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Leaf Rust Resistance of 35 Wheat Cultivars (Lines)

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Abstract

Wheat leaf rust caused by *Puccinia triticina* (*Pt*) is one of most severe fungal diseases threatening global wheat production. Rational utilization of the diversified leaf rust resistance (*Lr*) in different wheat cultivars is still the most efficient method to control this disease. Here in this study, 35 wheat cultivars collected from China main wheat growth region were tested for their seedling resistance to 16 different *Pt* races. Seedlings of 20 tested wheat cultivars showed high resistance phenotype to at least one *Pt* race. Various molecular markers closely linked to fifteen designated *Lr* genes were applied and only three *Lr* genes (*Lr1*, *Lr10* and *Lr26*) were identified in some of the tested cultivars. All cultivars were then inoculated with bulked *Pt* races mixed with even of 10 pathotypes at adult plant stage to detect the adult plant resistance (APR). Five cultivars including Liangxing 66, Hemai 9735, Luyuan 301, Jimai 17 and Taishan 027 showed typical APR reaction in the field. Our results indicate that the diversity of known *Lr* genes in the tested wheat cultivars is relative low, but 10 of them contained more durable leaf rust resistance and can be released in wheat production and can be used as resistance breeding resources.

Keywords: Wheat; Leaf rust resistance; Adult plant resistance

Introduction

Wheat leaf rust caused by *Puccinia triticina* Eriks (*Pt*) is one of the most severe fungal diseases on wheat worldwide. Yield losses caused by this disease range from 5% in resistance cultivars to 62.7% in susceptible one under favorable conditions [1], along with reduced protein level and softness equivalent scores in the grain [2]. There were several destructive epidemics of wheat leaf rust in 1969, 1973, 1975 and 1979 in China, especially caused sporadic but dramatic yield losses in the Yellow-Huai areas of China [3]. With the trend of global warming and high density of cultivation, wheat leaf rust has expanded its infection area to the major wheat planting region. The very recent epidemics of wheat leaf rust in China occurred in 2012 [4]. Rational utilization of the diversified leaf rust resistance (*Lr*) in different wheat cultivars, especially local ones, is still the most efficient method to control this disease. Hence, maintaining a level of resistance is an important goal in many wheat breeding programs. To date, 76 *Lr* genes have been formally cataloged in the wheat genome [5]. Many of the *Lr* genes were derived from alien species of wheat, including *Lr19* from *Agropyron elongatum* [6], *Lr26* from *Secale cereale* [7] and *Lr47* from *Aegilops speltoides* etc [8]. Majority of these genes showed race-specific resistance, whereas others showed adult plant resistance (APR). Race specific or so-called seedling resistance *Lr* genes are easy to lose their function during large scale cultivation due to the high selection pressure on *Pt* races. For example, after widely used in breeding program from the 1980s, *Lr26* gene was reported to be overcome by newly emerged virulent *Pt* races in China at the year of 1999 [8]. On the other hand, APR genes are more durable and normally show resistance to multiple fungal pathogens. Therefore, investigation on *Lr* genes in different wheat cultivars will greatly facilitate the wheat breeding program and utilization of the resistance in wheat leaf rust control.

Methods of detecting *Lr* genes in various wheat cultivars include traditional gene postulation and genetic co-segregation analysis, as well as molecular marker-assisted identification. Based on the gene-to-gene theory, gene postulation has been widely used in identification of both *Lr* genes from wheat and newly emerged races from *Pt*. [9-13]. However, the accuracy of gene postulation results may be influenced by several factors, including selection of pathogen races, inoculation conditions, genetic backgrounds of the plants and personal experience. During the last decade, molecular marker-assisted identification of *Lr* genes has been well established and various markers (STS, SCAR and CAPS) linked to different *Lr* genes have been developed. So far, more than 20 molecular markers closely linked to various *Lr* genes, including *Lr9*, *Lr10*, *Lr19*, *Lr21*, *Lr24*, *Lr26*, *Lr34*, *Lr37* and *Lr47 etc.* [14-18]. Combination of both traditional gene postulation and molecular markers has greatly improved the accuracy of *Lr* gene identification procedures [12,19,20].

The initial work of identifying *Lr* genes in released wheat cultivars was started from the 1980s in China [9,11,13,20,21]. Various *Lr* genes identified in different wheat cultivars provide valuable resources for wheat breeding program and resistance gene pyramiding. However, with the rapidly emerged high virulent *Pt* races and large number of newly released wheat cultivars, there are still urgent needs for identification of *Lr* genes. The 35 cultivars (lines) were from the main wheat growth region and some of them were used as the resistance breeding resources and some of them had been or will be released in China. Little information of wheat leaf rust resistance is known. Therefore, identifying *Lr* genes is important for sustainable gene deployment and for breeding new resistant cultivars. This study was designed to identify both gene postulation and molecular markers in 35 mainly cultivated wheat cultivars from China.

Materials and Methods

Wheat germplasm and *P. triticina* pathotypes

Thirty-five winter wheat cultivars were collected from mainly

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Test cultivars (lines)	Leaf rust pathotypes															
	FCGT	FCQT	FHGQ	FHHT	FHQT	FHST	NCRT	PBGP	PBQT	PCGT	PCLT	PHQT	RCHT	TCHT	TCQT	THST
TcLr1-RL6003	;1	;	;1	;1	2	;1	3	4	3	4	3	3	3	4	4	3
TcLr2a-RL6016	;	;	1	1	2	;1	;1	;1	;1	2	;	;1	3	4	4	3
TcLr2b-RL6019	;1	1+	3	4	4	1+	4	3	1	4	4	;	4	4	3	4
TcLr2c-RL6047	3	3	3	4	3	3	3	4	3	3	3	3	0	4	3	4
TcLr3-RL6002	3	3	3	4	4	3	1	4	3	3	3	3	3	3	3	4
TcLr3bg-RL6042	4	3	3	4	3	3	3	4	3	3	3	3	3	;	4	4
TcLr3ka-RL6007	2	3	1	1	3	3	3	;1	3-	;1	3	3	1	;	3	3
TcLr9-RL6010	;0	;	0	;	0	0	;	0	0	0	;0	0	0	;	1	;1
TcLr10-RL6004	3	3	4	4	3	3	4	;1	3	3	3	3	3	4	3	3
TcLr11-RL6053	3	3	3	4	3	3	3	4	3	3	1	3	3	4	3	4
TcLr14a-RL6013	3	3	2	4	4	3-	3	4	3	3	4	3	3	4	3	4;
TcLr15-RL6052	4	3	;1	;1	4	;	1	4	3	;1	3	3	1;	4	1	3
TcLr16-RL6005	;1	;	3	3	3	3	;1	;1	2	2+	1;	3	2	2	2	4
TcLr17-RL6008	1	2	2	1	2	3	2	;1	1;	1	;1	2+	2	1	1	3
TcLr18-RL6009	3	3	2	4	3	3-	3	4	3	3	3	4	4	4	4	4
TcLr19-RL6040	;1	;	;	;	0	;	;	;1	0	;1	0	;	1;	;1	0;	;1
TcLr20-RL6092	3	-	;1	;	4	;	4	4	3	3	3	3	3	2	4	4
TcLr21-RL6043	3	3	3-	4	3	2	3	4	-	3	2	1+	4	4	4	4
TcLr23-RL6012	;1	1	1+	;	1;	2	1;	;1	-	3	1;	1	1	;1	;1	3
TcLr24-RL6064	;1	;	;	;1	;	;1	;1	;1	;1	;1	;1	;1	;1	0	1;	;
TcLr25-RL6084	3	3	3	4	4	3	4	4	3	3	3	3	3	4	4	4
TcLr26-RL6078	4	3	3	4	4	3	3	2	2	3	3	3	3	4	3	3
TcLr28-RL6079	0	0	;	; 0	3	;	0	;	;	0;	0	0	0	;	4	;
TcLr29-RL6080	;1	1;	;1	;1	0	1;	;1	1	;	1	1	1;	;1	;	;1	;1
TcLr30-RL6049	0	1	2	4	;1	1	3	;1	2	1	2	;1	3	3	2	2
TcLr32-RL5497-1	3	2	3	2	4	2	;1	;1	2	2	2	3	3	;1	2	2
TcLr36-E84018	;1	1	1+	;1+	1;	1;	1	;1	;1	1	;1	2	1	;1+	1;	;1
TcLr38-RL6097	;1	;1	;1	;	;	1;	0	1	;	2	1	;1	2;	;1	;1	;
Lr39- KS86WGRC02	;	1+	2	4	3	1;	0	3	1	1	2	1;	3	3	3	3
Lr42- KS90WGRC11	;	;1	2	;	0	1	3	;	1;	0	0	1;	3	;	;1	;
TcLr44-RL6147	1	1+	1	2	3	3	1	;1	1	1	1	1	2	;1	1	;1
TcLr45-RL6144	;1	;1	1;	;	;1	1;	;1	0	;1	0	;12	1	1	3	1	;1
Pavon76-Lr46	;1	;1	;1	;	3	;	3	3	1;	;	3	1	3	;	;	4
Lr50- KS96WGRC36	;	3	4	4	4	3	3	1	3-	;1	;	3	1	4	2	4
TcLrB-RL5688	4	3	4	4	3	3	3	4	3	3	4	4	4	4	3	3
Liangxing 66	1;	3-	;1	;	2	3-	1	1	3-	2	2	3	2	;	2	3
PH01-24	;2	3	4	2	3	2	2	1	3	2	2	1	3	2	2	3
Zi6135	3	3	1	2	3	3-	2	3	3-	2	2+	3	2+	2	3	3
Hemai9735	2	3	2	1	2	3	3	1	3	3	3	3	3	1	;	3
Zhouyuan 187	;	;1	;1	;	3	;	3	1	3	3	3	3	3	;	0	3
Bin 02-47	3	3-	2	4	3	2+	1	4	2+	1	1	2+	2	4	1	2
Liao 9518	;1	;	;1	;	1	;1	;1	;1	3	3	1	2+	1	;2	1;2	2

Yan 896063	;	1+	;1	;1	1	2	1	1	2	1	1	3	2	;1	1	2
Linkang 5025	2	2	2;	;1	2+	2	3	1	3	1	2	3	1	;1	2	2
Taishan 269	3	3	2	4	3	3	3	4	3	3	3	3	3	4	3	4
Hemai 9803	2	3	2	4	3	3	2	4	3	2	3	3	2	4	3	4
Luyuan 301	2	3	4	4	3	2+	3	2	2	1	3	3	3	4	3	3
Taishan5024	3	3	3	4	3	3	3	4	3	4	3	3	3	4	4	4
Lainong 9214	;1	2	;1	;1	4	3	3	;	3-	2	2	3	3	;	2	2
Jimai17	3	3	2	4	2	2+	;12	2	2+	1	12	2	2	4	1	2
Zemai No.1	;	;	;1	;1	2	;1	3	2	2	1	3	3	3	;4	3	0
Yannong 15	;1	1+;	;1	;1	;	;1	12	;1	3	;	1	1	2	;1	3	2;1
Weimai No.8	0	;	;1	;1	1	;1	2	2	1	3	1	3	3	;	3	3
Yan 861601	;1	1+	2	1	1	1	1	;1	;1	;1	;1	2	1	;1	1	2
Linmai No.4	2	3	4	3	4	3	1	4	3	4	3	3	1	4	4	3
Laizhou 953	;1	1+	2	1	1	1	1	;1	;1	;1	;1	4	1	;1	1	2
Luyuan 9160471	2	3	4	3	4	3	1	4	3	4	3	3	1	4	4	3
Binzhou 98-6	3	1+	;1	;1	1	1	1	;	;1	1	;1	1;	2	;1	1;	3
Yan 5286	;1	;	;1	;1	;	;1	1	2	2+	3-	3	3	3	3	1	3
Liao 9629	3	1	2	4	4	1;	3	;	1	4	3	3	4	;1	1	3
Liao 9638	0	1+	;1	;1	3	1	2	1	2	2	2	2	2	1	1	2
Yan 5158	3	2+	2	4	3	2+	1	2	2+	3	2	2+	3	3	3	4
Taishan 027	0	3	2+	4	4	2+	3	;	3	3	;	3	3	4	3	3
Liao 9514	2	3	1	;1	3	1	2	4	;1	1	3	3	2	;1	2	3
Linmai No.6	3	3	2	;	4	1	3	;	3	1	3	3	3	;	4	4
Yan103	4	3	3	4	4	3	0	4	3	4	3	3	1	3	3	3
Yan C96	;1	3-	;1	;1	3	2+	0	2	2	1	2	2+	1	;1	1	2
Weimai No.6	4	3	4	4	3	3	3	4	3	2	3	3	3	4	2	4
Weimai No.7	0	3	4	4	4	3-	3	4	3	4	3	2	3	4	2	4
PH01-35-2	3	3	2	3	2	3-	1	;	3-	3	1	4	2	3	2	3
Thatcher	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Zhengzhou 5389	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

Note: 0: Immune Response, No Sign of Infection; ;: Hypersensitive Chlorotic or Necrotic Flecks; 1: Small Uredinia Surrounded by Necrosis; 2: Small Uredinia Surrounded by Chlorosis; 3: Moderate Size Uredinia without Necrosis or Chlorosis; 4: Large Uredinia without Necrosis or Chlorosis, + =: Uredinia Somewhat Larger than Normal, - =: Uredinia Somewhat Smaller than Normal.

Table 1: Infection types (ITs) of collected wheat cultivars and *Lr* near isogenic lines to 16 different *Puccinia triticina* races at seedling stage.

cultivated ones in China (Table 1). Thirty-five wheat near-isogenic lines (NIL) carrying different *Lr* genes in Thatcher background were kindly provided by the USDA-ARS Cereal Disease Laboratory and included in the gene postulation assay (Table 1). Thatcher and Zhengzhou 5389 were used as susceptible control. Sixteen isolated *Pt* races (FHHT, TCHT, PBGP, TCQT, PCGT, FHQT, RCHT, PCLT, NCRT, THST, FCGT, FHGQ, PHQT, FCQT, PBQT and FHQT) were designated following the naming system [22].

Postulation of leaf rust resistance genes at seedling stage

All the collected wheat cultivars, *Lr*-NILs and susceptible controls were inoculated by those of 16 *Pt* races at the seedling stage in the greenhouse with 3 biological replicates. Briefly, 8-10 seeds for each wheat material were sown in 25 cm × 18 cm × 7 cm iron trays with a pasteurized mixture of soil and compost for inoculation of each *Pt* race. Sixteen sets, each containing collected cultivars and *Lr*-NIL were grown separately with three biological replicates. Seven-day-old seedlings were inoculated separately with each of the sixteen *Pt* races. Inoculated plants were placed in a dew chamber overnight at 18°C to 20°C and then transferred to greenhouse chambers at 18°C to 22°C. Infection types (ITs) were recorded at 12 days after inoculation according to the 0 to 4 scales as described [23,24]. The presence of *Lr* genes in the seedlings of these collected cultivars was postulated by comparing the ITs according to the rules of gene postulation [25].

Evaluation of adult plant resistance (APR) in the fields

Field evaluations of APR to wheat leaf rust were conducted in cropping seasons of 2007-2008 and 2008-2009 at Baoding, Hebei, China. All the 35 collected wheat cultivars, as well as the susceptible cultivars Thatcher and Zhengzhou 5389 were grown in two rows, with 2 m length and 30 cm space, based on a randomized complete block design with three replicates. Spreader rows of cultivar Zhengzhou 5389

were planted perpendicular and adjacent to the rows of tested cultivars. Inoculation was conducted at the stem elongation stage by spraying an equivalent mixture of 10 *Pt* races (FHHT, TCHT, PBGP, TCQT, PCGT, FHQT, RCHT, PCLT, NCRT and THST) in urediniospore-water suspension with added Triton® (0.03% W/V). When leaves of the susceptible cultivar Zhengzhou 5389 were fully rusted, phenotype was recorded following the described scale [26] and the percentage of infected leaf area was visually estimated as infected leaf area index (LRS) according to the modified Cob scale (Supplemental Table S1).

Molecular markers-assisted identification of *Lr* genes

DNAs were extracted from leaves of 7-day-old seedling plants of collected wheat cultivars using CTAB method. Various molecular markers, including STS, SSR and SCAR markers, closely linked to specific *Lr* genes were derived from previous researches. Molecular markers of *Lr1* [27], *Lr9* [28,29], *Lr10* [30], *Lr19* [31], *Lr20* [32], *Lr21* [14], *Lr24* [33,34], *Lr26* [16], *Lr28* [35,36], *Lr29* [37], *Lr32* [38], *Lr34* [17,18], *Lr35* [39], *Lr37* [40], *Lr38* [41] and *Lr47* [8] were used in this study to screen all the collected 35 wheat cultivars. The sequences of all primers are provided in Supplementary Table S2**.** PCR reactions were performed in volumes of 20 μL containing 2.0 μL 10× buffer (containing Mg^{2+}), 0.4 μL 10 mmol/L dNTP, 50 ng of each primer, 50 ng of the extracted DNA and 0.8 U Taq DNA polymerase (Sangon, Shanghai). PCR programs were designed according to corresponding references (Supplemental Table S3). The PCR product was separated on a 0.8% agarose gel in 0.5 × TBE buffer and stained with ethidium bromide, then photographed under UV light.

Results

Postulation of seedling resistance genes

Infection types (ITs) of all the collected cultivars and *Lr*-NILs to 16 *Pt* races were recorded in Table 1. The most efficient seedling *Lr* genes with relative low ITs to major epidemic *Pt* races are *Lr9*, *Lr19*, *Lr24*, *Lr29*, *Lr36* and *Lr38*. However, after gene postulation, we could not detect any of these genes in the 35 collected wheat cultivars.

Five cultivars, including Liao 9518, Zemai No.1, Yannong 15, Weimai No.8 and Laizhou 953, were postulated to carry *Lr1* gene because TcLr1 displayed resistance to FHHT, FCGT, FHGQ, FCQT, FHST and FHQT pathotypes, while susceptible to the other tester races. These five cultivars displayed low infection type ";1" to pathotypes FHHT, FCGT, FHGQ, FHST, FCQT and FHQT, also displayed low infection types to other pathotypes (Table 1). Especially for Laizhou 953, it may also carry another two *Lr* genes (*Lr3* and *Lr32*). Four cultivars, including Binzhou 98-6, Bin 02-47, Linmai No.4 and Yan 5185 showed similar phenotype with *Lr*-NIL TcLr3. They may also carry other *Lr* genes due to the phenotype difference from TcLr3.

Lr26 was present in 11 cultivars, including Luyuan 301, Zemai No.1, Weimai No.8, Yan 861601, Laizhou 953, Binzhou 98-6, Yan 5286, Liao 9629, Liao 9638, Yan 5158 and YanC96. All these cultivars were postulated to carry *Lr26* and other *Lr* genes because they were but not only resistant with two *Lr26* avirulent pathotypes (PBGP and PBQT).

Other phenotypic correlation such as Yan 896063 with TcLr32, Yan 861601 with TcLr17 and TcLr32, Zhouyuan 187 and PH01-35-2 with TcLr10 and Taishan 269 with TcLr18, have been demonstrated based on their resistant responses in Table 1.

Identification of *Lr* genes by molecular markers

Various molecular markers, including STS, SSR and SCAR markers, closely linked to specific *Lr* genes were derived from previous researches. Molecular markers of *Lr1*, *Lr9*, *Lr10*, *Lr19*, *Lr20*, *Lr21*, *Lr24*, *Lr26*, *Lr28*, *Lr29*, *Lr32*, *Lr34*, *Lr35*, *Lr37*, *Lr38* and *Lr47* were used to screen the genome DNAs extracted from the collected 35 wheat cultivars. Corresponding PCR segments for three molecular markers linked to *Lr1*, *Lr10* and *Lr26*, respectively, were amplified in some of the cultivars.

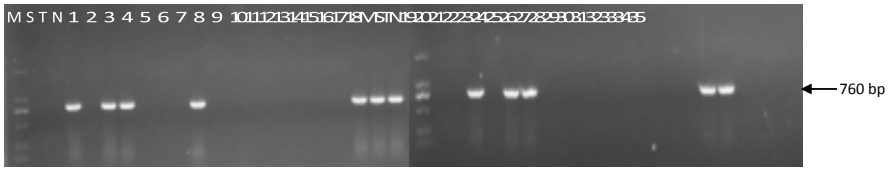

Figure 1: PCR amplification using specific primer linked to *Lr1* in the test cultivars M: DL2000 Marker, N: TcLr1, T: Thatcher, S: water. The cultivars of carrying *Lr1*: 2 (PH01-24), 3 (Zi 6135), 7 (Liao 9518), 16 (Zemai No.1), 17 (Yannong 15), 18 (Weimai No.8), 20 (Linmai No.4), 21 (Laizhou 953), 31 (Yan 103), 32 (Yan C96).

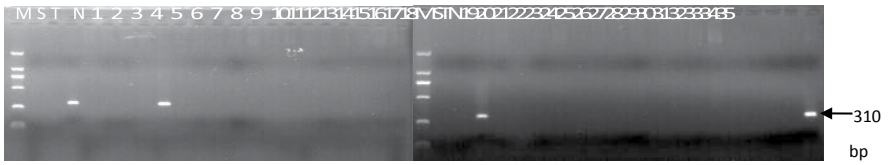

Figure 2: PCR amplification using specific primer linked to *Lr10* in the tested cultivars M: DL2000Marker, N: TcLr10, T: Thatcher, S: water. The cultivars of carrying *Lr10*: 5.

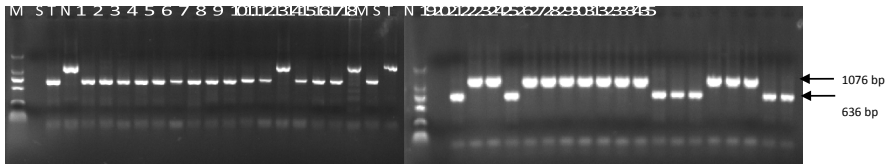

Figure 3: PCR amplification using specific primer linked to1BL/1RS in test cultivars M: DL2000Marker, N: TcLr26, T: Thatcher, S: water. The cultivars of carrying 1BL/1RS: 12 (Luyuan 301), 16 (Zemai No.1), 18 (Weimai No.8), 19 (Yan 861601), 21 (Laizhou 953), 22 (Luyuan 9160471), 23 (Binzhou 98-6), 24 (Yan 5286), 25 (Liao 9629), 26 (Liao 9638), 27 (Yan 5158), 31 (Yan 103), 32 (Yan C96), 33 (Weimai No.6).

Lr1 was identified using STS marker *WR003* (760 bp) in ten wheat cultivars, including Weimai No 8, Yannong 15, Zemai No.1, Liao 9518, Zi 6135, PH01-24, Yan C96, Yan 103, Laizhou 953 and Linmai No.4 (Figure 1). *Lr10* was identified using STS marker *Fl2245/Lr10-6/r2* (310 bp) in two wheat cultivars, including Zhouyuan187 and PH01-35-2 (Figure 2). *Lr26* or 1B/1R chromosome translocation of *Secale cereal* was identified using two markers *Glu-B3* (636 bp, *Lr26* negative) and *ω-secalin* (1076 bp, *Lr26* positive). Fourteen wheat cultivars including Luyuan 301, Zemai No.1, Weimai No.8, Yan 861601, Laizhou 953, Luyuan 9160471, Binzhou 98-6, Yan 5286, Liao 9629, Liao 9638, Yan 5158, Yan 103, Yan C96 and Weimai No.6, showed a positive band of *Lr26* (Figure 3). Information of *Lr* genes identified by both gene postulation and molecular markers were summarized in Table 2.

Germplasm	Pedigree	Adult stage		
		IT	Disease index (LRS)	Gene postulation
Liangxing 66	Ji 991102/Ji 935031	124	5	*Lr3*, APR, +
PH01-24	-	;13	15	+
Zi 6135	-	4	90	+
Hemai 9735	-	4	15	APR, +
Zhouyuan 187	-	;12	5	*Lr10*, +
Bin 02-47	-	;1	-	*Lr3*, +
Liao 9518	77115-1-2-9-1/Lumai 13	;1	-	*Lr1*, *Lr3*, +
Yan 896063	-	;1	-	*Lr32*, +
Linkang 5025	-	4	100	-
Taishan 269	Lumai18/Lumai14	4	75	*Lr18*
Hemai 9803	-	;2	10	+
Luyuan 301	Jiamai 16/121	4	25	*Lr26*, APR, +
Taishan 5024	-	4	55	-
Lainong 9214	Lumai No.7/YexuanNo.1	;1	-	+
Jimai 17	Linfen 5064/Lumai13	3	20	APR, +
Zemai No.1	Xu 9935/Yanyou 361	;1	-	*Lr1*, *Lr26*, +
Yannong 15	Youbao//ST2422/464	;13	5	*Lr1*, +
Weimai No.8	88-3149/Aus621108	;1	-	*Lr1*, *Lr26*, +
Yan 861601	-	;1	-	*Lr32*, *Lr17*, *Lr26*,+
Linmai No.4	Lumai23/Lin9015-	4	55	*Lr3*
Laizhou 953	Zao5/ Yexuan1/7832110-1	;13	15	*Lr1*, *Lr3*, *Lr26*, *Lr32*, +
Luyuan 9160471	-	4	95	+
Binzhou 98-6	-	;13	5	*Lr3*, *Lr26*, +
Yan 5286	Lumai14/945015	;1	-	*Lr3*, *Lr26*, +
Liao 9629	89B08-3-10-1-8/Lumai23	4	75	*Lr26*, +
Liao 9638	Shan160/Lumai 22	;1	-	*Lr26*, +
Yan 5158	Yanhangxuan No.2/ Yannong 15	;1	-	*Lr3*, *Lr26*, +
Taishan 027	-	4	25	APR,+
Liao 9514	-	4	90	+
Linmai No.6	86Jian22/84-346	;1	-	+
Yan 103	Lumai14// Wei132/87ren20	4	80	-
Yan C96	-	4	65	*Lr26*
Weimai No.6	77107-15-7-4/T770-5	4	45	+
Weimai No.7	Lin550/Qianni/Zhong 312	;1	-	+
PH01-35-2	-	4	40	*Lr10*, +
Zheng-zhou5389		4	100	-
A range of infection types is indicated by more than one infection type, with the predominant type listed first. e.g. 124.				

Table 2: Field evaluation for leaf rust resistance and gene postulation of 35 selected wheat cultivars.

Evaluation of adult plant resistance in the field

Adult plant resistance (APR) of all the 35 selected wheat cultivars were evaluated in the field by artificial inoculation of ten-mixed *Pt* races. The whole experiments were repeated twice in the crop seasons of 2007-2008 and 2008-2009 at Baoding, Hebei, China.

In total of 22 tested wheat cultivars showed a resistance or slow rusting phenotype in the field (Table 2). Relatively higher resistance (IT=0;1) was observed in 17 wheat cultivars, which might be associated with corresponding postulated *Lr* genes and/or other major resistance genes. Among these cultivars, three wheat cultivars including Lainong 9214, Linmai No.6 and Weimai No.7 may carry novel leaf rust resistance genes and/or other not tested major *Lr* genes. Five wheat cultivars including Liangxing 66, Hemai 9735, Luyuan 301, Jimai 17 and Taishan 027 showed very typical APR or slow rusting phenotype, thus highly susceptible (IT=3 or 4) during seedling stage, highly susceptible (IT=3 or 4) but low disease index (LRS<25) during adult plant stage. Since we have already ruled out the existence of the widely distributed APR gene *Lr34*, *Lr35* and *Lr37* in any of the selected wheat cultivars using molecular markers, we speculate that these five wheat cultivars may carry novel APR genes and/or other not tested APR genes.

Discussion

The first wheat leaf rust resistance gene *Lr1* derived from *Aegilops speltoides* was designated in 1946 and subsequent BAC-cloning research showed that this gene encode a CC-NBS-LRR protein [42]. *Lr1* is a major resistance gene following the gene-for-gene theory [43]. It still shows resistance to few of the *Pt* races with low virulence in China. In this study, *Lr1* has been identified in five wheat cultivars (Weimai No.8, Yannong 15, Zemai No.1, Liao 9518 and Laizhou 953) by both gene postulation and molecular markers.

Two cultivars were postulated to carry *Lr10*, and this postulation was further validated by molecular markers. *Lr10* was reported to encode a receptor-like protein kinase [44]. Currently, it showed resistance to only one *Pt* race PBGP in our study.

In the present study, *Lr26* was the most commonly identified *Lr* gene among the 35 tested cultivars. The presence of *Lr26* gene may relate more to its connotation with wide spread adaptability and higher yield rather than the resistance it confers to avirulent *Pt* pathotypes [45]. *Lr26* was introduced with the 1BL/1RS chromosome translocation wheat lines including Lovrin10, Lovrin13, Kavkaz, Aurora, Neuzucht and Predgornia from Europe to China in the 1970s, which were subsequently widely used in breeding programs for improving the quality and resistance of a large number of high yielding wheat cultivars in the 1980-1990s [46]. *Lr26* has lost its function toward most of the *Pt* races in China. During our investigation, 14 wheat cultivars were validated as *Lr26*-carrying or 1BL/1RS chromosome translocation wheat lines by molecular markers. Many of these wheat cultivars have 1BL/1RS ancestors in their pedigree. Three cultivars including Luyuan 9160471, Yan 103 and Weimai No. 6 were not postulated to carry *Lr26* at seedling stage, the reason may be the background of the cultivars effected the expression of *Lr26* or the environment effect the *Lr26* expression in the test cultivars.

Lr17 and *Lr32* still showed some resistant to few tested *Pt* races and these two genes have been postulated in only one and two cultivars, respectively. There was only one cultivar postulated to carry *Lr18*. However, this gene has already lost its function to major epidemic *Pt* races in China.

Conclusion

Based on all these results, we strongly recommend wheat breeders to introduce other functional seedling *Lr* genes such as *Lr9*, *Lr19*, *Lr24*, *Lr28*, *Lr29*, *Lr36*, *Lr38*, *Lr42*, *Lr45* and *Lr47 etc.* into mainly cultivated wheat cultivars in China.

Based on our evaluations for leaf rust resistance of these 35 selected wheat cultivars, 17 cultivars showed a high resistance and 5 showed a slow rusting phenotype. The other 13 cultivars were fully susceptible to the mixture of *Pt* races at adult plant stage. So far, thirteen APR genes (*Lr12*, *Lr13*, *Lr22a*, *Lr22b*, *Lr34*, *Lr35*, *Lr37*, *Lr46*, *Lr48*, *Lr49*, *Lr67*, *Lr68* and *Lr74*) have been identified in wheat. Utilization of APR genes will greatly expand the lifespan of rust resistance in the field condition. Since we did not detect any of the widely distributed APR gene *Lr34*, *Lr35* and *Lr37* in any of the selected wheat cultivars using molecular markers, we speculate that those five wheat cultivars may carry novel APR genes and/or other not tested ones. The ratio of wheat cultivars carrying APR genes is relative low, thus there are urgent needs to pyramid more APR genes in wheat cultivars planted in China.

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