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The identification of wheat leaf rust resistance genes and their utilisation value in 42 wheat cultivars

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Abstract: Leaf rust is an important wheat disease that considerably reduces the wheat production in most wheat growing regions worldwide. This study aimed to identify leaf rust resistance genes in 42 wheat varieties to find genetic sources with the broadest spectrum of resistance against leaf rust pathotypes, to enable effective breeding for disease resistance. In this study, 42 wheat cultivars were inoculated with 18 pathotypes of *Puccinia triticina* Eriks. at the seedling stage to postulate the *Lr* genes in the cultivars. Resistance to leaf rust at the adult stage was then tested in field trials under natural infection during the 2019 to 2020 cropping seasons at Baoding, Hebei Province. Gene postulation together with molecular marker detection identified ten *Lr* genes (*Lr1*, *Lr10*, *Lr14a*, *Lr26*, *Lr2a*, *Lr17*, *Lr20*, *Lr34*, *Lr37*, and *Lr46*) among the 42 accessions. *Lr1* was present in 16 accessions, *Lr14a* in three accessions, *Lr17* in five accessions, *Lr2a* in five accessions, *Lr34* in one accession, *Lr10* in two accessions, *Lr37* in two accessions and *Lr46* in 29 accessions. Additionally, 15 wheat accessions displayed adult-plant resistance or other unknown genes. These results suggest that a high level of leaf rust resistance can be achieved by combining known resistance genes and adult-plant resistance genes in wheat cultivars.

Keywords: adult plant resistance; gene postulation; leaf rust; slow rust; *Triticum*

Wheat is one of the most important food crops, providing about a quarter of the human dietary calories, and the emergence of novel and virulent virus strains, resulting in a spectrum of economically important diseases, is constantly affecting the global wheat yields. Wheat rusts pose a threat to wheat production in China, causing millions of dollars in production losses each year. The most widespread and damaging of the three wheat rusts is leaf rust, which accounts for 3.25% of the global wheat yield losses per year (Kolmer 2005; Savary et al. 2019).

In recent years, with the increase in temperature, fertiliser and irrigation amounts, wheat leaf rust has become increasingly severe. The temperature

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and moisture conditions in the future may be more conducive to the occurrence and spread of wheat leaf rust. Long-term planting of a single disease-resistance gene cultivar in a large area will lead to the rapid evolution of pathogen physiological races, which will cause the varieties to exert selective pressure on the pathogen of wheat leaf rust, resulting in the gradual loss of resistance of the varieties.

This research aims to pinpoint resistance within wheat cultivars and enhanced germplasm by integrating gene postulation with molecular-marker diagnostics. Characterising the resistance profiles of these wheats and uncovering previously unknown resistance sources in the germplasm will provide critical resources for diversifying rust resistance in future wheat-breeding programmes.

MATERIAL AND METHODS

Plant materials. Thirty-six near-isogenic lines each carrying a distinct *Lr* gene were employed as the differential set (Table 1). The Chinese landrace Zhengzhou 5389, which is universally susceptible to every *Puccinia triticina* (*Pt*) race throughout China at both the seedling and adult-plant stages, served as the susceptible check. For the field evaluation of the adult-plant resistance and for validating the *Lr34*/*Lr46* functional markers, the positive check was the cultivar Saar, known to possess both *Lr34* and *Lr46*. All the seed lots of the 42 candidate cultivars and of Zhengzhou 5389 were supplied by the Wheat Rust Research Unit of Hebei Agricultural University.

***Puccinia triticina* races.** Eighteen single-spore-derived *Pt* isolates were propagated and evaluated on a host panel consisting of 42 wheat cultivars and 36 differential lines with known *Lr* genes in Mianyang, Sichuan. The nomenclature followed the *Pt* coding scheme of Long and Kolmer (1989), extended by a fourth letter denoting virulence on the fourth differential set.

Seedling testing. In a greenhouse, 36 differential lines, 42 wheat cultivars plus Zhengzhou 5389 were tray-sown and inoculated with 18 *Pt* isolates: FHDS, FGJS, PGIS, FHJQ, FHLT, THMS, KJJK②, PHKQ, FKNQ, FHTQ, FHNT, FHBT, PHQT, FKDQ, FHJQ, THKP②, TGTS② and FHKT. When the first leaf was fully unfolded, spores taken from heavily sporulating susceptible plants were brushed onto the test seedlings. The inoculated plants were kept in plastic-covered cages at 18 °C and 100% relative humidity (RH) for 24 h, then moved to a growth room under an 18 h dark cycle at 18–20 °C and 65% RH. Approximately 14 days later, the infection types were scored with the 0–4 Stakman scale as modified by Roelfs, and the genes were inferred following the procedure described by Dubin et al. (1989).

Field trials. Forty-two wheat cultivars, among them Saar and Zhengzhou 5389, were field-grown in Baoding (Hebei Province) during the 2018–2019 and 2019–2020 growing seasons to assess the leaf rust response. The field trials were designed as randomised complete blocks with two replicates in each experimental field. Approximately 20 seeds of each variety were sown in a 1.2 m row with 50 cm between the rows. Zhengzhou 5389 was sown as a perpendicular border row beside the test plots to promote a uniform spore spread throughout the experiment. THTS, THTQ, PHPS and THTT were selected for the field inoculation on the basis of their high virulence on the wheat cultivars in the seedling trial than the other races. Equal amounts of urediniospores from THTS, THTQ, PHPS and THTT were mixed and suspended in 0.03% Tween 20 and then inoculated into rows of seed drills at the tillering stage. The final disease severity was scored following the protocol described by Li et al. (2010).

Statistical analysis. An analysis of variance was conducted using the Generalised Linear Model procedure (PROCMIX) in the SPSS Statistical Analysis System (Ver. 20, 2011). The varieties, growth environments (combinations of year and site), and their interactions were included as the fixed factors, with blocks nested within environments modelled as random effects. The treatment means of the final disease severity (FDS) were compared with Fisher's least significant difference (*LSD*) at $P = 0.05$ (Sokal & Rohlf 1989). Lines that produced high infection types against the mixed Pt isolates in the seedling assays yet displayed FDS scores below 60% in the field were classified as possessing slow-rusting resistance.

Molecular marker detection. Genomic DNA was extracted from wheat using the cetyl trimethyl ammonium bromide (CTAB) method. The DNA concentration was measured using the Nanodrop 2000 system and the concentration was diluted to 50 mg/L for polymerase chain reaction (PCR) detection. A panel of 12 markers that co-segregate with 10 characterised *Lr* loci was screened across every cultivar, amplifications being carried out with the PCR procedure described by Helguera et al. (2003). A total of 20 µL reaction mixture containing 10 µL 2×*Taq* PCR Master Mix, 6 µL ddH_2O, 2 µL (4 mol/µL) of the primers

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Table 1. Infection types[a] of 36 *Lr*-gene differential lines inoculated with 18 *Puccinia triticina* pathotypes

Line No.	*Lr* genes[a]	Infection types to *Pt* pathotypes																	
		FH DS	FG JS	PG IS	FH JQ	FH LT	TH MS	KJ JK②	PH KQ	FK NQ	FH TQ	FH NT	FH BT	PH QT	FK DQ	FH JQ	TH KP②	TG TS②	FH KT
1	RL6003 (*Lr1*)	2	1	3	;	;	3	2	3	1	1	0	2+	3	2	;	4	4	;
2	RL6016 (*Lr2a*)	1+	2	2+	2+	2C	4	4	2+	1+	2C	2	2+	2+	2C	2+	4	4	1
3	RL6047 (*Lr2c*)	4	4	3	4	4	4	4	3	4	4	4	4	4	4	4	4	4	3
4	RL6002 (*Lr3*)	3	3	3	3	4	4	4	3	4	4	3	4	4	4	4	4	3	4
5	RL6010 (*Lr9*)	0	;	;	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	RL6005 (*Lr16*)	3	3	3	3	4	3	4	3	4	3	4	4	4	4	4	4	4	4
7	RL6064 (*Lr24*)	0	0	2	;	0	1	0	0	2	0	2	1	0	2	1	2	2	;
8	RL6078 (*Lr26*)	4	2	2	4	4	2	4	4	3	4	4	4	4	3	3	4	2	3
9	RL6007 (*Lr3ka*)	2	2	2+	2+	2C	4	1+	2	2	3	3	1+	3	2	2	1	3	;
10	RL6053 (*Lr11*)	2	4	3	3	3	1	4	3	4	3	2	2	4	2	3	4	4	4
11	RL6008 (*Lr17*)	3	3	3	3	4	4	3	3	2	3	4	2	2+	3	3	4	4	3
12	RL6049 (*Lr30*)	1	1	;	1+	1+	;	2	3	2	3	1	2	1	2	1+	4	3	3
13	RL6051 (*LrB*)	3	4	3	3	3+	4	4	3	3	3	3	3	4	3	4	3	4	3
14	RL6004 (*Lr10*)	4	3	3	3	4	4	4	2	4	4	4	4	4	3	4	1	3	4
15	RL6013 (*Lr14a*)	3	3	3	2+	3	3	2	1	2	2+	3	3	3	2	2+	4	4	4
16	RL6009 (*Lr18*)	2+	1	2+	1+	3	1	2	1	2+	1	3	3	3	2	2+	4	;1	3
17	RL6019 (*Lr2b*)	2+	1+	1+	3	4	2	2	2	2	4	3+	2	2	2	1	4	2	3
18	RL6042 (*Lr3bg*)	3	3	4	4	4	3	4	4	4	4	3	3	4	4	4	4	4	3
19	RL4031 (*Lr13*)	3	2+	3	3	4	2	3	2	3	2	2	3	3	2+	3	4	4	3
20	RL6006 (*Lr14b*)	3	3	3	4	3	4	4	4	3	4	3	4	4	3	4	4	4	4
21	RL6052 (*Lr15*)	0	1	3	1	1	2	1	1	1	;	1	1	3	1	1	4	3	;
22	RL6040 (*Lr19*)	;	0,	0	0	;	2	0	0	0	0	0	1	2	2	0	0	0	0
23	RL6092 (*Lr20*)	1	2+	2	1+	2	2	2+	1	4	4	2+	4	4	2+	2	2	1	2
24	RL6043 (*Lr21*)	2+	3	3	3	3	4	3	3	3	3	3	1	3	3	4	3	4	4
25	RL6012 (*Lr23*)	3	3+	3	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4
26	RL6079 (*Lr28*)	;	1	;	1	1	2	;	1	2	;	0	2	2	;	;	0	0	0
27	RL6080 (*Lr29*)	1+	2	1+	1	1+	1	2	2	1	1	2	2	1	2	1	;0	2	1
28	RL6057 (*Lr33*)	3	4	3	3	4	3	3	4	3	4	3	4	4	4	4	4	4	4

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Table 1 to be continued

Line No.	*Lr* genes[a]	Infection types to *Pt* pathotypes																	
		FH DS	FG JS	PG IS	FH JQ	FH LT	TH MS	KJ JK②	PH KQ	FK NQ	FH TQ	FH NT	FH BT	PH QT	FK DQ	FH JQ	TH KP②	TG TS②	FH KT
29	E84018 (*Lr36*)	2+	2	1	1	2+	3	2	2	1	2	1	2	2+	3	2+	0	2	2
30	KS86NGRC02 (*Lr39*)	2	2	3	3	2	2	2	2	2	3	3	2	2	2	2+	4	2	3
31	KS91WGRC11(*Lr42*)	;	1	1+	1	;	0	1	1	1+	1	0	2	2	3	1	1	3	;
32	RL6147 (*Lr44*)	4	3	3	3	3+	4	4	3	3	3	3	3	3	3	1	3	3	3
33	RL6144 (*Lr45*)	3, 1	0	3	4	4	4	3	3	2	0	1	1	0	0	1	4	3	4
34	PAVON76 (*Lr47*)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	C78.5 (*Lr51*)	1	1	;	1	1	0	1	0	1	1	0	1	0	1	0	;	1	0
36	98M71 (*Lr53*)	0	0	;	0	0	2	0	0	;	0	;	0	0	;	0	0	0	0

[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, + – slightly larger uredinia; – indicates slightly smaller uredinia; C – more chlorosis than normal for the infection type

and 2 µL (4 ng/µL) of the template DNA was used. All the PCR amplification reactions were similar, except for the annealing temperatures, which are indicated in Table 2. Sequence-tagged site (STS) marker amplicons were resolved on 1.5% agarose and visualised by 20-min ethidium-bromide staining, whereas the simple sequence repeat (SSR) fragments were separated on 12% native polyacrylamide gels and revealed with silver nitrate.

RESULTS

Identification of the disease resistance gene and molecular marker detection at the seedling stage. Zhengzhou 5389 produced high infection types (IT 4) to all 18 *Pt* isolates. Among the differential lines, nine *Lr* genes – *Lr9*, *Lr18*, *Lr19*, *Lr24*, *Lr28*, *Lr29*, *Lr47*, *Lr51*, and *Lr53* – gave low ITs to every isolate, whereas another nine showed high ITs to the entire set. Consequently, the occurrence of these 18 *Lr* genes cannot be inferred from reactions to the tested *Pt* isolates.

The other 18 *Lr* genes among the 36 could be inferred from their infection-type profiles against the 18 *Pt* strains. According to the IT variation observed among the cultivars after inoculation with the 18 *Pt* races (Table 3), and molecular detection of ten *Lr* genes either singly or in combination, were identified in the 42 cultivars.

Lr1 showed low ITs to 12 *Pt* strains (FHDS, FGJS, FHJQ, FHLT, KJJK②, FKNQ, FHTQ, FHNT, FHBT, FKDQ, FHJQ and FHKT) and high ITs to the other six strains. Fifteen wheat accessions (Hybride du Jubile, Otofte 56, Professeur Journee, Mahndorfer Burgunder, Lada, Scipion, Milpain, Parade, Civic, Booty, Craftsman, Alka, 137, Carahu, and Barbu de Crussol) displayed avirulence/virulence profiles comparable to those of *Lr1* (Tables 2 and 3). All the cultivars with *Lr1* were also confirmed by the molecular marker for *Lr1*. Interestingly, the wheat cultivars Parade and Craftsman, also showed resistance to the non-toxic races of *Lr10* (THKP② and PHKQ) vector varieties, indicating that they may contain *Lr1* and *Lr10*. The molecular marker detection further confirmed that Parade and Craftsman contained *Lr1* and *Lr10*. Similarly, the Civic wheat cultivars, showed resistance to non-toxic races containing *Lr1* vector varieties and also showed low infection type to non-toxic races containing *Lr26* (FGJS, PGIS, THMS and TGTS②), which was inferred to contain *Lr26*. The molecular marker detection amplified the specific

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bands with the same positive and negative correlation as *Lr26*, consistent with the result of the gene derivation, and it was concluded that the cultivars carried *Lr1* and *Lr26*.

Lr2a showed low ITs to three *Pt* strains, PGIS, PHKQ and PHQT. Six cultivars (Professeur Journee, Parade, Booty, Perquenco, Gentil and Barbu de Crussol) also showed resistance to the *Pt* strains (PGIS, PHKQ and PHQT) and were inferred to contain *Lr2a*. The Professeur Journee, Parade, Booty, Perquenco, Gentil and Barbu de Crussol cultivars contained *Lr1* and *Lr2a*, consistent with the results of the molecular marker assays.

Lr14a showed low ITs to seven *P. triticina* strains FHJQ, KJJK②, PHKQ, FKNQ, FHTQ, FKDQ and FHJQ. Three cultivars (Professeur Journee, Craftsman and Barbu de Crussol) postulated in combination with *Lr1* and *Lr14a* or other *Lr*.

Lr20 was postulated in one cultivar (Rieti) because the cultivars were resistant to fourteen *Lr20* avirulent races (FHDS, FGJS, PGIS, FHJQ, FHLT, THMS, KJJK②, PHKQ, FHNT, FKDQ, FHJQ, THKP②, TGTS② and FHKT) (Table3). Rieti contained *Lr20* based on its low responses to all the avirulent races of these *Lr20*. *Lr20* and unknown *Lr* were present in Rieti. The molecular markers revealed the identical target band in the test variety Rieti as in the vector variety *TcLr20*, demonstrating that the *Lr20* disease resistance gene is present in Rieti.

Lr17 showed low infection to the *Pt* strains FKNQ, FHBT, and PHQT. Five wheat cultivars (Hybrid du Jubile, Sv 60504, Carahue, Perquenco and Rimbaus) are thought to contain *Lr17* or have a broader range of resistance than *Lr17*. It is assumed that the wheat cultivar Hybrid du Jubile has *Lr1* and *Lr17*, whereas the wheat cultivar Perquenco contains *Lr2a* and *Lr17*,

Table 2. Primer sequences and polymerase chain reaction (PCR) annealing temperatures for different primer combinations

Lr gene	Marker type	Primer	Size (bp)	Sequence of primer (5'-3')	Annealing temperature (°C)	Reference
Lr1	STS	*WR003F* *WR003R*	760	GGGACAGAGACCTTGGTGGA GACGATGATGATTTGCTGCTGG	65	Wang et al. (2022)
Lr9	STS	*J13/1* *J13/2*	1 100	TCCTTTTATTCCGCACGCCGG CCACACTACCCCAAAGAGAG	68.5	Meng et al. (2022)
Lr10	STS	*Lrk10D1* *Lrk10D2*	282	GAAGCCCTTCGTCTCATCTG TTGATTCATTGCAGATGAGATCACG	60	Wang et al. (2022)
Lr19	SCAR	*SCS265 F* *SCS265 R*	512	GGCGGATAAGCAGAGCAGAG GGCGGATAAGTGGGTTATGG	65	Meng et al. (2022)
Lr19	SCAR	*SCS253 F* *SCS253 R*	750	GCTGGTTCCACAAAGCAAA GGCTGGTTCCTTAGATAGGTG	60	Helguera et al. (2003)
Lr20	STS	*STS638 F* *STS638 R*	542	ACAGCGATGAAGCAATGAAA GTCCAGTTGGTTGATGGAAT	60	Zhu et al. (2023)
Lr24	STS	*Lr24 J 9/1* *Lr24 J 9/2*	310	TCTAGTCTGTACATGGGGGC TGGCACATGAACTCCATACG	60	Meng et al. (2022)
Lr26	STS	*Glu-B3F* *Glu-B3R*	636	GGTACCAACAACAACAACCC GTTGCTGCTGAGGTTGGTTC	65	Helguera et al. (2003)
Lr26	STS	*ω-secalin F* *ω-secalin R*	1 076	ACCTTCCTCATCTTTGTCCT CCGATGCCTATACCACTACT	65	Wang et al. (2022)
Lr34	STS	*csLV34 F* *csLV3R*	150	GTTGGTTAAGACTGGTGATGG TGCTTGCTATTGCTGAATAGT	55	Wang et al. (2022)
Lr37	STS	*VENTRIUP LN2*	259	AGGGGCTACTGACCAAGGCT TGCAGCTACAGCAGTATGTACACAAAA	60	Helguera et al. (2003)
Lr46	CAPS	*csLV46G22F* *csLV46G22R*	520	TCGACTTTGGAATGGAGTTGC GGCGAAGATGCCATCATCCACCAG	65	Wang et al. (2022)

STS – sequence tagged site; SCAR – sequence characterized amplified regions; CAPS – cleaved amplified polymorphic sequences

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Table 3. Infection types[a] of 42 wheat accessions produced with 18 *Puccinia triticina* strains in seedling stage

Line No.[b]	Resistance type[c]	Cultivars/ germplasms	*Lr* genes[a]	FH DS	FG JS	PG IS	FH JQ	FH LT	TH MS	KJ JK②	PH KQ	FK NQ	FH TQ	FH NT	FH BT	PH QT	FK DQ	FH JQ	TH KP②	TG TS②	FH KT
1	APR	Wa 4877	*Lr46*[f]	4	4	4	4	4	4	4	3	4	2	2	4	4	4	4	2	4	4
2	ASR, APR	Hybride du Jubile	*Lr1*[d], *Lr17*[d], *Lr46*[f]	2	1	4	0	0	3	2	3	1	1	0	2	2	2	0	;	;	0
3	ASR	Otofte 56	*Lr1*[d]	1	1	3	0	0	3	2	4	1	1	0	2	3	2	;	3C	4	0
4	ASR	Professeur Journee	*Lr1*[d], *Lr2a*[d], *Lr14a*[d]	1	2	1+	0	0	3	1	2	1	2	0	2	2	2	;	3	3	;
5	ASR, APR	Mahndorfer Burgunder	*Lr1*[d], *Lr46*[f]	1	2	2	;	;	4	2	4	;	1	0	2	3	2	;	2	3	;
6	APR	Slazaczka	*Lr46*[f]	4	4	4	4	3	4	4	4	3	4	4	3	4	3	4	3	1+	2
7	ASR, APR	Lada	*Lr1*[d], *Lr46*[f]	2	1	2	0	0	0	2	3	1	1	0	2	3	2	;	0	3	0
8	APR	167	*Lr46*[f]	4	4	3	2+	3	4	4	4	3	2+	3	3	4	2	3	2	1	1
9	ASR, APR	Sv 60504	*Lr46*[f], *Lr17*[d]	3	4	2+	;	;	4	;	3	1	2	2	2	;	2	3	;	4	;
10	APR	Kubb GLlatst Comp	*Lr46*[f]	1	4	4	2+	2	0	3	3	2	2	2	0	4	4	3	2	1+	2
11	ASR, APR	Scipion	*Lr1*[d], *Lr37*[f], *Lr46*[f]	2	1	2	0	0	2+	2	4	1	1	0	2	3	2	;	2	3	;
12	ASR, APR	Milpain	*Lr1*[d], *Lr37*[f], *Lr46*[f]	2	1	1	0	0	2	2	4	1	1	0	1	4	1	;	1	3	;
13		S15	–	4	3	3	3	4	0	4	3	3	3	3	3	3	3	3	3C	3C	4
14		87Wtr-6	–	4	3	4	4	4	4	4	4	4	4	4	4	4	3	4	2	;	4
15		Rooster	–	4	4	4	4	4	4	4	4	4	3	3	4	4	4	4	;	;	2
16	APR	Boxer	*Lr46*[f]	4	2+	4	4	3	2	4	3	4	2	4	4	4	4	4	3	3	;
17	ASR, APR	Parade	*Lr1*[d], *Lr2a*[d], *Lr10*[d], *Lr46*[f]	1	1	1+	;	;	3	;	2	1	1	0	2	2	2	;	2	3	;
18	ASR, APR	Civic	*Lr1*[d], *Lr26*[d], *Lr46*[f]	1	2	2	1+	;	0	1	3	1	1	0	1	4	2	;	;	2	;
19	ASR, APR	Booty	*Lr1*[d], *Lr2a*[d], *Lr46*[f],	1	2	2	;	;	4	2	1	;	1	0	2	1	2	;	4	4	;
20	ASR, APR	Craftsman	*Lr1*[d], *Lr10*[d], *Lr14a*[d], *Lr46*[f],	2+	1	3	;	;	4	2	1	1	1	0	1	4	2	;	1	4	;
21	ASR, APR	Alka	*Lr1*[d], *Lr46*[f]	2	2	3	;	;	4	2	4	1	1	0	2	4	2	;	0	2+	;
22	ASR, APR	Carahue	*Lr1*[d], *Lr17*[d], *Lr34*[f], *Lr46*[f]	1	1	2	;	;	4	2	3	1	1	0	1	2	2	;	3	3	;
23	ASR, APR	Perquenco	*Lr46*[f], *Lr2a*[d], *Lr17*[d]	1	2	1	2	2	3	2	2	1	2	1	2	2	2	2	3	2	1
24	APR	Conco	*Lr46*[e], +	3	3	4	3	;	4	3	2	3	3	2	2	2	2	2	3	;	;
25		Car 735	–	3	;	3	4	3	4	3	3	3	4	3	4	4	3	4	2	2	3
26	ASR, APR	Rieti	*Lr20*[d], *Lr46*[f]	1	2	1+	1	1+	1	2	1	3	3	2	4	2	2	2	1	1	1
27	ASR, APR	Gentil	*Lr2a*[d], *Lr46*[f], +	1	2	2+	;	;	4	;	1	1	2	2	2	;	2	2	2	2	1
28	ASR, APR	Rimpaus	*Lr17*[d], *Lr46*[f]	1	3	3	2+	2	0	3	3	2	2	2	2	1	4	3	2	2	3
29	ASR, APR	Barbu de Crussol	*Lr1*[d], *Lr2a*[d], *Lr14a*[d], *Lr46*[f]	1	1	2	;	;	3	2	2	1	1	0	2	2	2	;	3	1+	;

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Table 3 to be continued

Line No.[b]	Resistance type[c]	Cultivars/ germplasms	*Lr* genes[a]	FH DS	FG JS	PG IS	FH JQ	FH LT	TH MS	KJ JK②	PH KQ	FK NQ	FH TQ	FH NT	FH BT	PH QT	FK DQ	FH JQ	TH KP②	TG TS②	FH KT
30	APR	Eroica	*Lr46*[f]	4	4	4	4	3	4	3	3	4	3	4	4	3	4	4	;	;	;
31		1665	–	4	4	4	4	4	4	4	3	4	4	4	4	4	4	3	;	1	2
32	APR	Heines 476	*Lr46*[f]	4	4	4	4	4	4	4	3	4	4	4	4	4	4	3	3	1	1
33		Reichersber Kolben	–	4	4	3	4	4	4	4	3	4	4	4	3	4	3	4	;	2+	3C
34	APR	Chitral	*Lr46*[f], +	3	4	2+	;	;	4	;	3	1	2	2	3	;	3	3	0	3	2
35	APR	Riddar	*Lr46*[f], +	1	4	4	2+	2	0	3	3	2	2	2	3	4	4	3	4	3	2
26	ASR, APR	Rieti	*Lr20*[d], *Lr46*[f]	1	2	1+	1	1+	1	2	1	3	3	2	4	2	2	2	1	1	1
37		Victor Hvid	–	4	3	4	3	4	3	4	4	4	4	3	4	4	4	4	4	4	4
38		Wiwatka 4	–	4	4	4	4	4	3	4	4	4	3	4	3	4	4	4	2+	3	3
40	ASR	137	*Lr1*[e]	4	4	4	4	4	4	4	4	4	4	4	4	3	3	4	3C	3C	2+
41		Avocets	–	3	3	3	4	4	4	4	4	3	3	4	3	4	3	4	4	4	4
42		N. Strampelli	–	4	4	4	4	3	4	4	4	4	2	4	3	4	4	3	4	3	3

[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 – 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]postulation of *Lr* genes based on the gene postulation and molecular marker; [e]postulation of *Lr* genes based on the gene postulation; [f]detection of *Lr* genes based on the molecular marker; – no *Lr* gene postulated; + unidentified *Lr* gene

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Table 4. Analysis of variance of the final disease severity (FDS) in 42 wheat genotypes, including slow rusting cultivar Saar and susceptible Zhengzhou 5389 checks, tested in the 2019 to 2020 growing seasons

Source of variation	SS	*df*	MS	*F*-value[a]	*P*-value
Cultivar	185 364	43	4 310.8	6.040**	< 0.0001
Environment	2 240.818	1	2 240.8	3.120**	< 0.0001
Cultivar × environment	8 411.182	43	195.6	2.720**	< 0.0001
Error	3.16	44			

SS – sum of squares; MS – mean square; *df* – degree of freedom; [a]significant at 0.05 level of probability

implying that it may have other undiscovered leaf rust resistance genes in addition to *Lr17*.

Based on the seedling stage analysis, 32 of 42 international wheat materials demonstrated variable resistance to 18 physiological races, and the 42 cultivars were screened with 12 diagnostic markers targeting 10 characterised *Lr* genes. *Lr1*, *Lr10*, *Lr26* and *Lr20* were verified by both the seedling tests and linked markers. Because the adult-plant resistance (APR) genes *Lr37*, *Lr34* and *Lr46* are ineffective in seedlings, they were tracked only with diagnostic markers: *Lr37* appeared in *Scipion* and *Milpaln*, *Lr34* solely in Carahue, and *Lr46* – alone or combined – in 29 entries. Seedling assays plus marker screens failed to detect *Lr9*, *Lr19* or *Lr24* in any cultivar. Ten genotypes gave reaction patterns that did not match any reference line, leaving their resistance genes unresolved.

Identification of adult plant resistance in field. The FDS variance analysis ($P = 0.05$) revealed significant cultivar effects and cultivar × environment interactions (Table 4), confirming that the slow-

Table 5. Infection types (ITs) of mixed *Puccinia triticina* races in the seedling test and final disease severity (FDS) in the adult plant stage on wheat cultivars/germplasms with slow-rusting resistance in field trials in Baoding, Hebei during 2019 to 2020

Line No.[c]	Cultivar	Genes[d]	ITs in seedling[a]	FDS mean (%)	
				2019	2020
4	Professeur Journee	+	3+	5	15
7	Lada	*Lr46*	3	15	5
11	Scipion	*Lr37*, *Lr46*	3+	10	15
13	S15	–	3+	10	10
16	Boxer	*Lr46*	3+	7.5	1
17	Parade	*Lr46*	4	5	1
18	Civic	*Lr46*	3	10	1
22	Carahue	*Lr34*, *Lr46*	3	7.5	10
23	Perquenco	*Lr46*	4	3	5
24	Conco	*Lr46*+	4	5	5
25	Car 735	–	4	3	5
26	Rieti	*Lr46*	4	10	20
32	Heines 476	*Lr46*	4	5	5
38	Wiwatka 4	–	4	5	5
42	N. Strampelli	+	4	5	5
	Saar[b]		4	3	5
	Zhengzhou 5389[b]		4	90	90

[a]According to the 0 to 4 Stakman scale; 3 – moderate size uredinia; 4 – large uredinia; [b]susceptible check: Zhengzhou 5389 and resistant check: Saar for adult-plant resistance; [c]line numbers correspond to those in Table 2; [d]adult-plant resistance genes based on the molecular marker; +indicates unidentified all-stage gene postulated by the seedling test in the greenhouse as in Table 2

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rusting expression depends on both the genotype and its interaction with the environment. The environment, replication and cultivar × replication effects were non-significant. Across the two seasons, the susceptible check Zhengzhou 5389 reached 90% severity, whereas Saar remained at 3–5%, confirming the uniform epidemic pressure. Fifteen entries combined high seedling ITs to the mixed races with low field FDS scores (Table 5), classifying them as slow-rusting types.

DISCUSSION

The primary work to understand wheat rust resistance breeding is to screen resistance sources. Gene derivation, molecular marker detection and APR identification cannot only directly reflect the disease resistance level of the selected materials, but also provide excellent wheat resistance sources for practical applications (Kaur et al. 2023). The results showed that most of the 42 foreign wheat cultivars contained single resistance genes or gene combinations, and 15 varieties with non-race specific resistance were identified, which could be used for the genetic analysis and screening of effective resistance sources.

The seedling identification detected possible carriers of *Lr10*, *Lr1*, *Lr2a* and *Lr14a* in two cultivars Parade and Craftsman. The marker detection amplified the same target band as *Lr10*. This indicates that these two cultivars have resistance to 18 *Pt* physiological races and the severity of the field phenotypes is low. Due to the gradual loss of resistance in *Lr10*, it is speculated that they may contain other resistance genes or may be a common resistance expressed by multiple gene aggregates and further research is needed. Similarly, Wang et al. (2020) found a low detection rate of wheat cultivars carrying the *Lr10* gene when identifying foreign wheat cultivars, suggesting that the gene is not widely used in production. However, when combined with other disease resistance genes, this gene can show different levels of disease resistance and have some value in breeding. In addition, *Lr14a* was derived from the Craftsman cultivars, which were first discovered in the Hope common wheat and are located on chromosome 7BL of wheat (Kolodziej et al. 2021). *Lr14a* was also found to be resistant to the non-toxic race of the leaf rust fungus, like *Lr10*, indicating that the material carries both *Lr10* and *Lr14a*. *Lr14a* and *Lr2a* were also found in the Professeur Journee and Barbu de Crusol cultivars.

Lr26, *Lr1* and *Lr46* were derived from the Civic wheat cultivar, showing that the materials carrying the *Lr1*, *Lr26* and *Lr46* genes all had different levels of resistance to the non-toxic race of *Lr26*. The variety showed high levels of resistance in the field. As *Lr26* gradually lost resistance, it was speculated that the variety existed as a combination of genes. Studies showed that *Lr1*, *Lr26, Lr34*, *Lr37*, *Lr46* and *Lr67* could improve their own disease resistance when combined (McCallum & Hiebert 2022).

Lr34, *Lr37*, *Lr46*, *Lr67* and *Lr68* are very important durable resistance genes (Malysheva et al. 2023). *Lr34* is also a multiple-effect adult plant minor disease resistance gene, which is the most widely used resistance gene so far (Fang et al. 2017). However, the association of *Lr34* with severe flag leaf tip burning has limited its application. In the present work, *Lr34* was detected only in the Carahue variety and showed good resistance in the field. The resistance expression of *Lr1* and *Lr46* was detected by molecular markers and gene polymerisation.

Lr37 was first detected in the wheat variety VPM1, derived from the *T. ventricosa* variety, which is located on chromosome 2AS (Omara et al. 2021), and is the most important adult plant resistance gene in wheat breeding. It is closely related to *Sr38* and *Yr17*, but repels *Lr17* (Derive et al. 2012). In this study, *Lr37* was detected in the Scipion and Milpain wheat lines and *Lr1*. As *Lr1* and *Lr46* gradually lost resistance, it was suggested that *Lr37* could confer resistance in the field. However, *Lr37* has been overcome in Europe and is only effective in limited genetic backgrounds.

Lr46 is derived from the Mexican variety Pavon76 and is located on chromosome 1BL. It is linked to the stripe rust (*Yr29*) and powdery mildew (*Pm39*) resistance genes and has good resistance (Tomkowiak et al. 2020). In this study, *Lr46* was detected in 29 out of 42 foreign wheat varieties, representing 69% of the varieties tested, and 11 of them carried *Lr46* alone. In China, Zheng (2019) detected fewer materials containing *Lr34* and *Lr37* when identifying 70 foreign wheat varieties and the materials containing *Lr46* accounted for more than half of the tested cultivars. The results indicated that *Lr34* and *Lr37* had a low detection rate and were not widely used in production. However, *Lr34* and *Lr37* have some physiological resistance to leaf rust, so they have some value in breeding. The adult plant disease resistance gene *Lr46* has been detected many times in wheat varieties in China and abroad. The long-term cultivation of a variety alone caused the *Lr46* gene to exert

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selection pressure on the physiological races of leaf rust and gradually lost resistance. Studies have shown that *Lr34*, *Lr37* and *Lr46* are very important slow rust resistance genes in breeding. However, when they exist alone, they cannot fully confer durable resistance, but when they are combined with genes that are almost losing resistance, they can improve their own resistance (Wu et al. 2020).

Because these materials are from different parts of the world, the genetic background, gene interaction, leaf rust species and species differences in a limited number of factors, the results are unlikely to be fully consistent (Prasad et al. 2020). In this study, the phenotypes of Gentil, Rimpaus, Eroica, Chitral, Riddar and Lading Skaeghvede were susceptible to leaf rust. The molecular marker detection showed that *Lr46* is an adult plant resistance gene, indicating that the resistance of *Lr46* to mixed races of Chinese leaf rust was gradually weakening. This might be due to a few molecular markers that have been developed for identification. When there are many genes in the tested materials, errors can easily occur. It is speculated that the analysed cultivars may contain some unknown genes, which need further investigation. In this study, 15 foreign wheat materials were identified that showed different levels of resistance in the field, suggesting that they contained many resistance genes, most of which were in the form of polygenic polymerisation, which could be used in wheat breeding to enrich the wheat gene pool.

In this study, 42 wheat cultivars were analysed. It was found that 16 wheat cultivars contained *Lr1*, 3 wheat cultivars contained *Lr14a*, 5 wheat cultivars contained *Lr17*, 5 wheat cultivars contained *Lr2a*, the Parade and Craftsman wheat cultivars contained *Lr10*, the Rieti wheat cultivar contained *Lr20*, the Civic wheat cultivar contained *Lr26*. Furthermore, Carahue contained *Lr34*, Scipion and Milpain contained *Lr37*, 29 wheat cultivars contained *Lr46* and accounted for 69% of the tested cultivars. Fifteen slow rust cultivars were screened, most of which were single gene or gene polymerisation. They can be used for the genetic analysis as the next step and provide a rich source of genetic resistance data for wheat breeding in the future.

CONCLUSION

By analysing 42 wheat varieties for leaf rust resistance genes, the researchers identified genotypes with durable resistance, offering valuable genetic resources for breeding disease-resistant wheat cultivars. The integration of both all-stage resistance (ASR) and adult-plant resistance (APR) genes enables the development of wheat varieties with enhanced and long-lasting protection against leaf rust.

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