Identification of Leaf Rust Resistance Genes in Bread Wheat Cultivars from Ethiopia

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Abstract

Wheat leaf rust, caused by *Puccinia triticina* (Pt), is a widespread disease of bread wheat worldwide. In the present study, 50 wheat cultivars from Ethiopia and 34 differential lines, mostly near-isogenic lines (NILs) in the background of Thatcher with known resistance genes to leaf rust (*Lr*), were tested with 14 Pt races in the greenhouse to postulate *Lr* genes at the seedling stage. Field experiments were also conducted to identify adult plant responses to leaf rust in Baoding in the 2017–2018 and 2018–2019 growing seasons and in Zhoukou in the 2018–2019 growing season. Thirteen *Lr* genes (*Lr1*, *Lr18*, *Lr3ka*, *Lr15*, *Lr26*, *Lr20*, *Lr14a*, *Lr30*, *Lr2a*, *Lr11*, *Lr34*, *Lr46*, and *Lr68*) either singly or in combination were found in 39 cultivars. Known *Lr* genes were not present in the remaining 11 cultivars. *Lr1* and *Lr46*, each in 13 cultivars, and *Lr34* in

12 cultivars were the most commonly identified resistance genes. Less frequently identified genes included *Lr26* (five cultivars); *Lr30* and *Lr18* (each present in four cultivars); *Lr15*, *Lr3ka*, and *Lr2a* (each identified in three cultivars); and *Lr68* (two cultivars). Evidence for the existence of *Lr11*, *Lr20*, and *Lr14a* (each in one cultivar) was also obtained. Twenty-one cultivars were found to have slow rusting resistance to leaf rust in the field tests. The results should be valuable for cultivar selection with combinations of effective *Lr* genes and used in breeding new cultivars with improved resistance to leaf rust in Ethiopia and China.

Keywords: wheat leaf rust, gene postulation, adult-plant resistance, molecular marker

Wheat (*Triticum aestivum* L.) is the second major food crop next to rice produced worldwide. Ethiopia is the most important wheat growing country in sub-Saharan Africa with about 1.7 million hectares and 2.7 t grain yield/ha productivity (CSA 2017). Wheat is ranked fourth next to teff (*Eragrostis tef* [Zucc.] Trotter), maize (*Zea mays* L.), and sorghum (*Sorghum bicolor* [L.] Moench) in terms of production in Ethiopia (CSA 2017). However, wheat production in Ethiopia is disposed to diverse biotic and abiotic stresses, among which rusts are most important.

Leaf rust, caused by the fungus *Puccinia triticina* Erikss. (Pt), is an important disease of wheat in Ethiopia (Badebo et al. 2008; Hei 2017). The potential yield loss for leaf rust-susceptible cultivars can be more than 60% (Chen 2014). Resistant cultivars are considered the most cost-effective and eco-friendly approach to control leaf rust. Genetic resistance to wheat rust pathogens is usually categorized as either race-specific seedling resistance or race-non-specific resistance (Chen 2013). Race-specific resistance is usually controlled by major genes and is effective against some races of the pathogen with corresponding avirulence genes (Flor 1942). On the other hand, race-non-specific resistance is considered as a broad spectrum effective against all races of the pathogen. Race-non-specific resistance is considered as more durable and generally expressed at the adult plant stage governed by a number of minor-effect resistance genes. This

type of resistance is commonly called slow rusting or adult plant resistance (APR) (Caldwell 1968).

Identification of leaf rust resistance (*Lr*) genes existing in the released wheat cultivars helps in appropriate cultivar deployment to manage the disease. Multirace testing and molecular marker genotyping are useful approaches to gene postulation to understand the *Lr* genes present in wheat cultivars.

Up to now, about 79 *Lr* genes have been reported in wheat (McIntosh et al. 2017). Most of the reported *Lr* genes are race-specific conferring hypersensitive reactions and interact with the pathogen in a gene-for-gene manner (Flor 1956). However, *Lr34* (Lagudah et al. 2006), *Lr46* (Singh et al. 1998), *Lr67* (Herrera-Foessel et al. 2011), and *Lr68* (Herrera-Foessel et al. 2012) confer slow rusting resistance despite a susceptible reaction.

The gene-for-gene hypothesis by Flor (1956) has been used by researchers to identify *Lr* genes present in wheat cultivars. For example, Gao et al. (2019) reported nine *Lr* genes (*Lr2a*, *Lr3ka*, *Lr1*, *Lr30*, *Lr26*, *Lr15*, *Lr36*, *Lr37*, and *Lr46*) in 86 wheat cultivars using 14 Pt races. Gebrewahid et al. (2017) postulated 12 *Lr* genes (*Lr3ka*, *Lr11*, *Lr10*, *Lr2b*, *Lr1*, *Lr26*, *Lr13*, *Lr21*, *Lr34*, *Lr37*, *Lr44*, and *Lr46*) from 83 wheat cultivars using 18 Pt races. Fourteen *Lr* genes (*Lr2a*, *Lr3bg*, *Lr3ka*, *Lr14a*, *Lr1*, *Lr16*, *Lr17a*, *Lr23*, *Lr24*, *Lr26*, *Lr18*, *Lr20*, *Lr34*, and *LrZH84*) were identified in 102 wheat cultivars using 24 Pt races (Li et al. 2010). Mebrate et al. (2008) stated that 18 *Lr* genes (*Lr2c*, *Lr3*, *Lr3ka*, *Lr9*, *Lr10*, *Lr14a*, *Lr14b*, *Lr1*, *Lr13*, *Lr20*, *Lr21*, *Lr23*, *Lr27+31*, *Lr16*, *Lr18*, *Lr30*, *Lr37*, and *Lr44*) were identified in 36 wheat cultivars using 31 Pt races. Pathan and Park (2006) reported *Lr3a*, *Lr1*, *Lr17b*, *Lr10*, *Lr3ka*, *Lr13*, *Lr20*, *Lr26*, *Lr37*, and *Lr14a* in 105 cultivars using five Pt races. Maarroof et al. (2005) reported *Lr13*, *Lr16*, *Lr1*, *Lr3*, *Lr10*, *Lr23*, *Lr17*, and *Lr26* in 22 wheat cultivars with 13 Pt races. *Lr3bg*, *Lr14a*, *Lr1*, *Lr3*, *Lr10*, *Lr16*, *Lr13*, *Lr23*, and *Lr26* were reported in 163 wheat cultivars using 14 Pt races (Singh et al. 1999).

Molecular markers linked to some *Lr* genes have been developed to detect specific genes. For instance, *Lr1* (Cloutier et al. 2007), *Lr9* (Schachermayr et al. 1994), *Lr10* (Schachermayr et al. 1997), *Lr19* (Gupta et al. 2005), *Lr20* (Neu et al. 2002), *Lr24* (Schachermayr et al. 1995), *Lr26* (Chai et al. 2006), *Lr37* (Helguera et al. 2003),

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Lr46 (Singh et al. 1998), *Lr67* (Herrera-Foessel et al. 2011), and *Lr68* (Herrera-Foessel et al. 2012) can be identified using closely linked molecular markers. Allele-specific markers for APR genes like *Lr34* (Lagudah et al. 2009) have been developed and used for marker-based detection.

In Ethiopia, information on *Lr* genes in released wheat cultivars is limited, which makes it difficult to exploit resistant cultivars for managing leaf rust. Ethiopia is a hot spot for three wheat rusts: stem, stripe, and leaf rusts. Therefore, identifying *Lr* genes in the presently grown wheat cultivars is enormously useful for managing leaf rust using gene deployment schemes and for breeding new resistant cultivars. Hence, the objective of this study was to identify *Lr* genes in 50 bread wheat cultivars from Ethiopia.

Materials and Methods

Plant materials and *Pt* races. Fifty wheat cultivars from Ethiopia and 34 differential lines, mostly near-isogenic lines (NILs) in the background of Thatcher with known *Lr* genes kindly provided by CIMMYT, were used to test at the seedling stage in the greenhouse to 14 *Pt* races collected from China (Tables 1, 2, and 3). Fresh urediniospores of *Pt* races from the Department of Plant Pathology, Hebei Agricultural University, China, were obtained by multiplying urediniospores on the susceptible cultivar Zhengzhou 5389 in a greenhouse. These *Pt* races were named following the *Puccinia recondita* f. sp. *tritici* code system described by Long and Kolmer (1989), with adding a fourth letter for the reactions to the fourth quartet of differentials (http://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Cereal

Table 1. The pedigree and origin of Ethiopian bread wheat cultivars tested for leaf rust response^a

No.	Cultivar	Origin	Pedigree
1	Abola	CIMMYT/Ethiopia	BOBWHITE/BUCKBUCK
2	Alidoro	Ethiopia/U.S.A.	HK-14-R251
3	Biqa	NA	NA
4	Bobicho	CIMMYT	PEREGRINE/PF-70354/4/KALYANSONA/BLUEBIRD//ALONDRA/3/MARINGA
5	Bolo	CIMMYT	VEE/LIRA//BOW/3/BCN/4/KAUZ
6	Dandaa	CIMMYT	KIRITATI//2*PBW-65/2*SERI-82
7	Dereselgn	CIMMYT	300-SM-501-M/HAR-1709
8	Dashen	CIMMYT	VEE17KVZ/BUHO“S”//KAL-BB
9	Digelu	CIMMYT	SHANGHAI-7/KAUZ,MEX
10	Dinknesh	CIMMYT	CARACARA/4/CORYDON/3/PELOTAS-72380/ARTHUR-71*2/H-567-1
11	Dure	CIMMYT	BOBWHITE/YDING//ZENZONTLI
12	Enkoy	Kenya	HEBRARD//WISCONSIN245/SUPREMO51/3/2*FROCOR/FRONTANA//YAQUI
13	ET-13A2	Ethiopia	UQ105 × ENKOY
14	Galil	Israel	HORK/YAMHILL//KALYANSONA/BLUEBIRD/3/BOBWHITE
15	Gambo	CIMMYT	AVRORA//KALYANSONA/BLUEBIRD/3/(SIB)WOODPECKER
16	Gassy	CIMMYT	PFAU/SERI//BOBWHITE
17	Hawi	CIMMYT/Ethiopia	CHILERO/PARULA
18	Hidasse	CIMMYT/Ethiopia	YANAC/3/PARULA/ICTA-SARA-82//TESIA-79/VEERY-5/4/CROC-1/(224) AE.SQ//OPATA
19	Hobora	NA	NA
20	Hogona	NA	PYN/BAU//MILLAN
21	Huluka	ICARDA/Ethiopia	UTIQUE-96/3/PAYNE/BAGULA//MILAN
22	K6295-4A	Kenya	ROMANY × GB-GAMENYA
23	Kakaba	CIMMYT	KIRITATI//SERI-82/RAYON-89
24	KBG-01	Ethiopia/Germany	300-SM-501-M/HAR-1709
25	Katar	CIMMYT	COOK/VEE//DOVE/SERI/3/BJY/COC
26	Kingbrid	NA	NA
27	Kubsa	CIMMYT	NORD-DESPREZ/VG-9144//KALYANSONA/BLUEBIRD/3/YACO/4/VEERY-5
28	Kulkulu	ICARDA	PYN/BAU//MILAN
29	Lakech	NA	NA
30	Lemu	NA	NA
31	Mandoyu	CIMMYT	WORRAKATTA/PASTOR
32	Mekelle-01	NA	CPAN-1962/TONICHI-81//LIRA/PARULA
33	Mekelle-02	NA	HI-999/HI-601
34	Mekelle-03	NA	NA
35	Mekelle-04	NA	NA
36	Millennium	CIMMYT	ALONDRA/CEP 75630//CEP 75234/PAT 7219/3/BUCKBUCK/BIY/4/
37	Mitike	Ethiopia	BOBWHITE/ROMANY-BC
38	Monqolle	NA	NA
39	Ogalcho	CIMMYT	WORRAKATTA/2*PASTOR
40	Pavon 76	CIMMYT	VICAM-71//CIANO-67/SIETE-CERROS-66/3/KALYANSONA/BLUEBIRD
41	Shorima	ICARDA	UTIQUE-96/3/PAYNE/BAGULA//MILAN
42	Simba	CIMMYT	PARULA/VEERY-6//MYNA/VULTURE
43	Sirbo	CIMMYT	VS73.600/MRL/3/BOW//YR/TRF
44	Suf-Omer	CIMMYT	4777*2/4/FRONTANA/KENYA-58//NEWTATCH/3/(GB)GABO/5/PAVON-76
45	Sula	CIMMYT	MAIORAL(SIB)/BUCKBUCK(HAR-7140)//REIHENBACHII
46	Tay	NA	NA
47	Tossa	CIMMYT	ATTILA
48	Tsehay	NA	VEERY-5/ICTA-SARA-82//DUCULA
49	Tusie	CIMMYT	COOK/VEE“S”//DOVE“S”/SERI
50	Wabe	NA	MRL“S”-BUC“S”

^a NA = not available.

rusts/pt_nomen.pdf). Infection types (ITs) exhibited by the cultivars and NILs at the seedling tests were used for postulating *Lr* genes (Tables 2 and 3).

Seedling testing. Thirty-four NILs, 50 wheat cultivars, and Zhengzhou 5389 were grown in a plastic growth chamber (4 × 3 cm) in the greenhouse and inoculated with 14 *Pt* races (TGPQ, FHJQ, PHTS, KHSS, FGSS, KHSQ, FHNQ, KHJS, FHSQ, FHTQ, FGSD, FGGs, FGSR, and PHJT) (Tables 2 and 3). Inoculation at the seedling stage was done by brushing urediniospores from sporulating Zhengzhou 5389 seedlings onto test seedlings when the first leaves were fully expanded. Inoculated seedlings were then placed in plastic-covered cages and incubated at 18°C and 100% relative humidity (RH) for about 24 h. They were then placed into a growth chamber with 12 h light/12 h darkness at 18 to 20°C with 70% RH. ITs were scored based on the 0 to 4 scale described by Roelfs et al. (1992) 14 days postinoculation. The gene postulation was then done based on the method described by Dubin et al. (1989).

Field testing. All the wheat cultivars, including Saar (resistant control) and Zhengzhou 5389 (susceptible control), were planted in the field at Baoding (Hebei province) in the 2017–2018 and 2018–2019 growing seasons, and at Zhoukou (Henan province) in the 2018–2019 growing season for evaluating leaf rust reaction. Field experiments were managed in randomized complete blocks replicated twice. About 60 to 70 seeds of each cultivar were sown in a row with 1.5-m length and 30 cm between rows. Zhengzhou 5389 was grown perpendicularly and adjacently to the test rows to help spread of spores within the experiments. Races TGPQ, PHTS, and PHJT were selected for field inoculation based on their wider virulence on the wheat varieties in the seedling test than other races.

Equal amounts of urediniospores from TGPQ, PHTS, and PHJT were mixed and suspended in 0.03% Tween 20 and were then inoculated onto spreader rows at the tillering stage. One-month postinoculation, maximum disease severity (MDS) was recorded using the modified percentage scale described by Cobb (1948) when Zhengzhou 5389 had 90% disease severity.

Molecular marker detection. Genomic DNA of all 50 wheat cultivars was extracted using the cetyl trimethylammonium bromide method (Gill et al. 1991). Molecular markers closely linked or allele-specific to 12 known genes (*Lr1*, *Lr9*, *Lr10*, *Lr19*, *Lr20*, *Lr24*, *Lr26*, *Lr34*, *Lr37*, *Lr46*, *Lr67*, and *Lr68*) were used to test all cultivars. Polymerase chain reaction (PCR) was conducted according to the technique described by Helguera et al. (2003). A total of 20 µl of reaction mixture contained 2 µl (4 ng/µl) of template DNA, 2 µl (4 mol/µl) of primer, 6 µl of ddH₂O, and 10 µl of 2× Taq PCR Master Mix (Tiagen Biochemical Incorporation, Beijing, China). All PCR annealing temperatures, allele sizes, and primer sequences are provided in Table 4. The amplified products were separated by 1.5% agarose gel electrophoresis for all of the markers except 12% nondenaturing polyacrylamide gel electrophoresis for the cleaved amplified polymorphic sequence (CAPS) marker *csLV46G22* specific to *Lr46*.

Statistical analysis. Analysis of variance was performed using the generalized linear model procedure (PROC GLMMIX) of Statistical Analysis System (version 9.1; SAS Institute, Cary, NC). Environments (the combination of location and year), cultivars, and their interaction were taken as fixed effects, and replications nested in environments were taken as random. Fisher's least significant difference test at *P* < 0.05 was used to separate the treatment of MDS

Table 2. Infection types^a of 34 tester lines inoculated with 14 *Puccinia triticina* races

No.	Leaf rust resistance (<i>Lr</i>) genes	Infection types to <i>Pt</i> races													
		TGPQ	FHJQ	PHTS	KHSS	FGSS	KHSQ	FHNQ	KHJS	FHSQ	FHTQ	FGSD	FGGS	FGSR	PHJT
1	RL6003 (<i>Lr1</i>)	4	1	4	;1	1	0	1	1	;1	1	;1	;1	;1	3
2	RL6016 (<i>Lr2a</i>)	4	2	2	3+	0	3	2	3	1	1	;1	;	2	1
3	RL6047 (<i>Lr2C</i>)	3+	4	4	4	4	4	4	3+	4	3	3+	3+	3+	3+
4	RL6002 (<i>Lr3</i>)	4	4	4	4	4	4	4	4	4	4	3+	3+	3+	3+
5	RL6010 (<i>Lr9</i>)	0	0	0	;	0	0	0	0	0	0	0	0	0	0
6	RL6005 (<i>Lr16</i>)	3+	4	4	4	4	4	4	4	3+	4	3+	3+	3+	3+
7	RL6064 (<i>Lr24</i>)	0	0	0	0	0	1	0	1	0	0	;1	;	;1	;
8	RL6078 (<i>Lr26</i>)	1	3+	3	3	2	3+	3	3	3	3	2	1	2	3
9	RL6007 (<i>Lr3ka</i>)	3	2	4	3	3	3	3	2	3+	4	3	2	4	2
10	RL605 (<i>Lr11</i>)	2	4	4	4	3+	3	2	4	4	3+	3+	3	3+	3+
11	RL6008 (<i>Lr17</i>)	3	3	3+	3+	3+	3+	3+	3	3	3+	3	2+	3	3+
12	RL6049 (<i>Lr30</i>)	4	12	4	;	1	2	12	1	2	3	1+	1+	1	2
13	RL6051 (<i>LrB</i>)	3+	4	4	4	4	4	4	4	4	3+	3C	3+	3+	3+
14	RL6004 (<i>Lr10</i>)	4	4	4	4	4	4	4	3	3+	4	3C	3	3+	4
15	RL6013 (<i>Lr14a</i>)	1	2	3	3	3+	2	1+	3	2	2	3+	3	2	3
16	RL6009 (<i>Lr18</i>)	1	1	0	2	0	0	1	2	2	2	1	2	3	3
17	RL6019 (<i>Lr2b</i>)	3+	3+	4	4	2	2	3	3	3	2	2	3+	2	2
18	RL6042 (<i>Lr3bg</i>)	3+	4	4	4	3	3	3+	3	4	4	3	3	3+	3+
19	RL4031 (<i>Lr13</i>)	4	4	4	3	3+	3	4	4	4	3+	3	3	3+	3
20	RL6006 (<i>Lr14b</i>)	3	4	4	4	2+	4	4	3	4	4	3+	3+	3+	4
21	RL6052 (<i>Lr15</i>)	4	0	0;	1	0	1	0	0	0;	0	;	0	0	3+
22	RL6040 (<i>Lr19</i>)	;	0;	;	0;	0;	;	;	0	0;	0	;	;	0	0
23	RL6092 (<i>Lr20</i>)	3	3+	4	3+	;	3	4	4	;	2	;	;	1	;
24	RL6043 (<i>Lr21</i>)	3	4	2	3	2+	3	2	2	3	3	1+	2	2	3
25	RL6012 (<i>Lr23</i>)	3+	4	4	4	3+	4	4	3	3+	4	3	3+	3+	3+
26	RL6079 (<i>Lr28</i>)	;	0	;	0	0;	;	;	1	;	0	0	;	0	0
27	RL6080 (<i>Lr29</i>)	1	0;	2	12	1	1	1	2	1	2	;	2	2	1
28	RL6057 (<i>Lr33</i>)	3	4	4	4	4	4	3	3	2	3	3C	3	3+	3
29	E84018 (<i>Lr36</i>)	3	4	4	3+	3	3	2+	2	2	2	1+	1+	2	1
30	KS86NGRC02 (<i>Lr39</i>)	4	4	4	3+	4	4	4	3	3+	3	3	3+	3	3
31	KS91WGR11 (<i>Lr42</i>)	0;	12	1	;1	0	1	;	1	1	1	;	1+	1	2
32	RL6144 (<i>Lr45</i>)	4	4	4	3+	4	4	2	4	4	3+	3	1	3	3+
33	PAVON76 (<i>Lr47</i>)	0;	0	0	0	0	0	0	0	0	;	0	0	0	0
34	C78.5 (<i>Lr51</i>)	0;	0,1	;	1	;	1	1	;1	0;	;	;	;	;1	;

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

data. Cultivars that showed high ITs to mixed *Pt* races (TGPO, PHTS, and PHJT) at the seedling stage and had low MDS at the adult-plant stage (not significantly high MDS compared with the resistant control, Saar, in the field) were considered to have slow rusting resistance (Table 5).

Results

Lr genes based on gene postulation and molecular marker detection. A total of 34 differential lines, 50 wheat cultivars, and susceptible control (Zhengzhou 5389) were inoculated with 14

Pt races at seedling stage in the greenhouse (Tables 2 and 3). The differential lines for 11 *Lr* genes (*Lr2c*, *Lr3*, *Lr16*, *Lr17*, *LrB*, *Lr10*, *Lr3bg*, *Lr13*, *Lr14b*, *Lr23*, and *Lr39*) had high ITs to all *Pt* races. Low ITs to all *Pt* races were recorded for eight *Lr* genes (*Lr9*, *Lr24*, *Lr19*, *Lr28*, *Lr29*, *Lr42*, *Lr47*, and *Lr51*), which made it impossible to postulate these genes. However, 15 differential lines with *Lr1*, *Lr2a*, *Lr26*, *Lr11*, *Lr3ka*, *Lr30*, *Lr14a*, *Lr18*, *Lr2b*, *Lr15*, *Lr20*, *Lr21*, *Lr33*, *Lr36*, or *Lr45* had different ITs when inoculated with different *Pt* races and, therefore, could be postulated for presence or absence in other wheat cultivars. The results of the gene

Table 3. Seedling infection types^a and presence or absence of leaf rust resistance (*Lr*) genes in 50 Ethiopian wheat cultivars based on gene postulation using 14 *Puccinia triticina* races and molecular markers

Line no. ^b	<i>Lr</i> gene	Infection types to <i>Pt</i> races													
		TGPQ	FHJQ	PHTS	KHSS	FGSS	KHSQ	FHNQ	KHJS	FHSQ	FHTQ	FGSD	FGGS	FGSR	PHJT
1	<i>Lr30</i> ^c	4	2	3+	2	2	1	2	2	1	3+	1	1	2	2
2	<i>Lr18</i> ^c	1	1	1	1	;	1	1	1	;	1	0	2	3	3
3	...	2	;	;	0	2	1	0	;	;	1	2	3	1	0
4	...	4	4	4	4	4	4	4	4	4	4	3	4	3	4
5	<i>Lr3ka</i> ^c	3	2	4	3	3	4	3	1	4	3	3	1	4	2
6	<i>Lr15</i> , ^c <i>Lr46</i> ^d	3	1	0	0	1	0	2	1	1	1	1	2	2	4
7	<i>Lr26</i> , ^{c,d} <i>Lr46</i>	1	3+	4	4	0	4	3	4	4	3	2	1	0	2
8	...	2	2	4	2	2+	3	1	1	1	2	3	2	3	1
9	<i>Lr26</i>	2	3	4	3	2	4	3	4	3	3	1	1	1	3
10	<i>Lr1</i> ^{c,d}	3	1	4	1	0;	1	1	0;	1	1	1	1	2	3
11	<i>Lr30</i> , <i>Lr46</i>	3	1	4	2	2	;	1	;	1	3	2	2	1	1
12	<i>Lr1</i> , <i>Lr34</i> ^d	3	12	4	;	12	12	1	1	;	1	2	1	1	4
13	<i>Lr1</i>	3	;	4	;	1	;	;	0;	1	1	0	1	1	3+
14	...	;	1	4	2	;	1	0;	;	0	;	3	2	2	1
15	...	1	1	0;	1	;	;	;	;	0	1	3	1	1	2
16	<i>Lr2a</i> ^c	3	12	1	3	0	3+	1	3	1	2	0	2	1	2
17	<i>Lr34</i> , <i>Lr68</i> ^d	;	2	4	2	1	3	1	2+	1	1	2	3	1	2
18	<i>Lr3ka</i> , <i>Lr34</i>	3+	2	4	3	3+	4	3	2	4	3	3	1	4	1
19	<i>Lr1</i>	3	0;	3+	;	0;	;	0;	0;	;	;	2	1	1	4
20	...	0;	0	0;	0;	0;	0;	0;	0;	;	0	2+	1	2	1
21	<i>Lr18</i>	;	;	;	;	1	0	0;	0;	;	0	0	1	4	3
22	<i>Lr1</i> , <i>Lr34</i> , <i>Lr46</i>	3	1	4	;	1	1	1	0;	1	1	0	2	1	3
23	<i>Lr30</i> , <i>Lr34</i> , <i>Lr46</i>	3	2	4	;	2	1	1	1	2	3+	2	1	1	1
24	<i>Lr18</i>	;	1	2	;	1	1	2	1	1	1	2	1	3	3+
25	<i>Lr26</i> , <i>Lr34</i>	1	3	4	4	2	4	3	3+	3	4	1	1	1	2
26	<i>Lr15</i> , <i>Lr34</i>	3	0;	12	0;	1	1	0;	;	0	1	0	2	0	3+
27	<i>Lr1</i> , <i>Lr34</i>	4	;	4	;	;	2	1	;	;	1	0	0	2	4
28	<i>Lr1</i> , <i>Lr46</i>	4	0;	3	;	;	;	1	0	1	1	1	0	2	4
29	<i>Lr2a</i>	3+	2	1	3+	12	3	2	3+	1	2	1	1	2	2
30	...	0;	0;	;	;	;	;	;	0;	;	;	1	2	1	1
31	<i>Lr18</i>	;	;	0	0;	;	;	0	0;	1	0	1	0	4	3+
32	<i>Lr30</i> , <i>Lr34</i> , <i>Lr46</i> , <i>Lr68</i>	3	1	4	1	2	1	2	1	0	4	1	2	1	1
33	<i>Lr26</i>	2	4	4	3+	;	3	3	4	3	3	1	1	0	2
34	<i>Lr26</i>	1	3	4	3	2	3+	3	3	4	3	2	0	2	1
35	<i>Lr2a</i> , <i>Lr34</i>	3+	1	2	3+	1	3	2	4	;	1	2	1	2	2
36	...	0	0	;	0	0;	0;	0;	0	0	0	2	0	2+	0
37	<i>Lr1</i> , <i>Lr46</i>	3	;	3	;	;	;	;	;	;	1	2	2	0	3
38	...	1	12	;	0	1	1	1	;	1	1	4	1	1	3
39	<i>Lr1</i> , <i>Lr46</i>	3	;	4	;	1	1	;	;	;	0;	1	1	2	3
40	<i>Lr1</i> , <i>Lr46</i>	4	;	4	0;	1	0	1	0	;	0	0	1	2	3
41	...	0;	;	;	;	1	;	;	;	;	;	1	2	2	1
42	<i>Lr20</i> , <i>Lr46</i>	4	3	4	3+	1	3	4	3	0	1	1	2	1	1
43	<i>Lr3ka</i>	3+	12	4	3	3+	4	4	2	3	4	3	1	3	2
44	<i>Lr15</i> , <i>Lr34</i>	3	2	1	2	12	1	1	1	1	1	0	2	1	4
45	<i>Lr1</i>	3	;	4	;	01	1	1	;	1	0	0	2	2	3
46	<i>Lr14a</i> ^c	2	1	4	3	3	1	2	4	1	1	3	3	1	3
47	<i>Lr1</i> , <i>Lr46</i>	4	0;	4	;	1	0	0;	1	1	0	;	2	1	3
48	<i>Lr1</i>	3	0;	4	0	1	;	1	1	;	1	1	;	0	3
49	<i>Lr1</i> ^c , <i>Lr34</i> , <i>Lr46</i>	2	3	4	3	3	4	1	3	3	3	3	3	4	3
50	...	0;	;	0;	;	;	;	;	0;	;	0	0	1	1	2

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

^b Line numbers correspond to those in Table 1.

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

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

postulation and molecular detection showed that 13 *Lr* genes (*Lr1*, *Lr2a*, *Lr3ka*, *Lr11*, *Lr14a*, *Lr15*, *Lr18*, *Lr20*, *Lr26*, *Lr30*, *Lr34*, *Lr46*, and *Lr68*), either singly or in combination, were identified in 39 cultivars. Ten cultivars (Biqu, Dashen, Galil, Gambo, Hogona, Lemu, Millennium, Monqolle, Shorima, and Wabe) contained unknown resistance genes because they displayed low ITs to most of the *Pt* races, whereas only Bobicho showed high ITs to all the tested *Pt* races.

Lr1 was present in 13 cultivars. Eight cultivars (Enkoy, Hobora, Pavon-76, ET13A2, Sula, Dinknesh, Tsehay, and Kubsa) contained only *Lr1* because they were resistant to 11 *Lr1* avirulent races and susceptible to three *Pt* races (Tables 3 and 4). Four cultivars (Ogalcho, Kulkulu, Mitike, and Tossa) contained *Lr1* combined with *Lr46*. K6295-4A contained *Lr1*, *Lr34*, and *Lr46* in combination. All cultivars that carried *Lr1* based on gene postulation were confirmed by molecular marker detection.

Five cultivars were found to contain *Lr26* (Tables 2 and 3). Digelu, Mekelle-02, and Mekelle-03 contained only *Lr26*. Katar and Dere-selgn possessed *Lr26* in combination with *Lr34* and *Lr46*, respectively. The cultivars postulated to have *Lr26* were also confirmed by the specific molecular marker for *Lr26*. Four cultivars (Abola, Dure, Kakaba, and Mekelle-01) were postulated to have *Lr30*. Only *Lr30* was identified in Abola, because it was resistant to 11 *Pt* races but susceptible to the remaining three *Pt* races (TGPQ, PHTS, and FHTQ). The other three cultivars were found to have *Lr30* and *Lr46* in combination.

Mekelle-04, Gassy, and Lakech contained *Lr2a* because they were found with low reaction to 10 *Lr2a* avirulent *Pt* races (FHJQ, PHTS, FGSS, FHNQ, FHSQ, FHTQ, FGSD, FGGS, FGSR, and PHJT) (Tables 2 and 3). Only *Lr3ka* was postulated in Sirbo and Bolo, because they had low reactions to four *Lr3ka* avirulent *Pt* races (FHJQ,

KHJS, FGGS, and PHJT). *Lr3ka* in combination with *Lr34* was detected in Hidasse. Kingbird and Suf-Omer contained *Lr15* and *Lr34* in combination. *Lr15* and *Lr46* were detected in Dandaa. These cultivars had low reaction to 12 *Lr15* avirulent races. *Lr18*, ineffective to 12 *Pt* races but effective against only two *Pt* races (FGSR and PHJT), was postulated in Alidoro, Mandoyu, KBG-01, and Huluka. *Lr20* in combination with *Lr46* was detected only in Simba, because it was found with low ITs to seven *Lr20* avirulent *Pt* races (FGSS, FHSQ, FHTQ, FGSD, FGGS, FGSR, and PHJT). Simba was also confirmed to contain *Lr20* by the specific molecular marker for *Lr20*. Tusie was found to contain *Lr11*, *Lr34*, and *Lr46* in combination, because it had low ITs to two *Lr11* avirulent *Pt* races (TGPQ and FHNQ). Only *Lr14a* was postulated in Tay, because it was resistant to seven *Lr14a* avirulent races (TGPQ, FHJQ, KHSQ, FHNQ, FHSQ, FHTQ, and FGSR) (Tables 3 and 4).

Wheat cultivars that carried *Lr1*, *Lr20*, and *Lr26* were confirmed by molecular marker detection and gene postulation. All cultivars lacked *Lr9*, *Lr19*, and *Lr24* based on the molecular marker genotyping and seedling reaction. Postulation of *Lr34*, *Lr37*, *Lr46*, *Lr67*, and *Lr68* was not possible in the seedling tests because all are APR genes. Therefore, the closely linked molecular markers with these APR genes were used to test all the cultivars. The results indicated that *Lr46* and *Lr34* were detected in 13 and 12 cultivars, respectively. Two cultivars, Hawi and Mekelle-01, were found with *Lr68* and other known genes in combination. None of the cultivars contained *Lr37* and *Lr67*.

Overall, the combined results of molecular marker genotyping and gene postulation showed that 13 *Lr* genes (*Lr1*, *Lr2a*, *Lr3ka*, *Lr11*, *Lr14a*, *Lr15*, *Lr18*, *Lr20*, *Lr26*, *Lr30*, *Lr34*, *Lr46*, and *Lr68*) either singly or in combination were identified in 39 cultivars, whereas none of the known *Lr* genes could be found in the remaining 11 cultivars.

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

<i>Lr</i> gene	Marker type ^a	Primer	Sequence of primer (5'→3')	Annealing temp. (°C)	Size (bp)	Chr.	Reference
<i>Lr1</i>	STS	WR003F WR003R	GGGACAGAGACCTTGGTGGG GAC GATGATGATTGCTGCTGG	65	760	5DL	Cloutier et al. (2007)
<i>Lr9</i>	STS	J13/1 J13/2	TCCTTTTATCCGCACGCCGG CCACACTACCCAAAGAGAG	66	1,100	6BL	Schachermayr et al. (1994)
<i>Lr10</i>	STS	Lrk10D1 Lrk10D2	GAAGCCCTTCGTCTCATCTG TTGATTTCATTGCAGATGAGATCACG	58	282	1AS	Schachermayr et al. (1997)
<i>Lr19</i>	SCAR	SCS265 F SCS265 R	GGCGGATAAGCAGAGCAGAG GGCGGATAAGTGGGTATGG	65	512	7DL	Gupta et al. (2005)
<i>Lr19</i>	SCAR	SCS253 F SCS253 R	GCTGGTTCACAAAGCAAA GGCTGGTTCCTTAGATAGGTG	60	736	7DL	Gupta et al. (2005)
<i>Lr20</i>	STS	STS638-L STS638-R	ACAGCGATGAAGCAATGAAA GTCCAGTTGGTTGATGGAAT	60	542	7AL	Neu et al. (2002)
<i>Lr24</i>	STS	Lr24 J 9/1 Lr24 J 9/2	TCTAGTCTGTACATGGGGGC TGGCACATGAACCTCATACG	57	310	6BL	Schachermayr et al. (1995)
<i>Lr26</i>	STS	Glu-B3F Glu-B3R	GGTACCAACAACAACAACCC GTTGCTGCTGAGGTTGGTTC	65	210	1BL	Froidmont (1998)
<i>Lr26</i>	STS	ω-secalin F ω-secalin R	ACC TTCCTCATCTTTGTCCT CCGATGCCTATACCACTACT	65	1,076	1BL	Chai et al. (2006)
<i>Lr34</i>	CAPS	cssfr6-f cssfr6-r	CTGAGGCACTCTTCTCTGTACAAAG GCATTCAATGAGCAATGGTTATC	67.5	135 and 451	7DS	Lagudah et al. (2009)
<i>Lr37</i>	STS	VENTRIUP LN2	AGGGGCTACTGACCAAGGCT TGCAGCTACAGCAGTATGTACACAAAA	65	259	2AS	Bariana and McIntosh (1993)
<i>Lr46</i>	CAPS	csLV46G22F csLV46G22R	AGGGAAAAGACATCTTTTTC CGACCGACTTCGGGTTTC	58	335	1BL	Lagudah, E. S. (pers. comm.)
<i>Lr46</i>	SSR	Xwmc44	TCTTCTGGGCTTTGATCCTG TGTTGCTAGGGACCGTAGTGG	61	242	1BL	Lillemo et al. (2008)
<i>Lr67</i>	SSR	Xcfd23-4D	TAGCAGTAGCAGCAGCAGGA GCAAGGAAGAGTGTTCAGCC	60	187	4DL	Herrera-Foessel et al. (2011)
<i>Lr67</i>	SSR	CFD71-F CFD71-R	CAATAAGTAGGCCGGGACAA TGTGCCAGTTGAGTTTGCTC	60	216	4DL	Herrera-Foessel et al. (2011)
<i>Lr68</i>	STS	csGS-F1 csGS-R1	AAGATTGTTACAGATCCATGTCA GAGTATTCGGGCTCAAAAAGG	60	385	7BL	Herrera-Foessel et al. (2012)

^a STS = sequence-tagged site; SCAR = sequence characterized amplified region; CAPS = cleaved amplified polymorphic sequence; and SSR = simple sequence repeat.

APR to leaf rust. The analysis of variance results for MDS of the tested cultivars indicated that there were significant differences among cultivars, environments, and cultivar-environment interactions in the field ($P < 0.05$) (Table 5). This revealed that cultivar and cultivar-environment interactions influenced the APR for leaf rust. However, no significant differences were observed between replications and cultivar-replication interactions, as expected. The MDS values of Zhengzhou 5389 ranged from 80 to 95%, and that of Saar was 5%, at all environments, demonstrating adequate levels of leaf rust disease (Table 6). MDS values of all the cultivars including the resistant control, Saar, and susceptible control, Zhengzhou 5389, are presented in Supplementary Table S1. Twenty-one cultivars with high ITs to the mixed *Pt* races at the seedling stage but showing low MDS values in the field across all the environments were considered to have slow rusting resistance.

Discussion

Postulation of seedling-effective *Lr* genes performed based on the gene-for-gene hypothesis (Flor 1956) enables quick identification of genes present in wheat cultivars with diverse genetic backgrounds.

Lr1 was first described in Malakoff (Mains et al. 1926) and reported as *Lr1* in 1946 (Ausemus et al. 1946). Cloutier et al. (2007) mapped *Lr1* on chromosome 5DL. Mebrate et al. (2008) reported four cultivars with *Lr1* among a collection of 23 wheat cultivars from Ethiopia tested with 31 *Pt* races. In the present study, *Lr1* was the most commonly identified seedling *Lr* gene among the tested 50 cultivars (Table 3). In the same way reported by Singh and Rajaran (1991), Pavon-76 was found to contain *Lr1*. According to its pedigree analysis, *Lr1* in Pavon-76 and Kubsa might be derived from Vicam-71 (Singh 1993) and Bluebird (McIntosh et al. 1995), respectively. *Lr26*, originated from rye (*Secale cereal* L.), exists in wheat in the 1BL/1RS translocation. According to Rajaram et al. (1983), the 1BL/1RS translocation region also carried *Sr31*, *Yr9*, and *Pm8* genes for resistance to stem rust, stripe rust, and powdery mildew, respectively, and served as a basis of resistance genes used in wheat breeding. In this study, *Lr26* was identified in five cultivars (Table 3). *Lr26* in Digelu might be originated from Kauz or Mex according to the pedigree analysis (Singh and Rajaran 1991). *Lr30*, as a seedling recessive resistance gene originated from Terenzio, was mapped on chromosome 4AL (Dyck and Kerber 1981). *Lr30* was identified in four cultivars (Table 3). Similar to the present study, Mebrate et al. (2008) also reported *Lr30* in Abola. McIntosh and Baker (1968) cytologically mapped *Lr15* closely linked with *Sr6* on chromosome 2DS. *Lr15* is widely effective when combined with an APR gene like *Lr34* (Dholakia et al. 2013). In the present study, three cultivars contained *Lr15* in combination with other APR genes (Table 3). Based on the pedigree analysis, *Lr15* in Suf-Omer might be from Frontana (Bansal 1988).

Dyck (1977, 1987) characterized *Lr34* from the very beginning in Canada; however, *Lr34* had been used in the expansion of new wheat cultivars from the early 20th century. *Lr34* was the first cloned slow rusting resistance gene that has remained durable for more than 50 years (Krattinger et al. 2009; Lagudah et al. 2009). Cloning of *Lr34* enabled a gene-specific DNA marker to be developed (Dakouri et al. 2010; Lagudah et al. 2009), which provides a powerful tool for marker assisted selection (MAS) in wheat breeding. In this study, 12 cultivars were detected with *Lr34* (Table 3). The presence of *Lr34* in Hawi, Kakaba, and Kubsa might be derived from Parula (McIntosh et al. 2012), Rayon-89 (Singh 1993), and Bluebird (McIntosh et al. 1995), respectively, based on the pedigree analysis. *Lr34* in Enkoy and Suf-Omer might be derived from Frontana (McCallum et al. 2012), and that of Katar and Tusie might be from Cook (McIntosh et al. 1995).

According to William et al. (2003), *Lr46* has been found widely in wheat genotypes originating from CIMMYT. *Lr46* was originally identified from Pavon-76 on chromosome 1BL (Singh et al. 1998). *Xwmc44* marker, linked to *Lr46* (Lillemo et al. 2008), is being used in MAS. Although *Lr46* has a similar phenotype to *Lr34*, resistance to multiple pathogens (Lillemo et al. 2008; William et al. 2003), the

resistance conferred by *Lr46* is not as obvious as *Lr34* (Kolmer et al. 2018a; Lagudah 2011). In the present study, *Lr46* was found in 13 cultivars. According to the pedigree analysis, *Lr46* in Hawi, Simba, and Tossa might be derived from Rayon-89 (Kolmer et al. 2008), Parula (McIntosh et al. 2012), and Atila (McIntosh et al. 2008), respectively.

Lr68 is a slow rusting resistance gene mapped on chromosome 7BL (Herrera-Foessel et al. 2012). This gene originated from Frontana, which also possessed *Lr34* and *Lr46*. The dominant marker *csGS* was afterward developed for *Lr68* (Herrera-Foessel et al. 2012). *Lr68* in Hawi might be derived from Parula (McIntosh et al. 2012).

Table 5. Analysis of variance of maximum disease severity in 50 wheat genotypes, including slow rusting cultivar Saar and susceptible Zhengzhou 5389 checks, tested in the 2017–2018 and 2018–2019 growing seasons^a

Source of variation	df	Mean square	F value	P
Cultivar	51	1,310.39	67.03*	<0.0001
Environment	2	1,072.69	54.87*	<0.0001
Replication	1	3,459.73	176.97	1.9624
Cultivar × environment	102	125.49	6.42*	<0.0001
Cultivar × replication	51	72.27	3.70	2.8647
Error	104	19.55		

^a * = significant at 0.05 level of probability.

Table 6. Seedling infection types (ITs)^a with mixed *Puccinia triticina* (*Pt*) races in the seedling test and maximum disease severity (MDS, %) in field experiments in Baoding and Zhoukou in the 2017–2018 and 2018–2019 growing seasons for Ethiopian wheat cultivars with slow rusting resistance to leaf rust

Line no. ^b	APR ^c genes	Seedling ITs to mixed <i>Pt</i> races	2017–2018 Baoding MDS (%)	2018–2019 Baoding MDS (%)	2018–2019 Zhoukou MDS (%)
1	...	4	5	5	5
2	...	4	0	5	0
6	<i>Lr46</i>	3	1	5	0
8	...	4	5	5	5
11	<i>Lr46</i>	3	0	10	5
14	...	3	1	5	5
16	...	4	1	5	1
17	<i>Lr34</i>	3+	5	5	1
21	...	4	5	15	0
25	<i>Lr34</i>	3-	0	5	0
27	<i>Lr34</i>	3+	1	5	1
28	<i>Lr46</i>	3	1	5	0
35	<i>Lr34</i>	3	0	5	1
37	<i>Lr46</i>	4	0	5	0
38	...	3+	1	5	0
39	<i>Lr46</i>	3	5	1	5
40	<i>Lr46</i>	4	10	1	5
42	<i>Lr46</i>	4	1	5	5
47	<i>Lr46</i>	4	1	5	5
48	...	4	1	10	0
49	<i>Lr34</i> , <i>Lr46</i>	4	0	1	0
Saar ^d		3	5	5	5
Zhengzhou 5389 ^e		4	80	95	90
LSD ^f				5.06	

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

^b Line numbers correspond to those in Table 1.

^c Adult plant resistance genes based on molecular marker.

^d Slow rusting check.

^e Susceptible check.

^f LSD = least significant difference.

The seedling *Lr* genes in 11 cultivars out of the tested 50 cultivars could not be identified. It might be better to use different *Pt* races with various pathogenicity or additional differential lines with other resistance genes to postulate the genes in these cultivars.

Even though the seedling *Lr* genes were postulated based on the gene-for-gene approach, there was an observable limitation to this strategy. It was not able to identify all the *Lr* genes in the tested cultivars due to restrictions of virulence in *Pt* races. It is possible that five cultivars (Hogona, Lemu, Millennium, Shorima, and Wabe) might harbor unknown major resistance genes to leaf rust, because they were detected with low ITs to all the tested *Pt* races at the seedling stage in the greenhouse and showed resistant response to the mixed *Pt* races used in the field nurseries at the adult-plant stage. Genotyping the cultivars using molecular markers linked to the *Lr* genes was more effective than gene postulation. Identification of *Lr1*, *Lr20*, *Lr26*, *Lr34*, *Lr46*, and *Lr68* using molecular markers was reliable. Hence, the molecular markers linked with these *Lr* genes might be very useful in MAS for developing wheat cultivars for resistance to leaf rust.

Singh et al. (2000) stated that APR has proven to be durable, having been widely deployed in CIMMYT spring wheat cultivars. Twenty-five (50%) of the tested cultivars originated from CIMMYT genotypes, and APR genes were widely deployed in them (Tables 1 and 3). *Lr34*, *Lr46*, and *Lr68* either singly or in combination with other genes were detected in 21 cultivars. The remaining 10 cultivars might have unknown APR genes. The mixed *Pt* races were virulent to most of these cultivars at the seedling stage, and the APR in these cultivars might be obtained from *Lr34*, *Lr46*, *Lr68*, and other unknown APR genes.

In summary, the gene postulation and molecular marker detection results revealed 13 *Lr* genes (*Lr1*, *Lr2a*, *Lr3ka*, *Lr11*, *Lr14a*, *Lr15*, *Lr18*, *Lr20*, *Lr26*, *Lr30*, *Lr34*, *Lr46*, and *Lr68*) either singly or in combination in 39 cultivars. Ten cultivars might contain undetectable *Lr* genes, whereas only one cultivar, Bobicho, may contain no effective *Lr* gene. Additional *Pt* races with various pathogenicity or using more differential lines with other resistance genes might be better to postulate the genes in these cultivars. Twenty-one cultivars were found with slow rusting resistance. *Lr34*, *Lr46*, and *Lr68*, either singly or in combination with other genes, were detected in 21 cultivars. The results should be valuable for selecting cultivars to grow and used in breeding new cultivars with improved resistance to leaf rust in Ethiopia and China.

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