^f	3	3+	3	3	3	3+	3+	3+	3	3	3	3	3	3	3	3	3	3	3+	3
24	ASRAPR	<i>Lr1</i> ^g , <i>Lr46</i> ^f , +	;	4	3c	;	3+	3+	4	3	1	1	1	;	1	2	1	1	1	1	;	;
25		+	4	2+	3+	3+	3+	3+	3+	4	3	3+	3+	3+	3	3+	3	4	3	3+	3+	3
26	ASR	<i>Lr26</i> ^g	4	3-	4	4	3+	3+	4	3+	4	3+	4	3+	3	3	1	1	3+	3	3+	3+
27	ASR	<i>Lr26</i> ^g , +	4	2+	3+	3	3+	3+	4	3+	3+	4	3+	3	1	3	1	1	3+	3	3+	3
28	APR	<i>Lr34</i> ^f	4	3	3+	3+	3+	3+	3, 3c	3+	4	3+	3	3	3	3+	3	3	3+	3+	3+	3+
29	ASR	<i>Lr26</i> ^g , +	3+	2+	3+	3	3+	4	2, 3+	3+	3	3+	3	2+, 3+	3	3	1	1	3+	3+	3+	3-
30	ASR	<i>Lr26</i> ^g	3+	3	3+	3+	3+	4	3+	3+	3+	3	4	3	3	3+	1	1	3	3+	3+	3
31	APR	<i>Lr46</i> ^f , +	1	4	2	3	3+	3+	3	3+	2	2	3	3	3	3	2	1	3	3	2	1
32		+	3	3	4	23	3+	4	3	3	2	2	3	3, 2+	2+	3+	2	3	2	3	3	3-
33	ASR	<i>Lr26</i> ^g	3+	3	4	3+	3+	4	3	3	3	3+	3+	3	3	3+	1	1	3	3	4	3
34	APR	<i>Lr46</i> ^f	3+	3	4	3	3	4	3+	3	3+	3+	3+	3	3	3+	3+	3	3	3+	3+	3+
35	APR	<i>Lr46</i> ^f	4	3	4	3	3+	3+	4	3+	3+	4	3+	4	13	3+	3+	3+	3	3+	3+	4
36		+	3	3+	4	2	3+	4	3+	3+	2+	3	2, 3+	3+	3, 1	3+	2	3+	3+	3+	3	1, 3
37	APR	<i>Lr34</i> ^f , +	3	3+	4	3	3+	4	4	4	3	3	2, 3	3+	1	3+	3	3	2, 3	3+	3+	3;
38	APR	<i>Lr46</i> ^f	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	3	3	3
39		+	2	3	3	3	2, 3+	3	3	3	3	3	3	3	3	3	3	3	2, 3	3	3	1, 3
40	APR	<i>Lr34</i> ^f , <i>Lr46</i> ^f , +	1	3	3	3	3	3+	3	3+	3-	3	13	3	1	3	1, 3	3+	1, 3	1, 3	1, 3+	2
41	APR	<i>Lr46</i> ^f	3	3+	3+	3	3+	3+	3+	3+	3	3+	3	3	3	3+	3	3, 1	3, 1	3+	3+	1, 3
42	APR	<i>Lr34</i> ^f , +	2	3	1	1	2	3+	3	3	3	3	3	1	3	2	2	1	3	3	1	;
43		+	3+	4	4	3	3+	4	3	3	3+	3+	3+	4	1	3	3+	3+	3+	3+	4	4
44	APR	N	4	4	3	3	4	4	4	3+	3+	3+	4	3+	3+	3+	3	3+	3	3+	3+	3
45	APR	<i>Lr34</i> ^f	3+	4	4	3	4	3+	3+	4	3	3+	3+	3+	3+	3	3	3	3	3+	3+	3-
46		N	3+	4	3+	3	3+	3	3+	3	3+	3	3+	3	3+	3+	1, 3	3+	3	3+	3+	3+
47	ASR	<i>Lr26</i> ^g	3	3+	3+	3	3	3+	4	3+	3	3+	3	3	3	3+	1	1	3	3+	3+	3
48		N	3+	3+	3+	3	3+	3+	3+	3+	3	3	3	3	3	3	3	3	3	3	3+	3
49	APR	<i>Lr46</i> ^f , +	3	3	3	3+	3+	3+	3+	3+	3+	1	1, 3+	2, 3	1	3	1	1	3	3+	1, 3+	3, 1
50	APR	N	3	3+	4	3	3+	3+	4	3+	3	3	3	3	3	3	3	3	3	3	3	3
51		+	3	4	4	3-	3+	4	4	4	3+	3+	3	3+	1	3	3	3+	3+	3+	3+	3
52	ASR	<i>Lr26</i> ^g , +	3+	3+	2, 3+	3, 2	3+	3+	3	3	1, 3	1, 3+	1, 3+	1, 3	3, 1+	1, 3	1	1	1	1, 2	2	3
53		+	1	3	3	3	3+	3+	3+	4	3	3	1, 3	1, 3+	2+	3	2, 2+	3	2	3, 3c	2, 3	2
54		N	3+	3+	3+	3+	3+	4	3	3+	3+	3+	3+	3+	3	3	3	3	3	3+	4	3+
55		+	3	3	3	1	3+	4	3	3+	1	3	3+	3+	3	3	3	1	2	3+	2	3+

(Continued on next page)

^a According to the 0 to 4 Stakman scale. 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, - indicates slightly smaller uredinia, C indicates more chlorosis than normal for the infection type.

^b Line numbers correspond to those in Table 1.

^c Resistance type: APR, adult-plant resistance; ASR, all-stage resistance.

^d N: no *Lr* gene postulated.

^e +: unidentified *Lr* gene.

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

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

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

Table 4. (Continued from previous page)

Line no. ^b	Resistance type ^c	Lr genes	Infection types ^a																				
			KH	TH	PH	FC	PH	PH	PH	TH	FHJ	FHJ	FHJ	FHJ	FHJ	FHJ	FGJ	FGJ	FHS	FHS	FHJ	FHJ	
			JS	TS	SQ	JS	JS	TS	JQ	ND	Q-1	Q-2	Q-3	Q-4	Q-5	Q-6	Q-1	Q-2	Q-1	Q-2	S-1	S-2	
56		+	3	3+	2+	3	3	2	4	3+	;	3	3+	3	2	2, 3	3	3+	3	3+	3	1, 3	
57		N	3	4	3+	3+	3+	4	3+	3+	3+	3	3+	3	3+	3	3	3	3	3+	3		
58		+	3	3+	3+	3, 1	3+	3+	3	3+	3+	2	3	3	3	3	3-	3	3+	3	3		
59	APR	Lr34 ^f	3	3+	4	3+	3+	3+	3+	3	3+	3+	3+	3	3	3	3	3+	3+	3	3		
60		+	3+	2, 3	3	3+	2, 3	4	4	3	2, 3	3	3+	3	1, 2	2, 3+	1	1	2, 3+	3+	3	1	
61	APR	N	3+	4	3+	3	3+	3+	4	3	3	3+	3+	3+	3	4	3+	3	3	3+	3	3	
62		+	4	4	3+	4	3+	3+	3+	3+	4	3+	3+	3+	4	1	1	1	3+	3	3+	3	
63		N	3	4	3	4	3+	4	3+	3+	4	3	3+	3+	3	3+	3	3	3+	3+	3+	3	
64		+	3	2	3+	3+	3	4	3+	3	3	3	3+	3	3	3+	3	3+	3	3+	3	3+	
65		N	3	3	3+	3	3+	3+	3+	3	3+	1, 3	3+	3+	3	3, 3c	3+	3	3	3+	3	3+	
66		+	3	2	2+	1	3+	4	2, 3+	3+	3+	1+	3+	1	3	3	3	;	1	3	3+	3	3+
67	APR	N	3	4	3+	3+	3+	4	4	3+	3+	3+	3+	3+	3+	3+	3	3	3+	3+	3+	3+	
68		+	4	1	4	3	3+	3+	4	3	3	3	3+	3	3+	4	3	3	3+	3+	3+	2+, 3-	
69	ASR	Lr26 ^g , +	3+	2+	3+	3	3+	3+	4	3+	3+	3	3+	2+, 3+	2	3c	1	1	3+	3	3+	3	
70		+	3+	1+	3+	3	3	3+	3	3+	3+	3	3+	3	3+	3+	3	3	3	3+	3+	3	
71	APR	N	4	4	4	3+	3	3+	3+	3+	4	3+	3+	4	3+	3+	3+	3	3+	3+	3+	3	
72		N	4	4	3+	3	3	4	3+	3+	4	4	3+	4	3+	3	3+	4	3	3	3+	3	
73		+	3	3-	3	3	2, 3+	3	3+	2, 3	3+	3	3	2	2, 3	2, 3	3-	3	3	3	3-	3	
74		+	3+	3+	4	3	3+	4	4	3+	3	3	4	3+	2	3	3	3	3	3+	3+	2+	
75		N	4	4	4	3+	3+	4	4	3+	3+	3+	4	3	3+	3+	3+	3+	3	3+	3+	3	
76		N	4	4	4	4	4	4	3+	3+	3	4	3+	3+	3+	3+	4	3+	3+	3+	4	3	
77		N	4	4	3+	4	4	4	3+	3+	3+	4	3+	3+	3+	3+	3+	4	3+	3+	3+	3	
78		N	3+	4	3+	4	3+	4	3+	3+	3	3+	3	3+	3+	3	4	3+	3+	3+	3	3	
79		N	3	3+	3	3+	23+	3	3	23+	3+	3+	3	3+	3	3+	3	3	3+	3+	3	3	
80		N	3+	4	3+	3	3	3+	3+	3+	3	3+	3+	3+	3+	3+	3	3+	3+	3+	3+	3	
81		N	3	3+	3+	3	3+	3+	3	3	3	3	3+	2, 3	3	3	3	3+	3	3+	3+	3	
82		N	3+	3+	4	3+	3+	4	4	3+	3+	3+	3+	3+	4	3+	3	3+	3	3+	3+	3+	
83		+	3+	2	4	3+	3+	4	4	3	3	3	3	3+	3+	4	3+	3	3+	3	3+	4	3-
84		+	3	4	1	2	2	4	3	3	3+	2	3	3	3+	2	2	3c	1	;	1	3	3
85	ASR	Lr45 ^h	3+	4	3+	2	4	3+	4	3	3+	3	4	3+	3+	3+	3	3	3	3+	3	3	
86		+	3+	3+	2	2	3	2	4	3+	3	2	2	2, 3	3+	3	3	3	3	3+	3	3c	
87		+	3	2, 3+	3	2+	3+	3	2	3	3+	2	3	3	3	3+	1, 3	3	3+	3	3+	3	
88		+	3	1+	3	3+	3	3+	2	3	3	3	3+	3	2	3	3	3+	3	3	3	3	
89		+	4	3+	3	3+	4	3+	2+	3	3	2, 3	4	3+	3	3	3	3+	3	3	3+	3	
90		+	3+	2, 3	3	3	4	4	3+	3+	3	3	3	3+, 2+	2	3	3	2	3	3	4	3	
91	ASR	Lr45 ^h	4	3+	3+	2	3+	4	3+	3	3+	3	3+	3+	3+	3	3	3+	3	3	4	3+	
92		+	3	1	3	3	3	;	3+	3+	3+	3+	3+	3+	3	3	3	3	3+	3+	3	3	
93	APR	Lr34 ^f	3	4	3+	3+	3	4	3+	4	3+	3+	3+	3+	3+	3+	3	3	3	3+	3+	3	
94		N	3+	4	3+	3	3	4	3+	4	3+	3+	3+	3+	3	3+	3+	3	4	3+	3+	3	
95	ASR	Lr26 ^g , +	3	4	3-	3	2, 3+	4	3+	3+	3	3+	2	3+	3	3	1	1	3	3	3+	3	
96		+	1+	2	2+	3	3+	3	3+	3+	1	3	3	3	3	3	1	;	2	2	3	1	
97	ASR	Lr26 ^g , +	3	1	3-	3	3+	3+	1, 3+	3	3	1	3	3	3	3+	;	1	1	3+	3+	3	3
98		N	3	3	3	3+	3	3+	3+	3	3+	3	3+	3+	3	3+	3+	3+	3+	3+	3+	3	
99		N	3+	3+	3+	3	3+	4	3+	3+	4	3+	3+	3+	3+	3+	3	4	3+	3	3	3	
100		+	3	4	4	3	3+	4	4	4	4	3	3+	3+	3+	3	3+	3	3+	3	3	2	
101	ASR	Lr33 ^h	3+	4	3+	2	2	4	3	3+	4	4	3+	3+	3+	3+	3+	3+	3+	3+	3+	3-	
102		+	3+	2	3+	2+	1	4	2	3	2	3c	3+	3+	3	2+	3c	3	3+	3	3	2+	
103		+	3	1	2	2	1	3	2+	3	3+	3	3+	3c	3+	2+	3	3	3c	3c	2	3	
104		N	3	3c	3	3+	3	3+	3	3	3	3+	3+	3+	3+	3+	3	3	3+	3+	3+	3	
105		+	1	3	;	1	2	;	1	1	3	1	;	2	3	3	1	2	1	2	1	1	
106	ASR	Lr45 ^h	3	4	3+	2	3	3+	3	3	3+	3	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	
107		+	3	1, 3	2	2	;	1	1+	3	3+	2+	3	3+	3	2	3+	2	1	2	2	2	
108		+	2	3+	2	3	3	3	2	3	3	1	3	3	2	3	2	3	3+	3+	2	1+	
109		+	4	2+	3+	3+	3	4	4	4	3+	3	3+	3	3	3	3	3	3	3	3	3	
110	APR	Lr34 ^f	3+	3	3+	3	1, 3	4	3+	3	3+	3	3+	3	3	3	3-	3	3	3	3	3-	
111	APR	Lr46 ^f	3+	4	4	3+	3	4	4	4	4	3+	4	3+	4	3	3+	3+	3+	3+	3	3+	
112		N	4	3+	4	3	3	4	3+	3+	4	3+	3+	3+	3+	4	3	3+	3+	3+	3	4	
113	APR	Lr46 ^f	4	4	3+	3+	3, 2+	4	3+	3	4	3+	3+	3+	3+	4	3	3	3+	3+	3+	4	
114	ASRAPR	Lr1 ^g , Lr46 ^f	1	1+	3	;	1	2	3-	3	2	1	1	1	1	1	;	1	3	1	2	1	
115	ASRAPR	Lr33 ^h , Lr46 ^f	3+	4	3+	2+	2	3	3+	3+	4	3+	3+	3+	3+	4	3+	3+	3+	3+	3	3	
116		+	1	;	;	3	3	;	;	1	3	3	3	3	3	1	2	1	;	3	3	1	;
117		+	4	3+	3	2	3	3	3	4	2	3	3	3	2	3, 2	3	3	3	2	2+	3	3
118		+	3+	2	3	2+	3	3+	1, 3	1, 3+	3	3	3	3+	3c	3	2	3+	3+	3	3c	3	3, 2+

were postulated carrying *Lr45*. In field adult plant tests, the three wheat accessions were resistant in 2017, and PI 660073 was resistant in 2018. Since this gene has good resistance to leaf rust, if using new genetic techniques breaks the genetic lag, this gene can be used for improvement of leaf rust resistance in wheat cultivars.

Lr34 is the first cloned slow rusting resistance gene, which confers resistance to leaf rust, stripe rust, stem rust, and powdery mildew. The resistance had been kept for more than 50 years (Lagudah et al. 2009). It is widely present in wheat worldwide (Kolmer et al. 2008; Singh et al. 2007). In their evaluation of a larger number of

global collections of wheat landraces, improved germplasms, and current and historic cultivars, Kolmer et al. (2008) found the *Lr34* gene in modern wheat cultivars from North and South America, CIMMYT, Australia, China, and Russia, which can be tracked back to the cultivars Mentana, Ardito, Exchange, Frontana, and Chinese Spring. Chinese wheat landraces have a high frequency (30%) of *Lr34* (Kolmer et al. 2008). Yang et al. (2008) found 14 (6.1%) commercial cultivars and 359 (85.1%) landraces having the *Lr34* allele, and *Lr34* resistance in commercial cultivars was mostly from Nanda 2419, Ardito, or Xiannong 4 based on the pedigree. In this study, 14 accessions with *Lr34* showed good resistance in field conditions. The *Lr34* resistance in five Chinese cultivars (Jinghong 4, E'mai 9, Jinmai 20, Shuwan 8, and Weidong 4) is likely derived from Nanda 2419 (Mentana) and *Lr34* in Kechun 14 and Changwei 18 from Orofen, which are present in their pedigrees.

Lr46 was another important slow rusting resistance gene, first identified in a CIMMYT cultivar Pavon 76 and mapped on chromosome 1B (Singh et al. 1998; William et al. 2003). *Lr46* resistance was reported in wheat cultivars from CIMMYT and other countries (Basnet et al. 2013; Calvo-Salazar et al. 2015; Lan et al. 2014, 2015; Lillemo et al. 2008; Ponce-Molina et al. 2018; Ren et al. 2017; Rosewarne et al. 2012). It has remained effective for almost 30 years (William et al. 2006). The locus of *Lr46* cosegregated with *Yr29* (William et al. 2003), *Sr58* (Singh et al. 2013), and *Pm39*

Table 5. Analysis of variance of final disease severity (FDS) in 120 wheat genotypes, including slow rusting cultivar Saar and susceptible Zhengzhou 5389 checks, tested in the 2016 to 2018 growing seasons

Parameter	Source of variation	df	MS ^a	F value	P
FDS	Genotype	119	1,463.6	6.5 ^b	<0.0001
	Environment	1	153,005.9	674.6 ^b	<0.0001
	Replication	1	12,350.5	54.5	0.0601
	Genotype*environment	119	831.3	3.7 ^b	<0.0001
	Genotype*replication	119	250.5	1.1	0.2924
	Error	119	226.8		

^a MS = Mean square.

^b Significant at 0.05 level of probability.

Table 6. Infection types (ITs) of mixed *Puccinia triticina* races in the seedling test and final disease severity (FDS) in adult plant stage on wheat cultivars/germplasms with slow-rusting resistance in field trials in Baoding, Hebei during 2016 to 2018

Line no. ^a	Cultivar	Genes ^b	ITs in seedlings ^c	Mean FDS (%) in 2017	Mean FDS (%) in 2018
3	Zhongzuo 50009	<i>Lr34</i>	3	25	35
4	Yanda 1817	<i>Lr34</i>	3	10	20
7	Dexuan 1		3	45	35
10	Beijing 8	<i>Lr46</i>	4	5	10
13	54405	<i>Lr34</i> , + ^d	3	5	15
15	Pulin 5	<i>Lr34</i>	3	25	45
16	Luzhan 1	<i>Lr1</i> , <i>Lr46</i> , +	3	10	15
18	Changwei 18	<i>Lr34</i> , <i>Lr46</i> , +	3	15	5
19	Jingzuo 278	<i>Lr46</i>	3	5	10
23	Kechun 14	<i>Lr34</i>	3	10	30
24	Linfen 10	<i>Lr1</i> , <i>Lr46</i> , +	3	10	5
28	Fengkang 15	<i>Lr34</i>	4	35	20
31	Kedong 83	<i>Lr46</i> , +	3	10	5
34	Jing 411	<i>Lr46</i>	4	5	5
35	Anhui 3	<i>Lr46</i>	4	10	10
37	Jinghong 4	<i>Lr34</i> , +	3	10	35
38	Mianyang 62-31	<i>Lr46</i>	3	10	5
40	Shuwan 8	<i>Lr34</i> , <i>Lr46</i> , +	3	1	5
41	Wangmai 17	<i>Lr46</i>	4	10	10
42	Weidong 4	<i>Lr34</i> , +	4	5	5
44	Xuyue 6			15	40
45	Jinmai 20	<i>Lr34</i>	4	10	15
49	Linmai 6	<i>Lr46</i> , +	4	5	10
50	Ningmai 1		3	40	30
59	E'mai 9	<i>Lr34</i>	3	5	30
61	Xinyang 1		4	55	35
67	Yangmai 17		3	55	30
71	Xiaoyan 4		4	20	60
93	Pingliang 32	<i>Lr34</i>	4	5	5
110	PI 660092	<i>Lr34</i>	3	5	20
111	PI 660099	<i>Lr46</i>	4	5	5
113	PI 660110	<i>Lr46</i>	4	1	10
114	PI 660111	<i>Lr1</i> , <i>Lr46</i>	3	15	10
115	PI 660113	<i>Lr33</i> , <i>Lr46</i>	4	10	5
	Zhengzhou 5389 ^e		4	80	100
	Saar ^e		4	1	3

^a Line numbers correspond to those in Table 1.

^b Adult-plant resistance genes based on molecular marker.

^c According to the 0 to 4 Stakman scale. 3 = moderate size uredinia, 4 = large uredinia.

^d + indicates unidentified all-stage gene postulated by seedling test in the greenhouse as in Table 4.

^e Susceptible check: Zhengzhou 5389 and resistant check: Saar for adult-plant resistance.

(Lillemo et al. 2008), giving it more value in wheat breeding. Except Pavon 76, *Lr46* resistance has been reported in CIMMYT cultivars Pavon, Attila, Francolin#1, Saar, Pastor, Oligo, Kundan, Sujata, Kenya Kongoni, and Quaiu 3 (Basnet et al. 2013; Calvo-Salazar et al. 2015; Lan et al. 2015; Li et al. 2014; Lillemo et al. 2008; Ponce-Molina et al. 2018; Rosewarne et al. 2012), Uruguay landrace Amercano 25e (Kolmer et al. 2013), and Indian wheat Sujata (Lan et al. 2014) and Kundan (Ren et al. 2017). In this study, 16 wheat accessions were identified having *Lr46* and showing good resistance in the adult-plant stage in fields. None of the above *Lr46* carrying cultivars are present in the pedigrees of the accessions postulated as having *Lr46* in this study. *Lr46* probably has more distribution in the wheat gene pool, and the *Lr46* in our collections has other origins.

Generally, the combination of adult-plant resistance with one or more other genes can confer higher level resistance than any APR genes using singly (German and Kolmer 1992; Kolmer 1992). *Lr34* and/or *Lr46* together with other resistance genes significantly enhanced the resistance level to leaf rust in the reported cultivars (Kolmer et al. 2013; Lan et al. 2014; Singh et al. 1998). Similarly, we found the combination of *Lr46* with other genes in six cultivars conferred high level resistance to leaf rust in two-year field trials with mean FDS from 3 to 12.5%, and *Lr34* with *Lr46* together reached a mean FDS of 3 to 10%. Even if *Lr26*, *Lr1*, or *Lr13* is not effective to some *P. tritricina* races in China, the combination of these genes with *Lr34* or *Lr46* achieved better resistance in the fields as observed in this study. Cultivars Kedong 83, Weidong 4, Jinghong 4, Linmai 6, and 54405 carrying both APR and seedling resistance displayed very high-level resistance in fields. The combination of APR and seedling resistance can enhance the durability of leaf rust resistance in wheat cultivars; this approach can be considered in wheat breeding. Except for *Lr34* and *Lr46*, two other slow rusting resistance genes, *Lr67* and *Lr68*, are an important resource for leaf rust resistance (Herrera-Foessel et al. 2011, 2012). Since the seeds and DNA of *Lr67* and *Lr68* were not available in this study, whether these two genes exist in our collection will be assessed in the future.

In this study, we identified 34 wheat lines with slow rusting resistance for leaf rust; these wheat lines can be used as a resistance resource in Chinese wheat breeding, especially some germplasms from a diverse global landrace collection including PI 660068, PI 660069, PI 660072, PI 660073, PI 660078, PI 660087, PI 660092, PI 660099, PI 660110, PI 660111, PI 660113, and PI 660123. They also showed good stripe rust resistance in the U.S.A. (Wang et al. 2012) and China (Zhou et al. 2015), and displayed good agronomic traits, such as excellent tillering, plump spikes, lodge resistance, and early maturity (Wang et al. 2012), which can be used to develop multiple rust resistance, enrich resistance genetic background, and improve agronomic traits in wheat cultivars.

Our findings showed the usefulness of using molecular marker technology and gene postulation in identifying wheat leaf rust resistance genes. However, outside of 44 wheat lines identified with *Lr* genes, 40 wheat lines might contain unknown gene(s). Therefore, genetic analysis methods should be further used for gene identification. The next step is for genetic biparental populations to be constructed by crossing these resistant lines with other susceptible lines for gene identification. As the development of sequencing technology and high throughput molecular markers continues, this technology can be well used in gene mapping for rust resistance. This research finding could be beneficial to wheat resistance breeding.

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