Mapping Lr18: A leaf rust resistance gene widely deployed in soft red winter wheat.

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Abstract

Leaf rust caused by *Puccinia triticina* is a destructive pathogen of wheat (*Triticum aestivum*) in a majority of the world's wheat producing areas. Host resistance is the most economical solution for providing full season control and reducing damage due to leaf rust as opposed to use of multiple fungicide applications. The soft red winter wheat cultivar Jamestown is productive in the deep south, southern Corn Belt, and mid-atlantic regions, and this can be attributed in part to its resistance to multiple diseases including leaf rust. Jamestown is postulated to contain gene Lr18. Seedlings of 186 F5:9 recombinant inbred lines (RILs) from a cross of Pioneer '25R47'/Jamestown and 200 F2 seedlings from eight other crosses including Jamestown and/or the Lr18 host differential line RL6009 (Thatcher*6/Africa 43) were screened with *P. triticina* race TNRJJ. Genetic analysis of the populations was conducted to validate the presence of Lr18 in Jamestown. Linkage analysis conducted with SNP markers in the Pioneer 25R47/Jamestown population identified markers that were closely linked with Lr18, and these were validated in Jamestown/VA10W-21 and RL6009/VA10W21 F2 populations. Results of linkage analysis identified SNP maker IWB41960 linked within 5 cM of gene Lr18 in all three populations.

Keywords: Wheat, Lr18, Molecular mapping, *Puccinia triticina*.

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Introduction

Leaf rust (*Puccinia triticina* Eriks) is the most common type of rust of wheat (*Triticum aestivum* L.) and it occurs in most wheat growing areas worldwide [1]. It occurs in a wide range of climates wherever wheat is grown and causes yield losses up to 65% under favourable conditions [2]. Resistance to leaf rust can be classified into two types, qualitative resistance conferred by single resistance genes and quantitative resistance, facilitated by multiple genes or quantitative trait loci (QTL) [3]. There are 72 leaf rust resistance genes that have been permanently catalogued, and numerous temporarily designated leaf rust resistance genes [4].

Most leaf rust (Lr) resistance genes operate on a gene for gene basis [1] and their effectiveness is race-specific. Genetic resistance to leaf rust can be exploited best when knowledge of the specific resistance genes present in commonly used parental germplasm and cultivars is available. Identification of diverse leaf rust resistance genes and effective combinations allows for effective integration and pyramiding of different and complementary genes into breeding populations, and thus aids in the release and deployment of cultivars that are not genetically uniform [5]. High levels of variation and mutation within *P. triticina* populations highlights the need for identification, incorporation, and pyramiding of novel and complementary resistance genes [1].

The leaf rust resistance gene Lr18, derived from *Triticum timopheevii* Zhuk, is known to be located on the long arm of the 5B chromosome [6]. It is the only documented leaf rust resistance gene derived from *Triticum timopheevii*. Seedling resistance governed by Lr18 is most effective between 15°C and 18°C, and as temperatures increase the gene becomes less effective, and at 25°C it becomes ineffective [6]. While the initial source of Lr18 in North American wheat is unknown [7], it has been prevalent and maintained in SRW wheat varieties for more than half a century. Leaf rust genes postulated from seedling tests using differential races of *P. triticina* at the Cereal Disease Lab were first reported for SRW wheat lines in the 1987-88 USDA-ARS Uniform Southern and Eastern SRW Wheat Nurseries. In the 1999-2016 nurseries (https://www.ars.usda.gov/pacific-west-area/aberdeen-id/small-grains-and-potato-germplasm-research/docs/uniform-nurseries/), 44 lines in the Uniform Southern and 34 lines in the Uniform Eastern nurseries were postulated to carry Lr18. One or more lines in all but five of the 18 years were postulated to carry Lr18.

The SRW wheat cultivars Bledsoe (CItr 13238) and GA1123 (CItr 13292) developed at the University of Georgia in 1956

and 1961, both have *Triticum timopheevii* in their pedigrees. Cultivar Holley (CItr 14579) having both of the former cultivars in its pedigree has been postulated to carry Lr18 based on the presence of this gene in subsequent lines derived from the cross 'Massey' (CItr 17953)/Holley. Massey is very susceptible to leaf rust and does not carry gene Lr18. Subsequent cultivars postulated to carry gene Lr18 on the basis of ancestry and/or via seedling reaction to differential races of *P. triticina* conducted at the USDA-ARS Cereal Disease Lab are listed in Table 1. In the current study, gene Lr18 was mapped in the SRW wheat cultivar Jamestown, which was postulated to contain Lr18 [8].

Table 1. *Soft red winter wheat cultivars postulated to carry gene Lr18 and prospective parental donor ancestors.*

Release	Cultivar	Pedigree	TNRJJ	Lr18[a]
1961	GA1123 (CItr 13292)	Trumbull/Red Wonder//Steinweidel/*T. timopheevii*/3/W38/Illinois No. 1/ Hope// Purplestraw/Thatcher// Leap/4/ Chancellor	12;	Present
1960	Redcoat (CItr 13170)	Surpreza/Fultz sel. CI 11845/7/Kawvale/5/Fultz/Hungarian //W38 /3/ Wabash/4/ Fairfield/6/Trumbull*3//Hope/Hussar	3	Absent
1970	Holley (CItr14579)	Georgia 1123*2//Knox 62/Suwon 92/3/Redcoat/Bledsoe	12;	Present
1970	McNair 2203 (CItr15228)	Redcoat sib//Norin 10/Brevor/6/Seneca/5/Asosan /3/ Supreza/Redhart // Chancellor/4/Transfer	3	Absent
1975	McNair 1813 (CItr15289)	Seneca/6/Redcoat sib/5/Redcoat sib/Kenya 338/3/(Purple Straw/Trumbull / Steintin/Thorn)//Kenya 338/4/Norin 10/ Brevor	3	Absent
1977	McNair 1003 (PI552975)	McNair 2203/Blueboy	3	Absent
1980	Stacy (CItr17861)	Purdue 4946/McNair 1813	3	Absent
1980	Coker 797 (CItr17722)	Coker 68-15/5/Coker 57-6//Hadden/4/Nadadores 63/3/Hadden/Purdue 4946A4-18-2-10-1//Coker 57-6*2/Purdue 4946A4-18-2-10-1	23	Absent
1980	Coker 916 (PI600974)	Purdue 6028A2-5-9/3/Coker 61-19*3/Purdue 4946A4-18-2-10-1//Blueboy	3	Absent
1983	Coker 983 (PI601076)	Coker 68-15/4/Potomac/3/Coker 61-19*3/Purdue 4946A4-18-2-10-1 // Blueboy	12;	Present
1990	GA-Gore (PI561842)	Coker 797 / Stacy	12;	Present
1990	Coker 9803 (PI548845)	McNair 1003/Coker 916	2;/3	Present
1993	2684 (PI566923)	Pioneer Line W9057B/Caldwell//Hunter	12;	Present
1994	2643 (PI583739)	Pioneer line W9032B/Pion. line W1074B//Pion. line W1039B/Coker 983	12;	Present
1999	26R24 (PI614110)	WBA084D5(Aurora/Tyler//2550sib/Coker87-13)/Coker 983//Coker 87-13	12;	Present
2000	38158 (PI619052)	FFR555W/GA-Gore	2;	Present
2000	Renwood3260 (PI635148)	SC861562/Coker 9803	23	Absent
2004	Choptank (PI639724)	Coker 9803/Freedom	12;	Present
2007	Jamestown (PI653731)	Roane/Pioneer 2691	12;	Present
2015	Hilliard (PI66271)	Pioneer 25R47/Jamestown	12;	Present

[a]Indicates presence of Lr18 based upon IWB41960 marker with Jamestown used as the positive control

Materials and Methods

Pathogen material

Puccinia triticina race TNRJJ was used throughout this study. The original four letter code used to denote race identity is based on reaction of a given race to a set of wheat host differential lines having known resistance genes [9]. Race TNRJJ is avirulent to gene Lr18, but contains virulence for genes Lr1, Lr2a, Lr2c, Lr3, Lr9, Lr24, Lr3ka, Lr11, Lr30, Lr10, Lr14a, Lr28 and Lr39.

Host material

One hundred and eighty six recombinant inbred lines (RILs) were derived from a cross of Pioneer '25R47' (PI 631473) [10] by Jamestown (PI 653731) [8]. The varieties are adapted to the soft red winter wheat growing regions of the eastern United States. The cultivar Jamestown is postulated to possess Lr18. In addition to the initial mapping population, 1600 individuals from eight other F2 populations (200 individuals per population) were evaluated to validate the presence of Lr18 in soft red winter wheat cultivars. These validation populations included Jamestown/'MCIA Venus' (PI 669575), Jamestown/VA10W-21(PI 676295), RL6009 (Thatcher*6/Africa43)/Jamestown, RL6009/VA10W-21, RL6009/VA09W-110

(GA931241E16/VA01W-303), RL6009/VA10W-119 ((KY97C-0540-04/GF951079-2E31 (PI 644020)), RL6009/ Shirley (PI 656753), Jamestown/P0537A1-12 (IN0411/2754// IN0412/98134), and VA08MAS-369 (McCormick/ GA881130LE5)/Jamestown. VA10W-21 and MCIA Venus are susceptible to TNRJJ. Near isogenic line RL6009 (CItr 15242) is the host differential with gene Lr18. VA09W-110, VA10W-119, and Shirley were postulated to contain Lr18. P0537A1-12 and VA08MAS-369 were postulated to lack Lr18.

Inoculation and *P. triticina* evaluation

Race TNRJJ was maintained and increased on the cultivar Tribute (PI 632689) [11]. The Pioneer 25R47/Jamestown population (186 RIL), two parental lines, and a set of leaf rust host differentials consisting of 24 'Thatcher' wheat near isogenic lines having different Lr genes (Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17, Lr30, LrB, Lr10, Lr14a, Lr18, Lr21, Lr28, Lr41, Lr42, Lr3bg, Lr14b, Lr20, Lr23) were evaluated for reaction to race TNRJJ. Seedlings were inoculated with urediospores of TNRJJ using a light mineral oil Soltrol 170 (Phillips Petroleum Co. Itex Plant, Borger, TX). The inoculated seedlings were then allowed to air dry for ten minutes and then were placed in a Percival Scientific (Perry, Iowa 50220) dew chamber (Model No. I-36DLC8) held at 99% relative humidity and 18-20°C for approximately 16 hours. The seedlings were then transferred and incubated in a Conviron (Winnipeg, Manitoba, Canada R3H 0R9) growth chamber (Model CMP5000) at 18°C, 55% relative humidity, and 16 hours of light (227 µMol). Disease assessments were made at 10 to 14 days after inoculation, using a 0-4 rating system described by Roelfs et al. [12]. Infection types 0-2 were classified as resistant and infection types 3-4 were classified as susceptible.

DNA extraction

Tissue of each RIL was collected when seedlings reached the three-leaf stage and placed into 2 ml test tubes, each containing two stainless steel beads for tissue grinding. Tissue samples were frozen in an ultra-low temperature (-80°C) freezer and then subsequently ground using a Spex CertiPrep 2000 Geno-Grinder (Metuchen, NJ, USA) for 15 s or until finely ground. DNA was extracted using a modified CTAB method [13].

Microsatellite assay

Over 400 Simple Sequence Repeat (SSR) markers were analyzed using bulk segregant analysis [14] of 142 samples including 71 of each extreme phenotype (most resistant and most susceptible) from the Pioneer 25R47/Jamestown population. Simple sequence repeat (SSR) primer were either directly labeled with a fluorescent dye or indirectly labelled with an M13 tail sequence (5'-ACGACGTTGTAAAACGAC-3'or 5'-CACGACGTTGTAAAACGAC-3'). Fluorescent dye labels included FAM (blue), NED (yellow/black), VIC (green), HEX (green) and PET (red). A single Polymerase Chain Reaction (PCR) with four direct-labeled fluorescent SSR primers were conducted in a volume of 14.0 µl containing 4 µl of DNA (50 ng), 1.0 µl of 10 × buffer and $MgCl_2$ (50 mM/µl), 0.20 µl of dNTP (25 mM/µl), 0.20 µl of each pair (forward and reverse) of direct-labeled primers (10 µM/µl), 0.03 µl of Taq polymerase (5 units/µl) and 8.37 µl of molecular grade water. A single PCR for each M13-labelled tailed primer pair was used in a volume of 14.0 µl that contained 4.0 µl of DNA (50 ng), 1.0 µl of 10 × buffer and $MgCl_2$ (50 mM/µl), 0.2 µl of dNTP (25 mM/µl), 0.3 µl of M13-tailed forward primer (1 µM/µl), 0.2 µl of reverse primer (10 µM/µl), 0.2 µl of M13 dye-labelled primer (10 µM/µl), 0.03 µl of Taq polymerase (5 units/µl) and 3.47 µl of molecular grade water. The PCR products of four separate M13-PCRs were combined for analysis in an Applied Biosystems 3130 × l Genetic Analyzer (Foster City, CA, USA). The PCR products were transferred to a 96-well PCR plate with each well containing 9.9 µl of Hi-Di formamide and 0.1 µl of size standard. Samples were denatured at 95°C for 5 min. PCR products were visualized on an applied biosystems 3130 × l Genetic Analyzer, and the generated data were analyzed using the genotyping software Genemarker version 1.70 designed by Soft Genetics [15].

Single Nucleotide Polymorphism (SNP) array

A 90K iSelect SNP genotyping assay was performed on the Pioneer 25R47/Jamestown population at the USDA-ARS small grains genotyping laboratory in fargo, ND. Genome Studio v2.0 software [16] was used to cluster the SNPs based on genotypes. Procedures for calling SNP(s) were similar to the procedures used in Cavanagh et al. [17] and Wang et al. [18]. In summary, there were multiple parental clusters called, similar to Liu et al., which were then converted to the A, B, H format (Supplemental Table S1). Manual scoring was assessed for each SNP cluster that could not be categorized by the default algorithm. Each SNP cluster was manually authenticated by visually assessing each cluster. Oligos for Kompetitive allele specific (KASP) PCR assays were developed from source sequences of iSelect SNP (s) linked to Lr18 in the Pioneer 25R47/Jamestown population, with primers carrying standard FAM or VIC compatible tails (Table 2). Reaction was performed baseon upon manufacturer's instructions (LGC Genomics). KASP primers were used for validation of results found in the initial linkage analysis.

Table 2. *KASP primers developed for SNP loci polymorphic among all populations linked with Lr18 through genotyping using the iSelect 90K wheat assay.*

Polymorphic KASPar SNP		**Primer sequence**		
SNP ID	iSelect 90K SNP Names	Allele 1a	Allele 2b	Reverse

IWB	BobWhite_s66049_223	gccgaccccaagaaaaca	gccgaccccaagaaaacg	gctttgaacaagtgcagccta
5688				
IWB	Kukri_c18410_193	tgaacatctcaacaccagtaact	tgaacatctcaacaccagtaacc	tggtcacagagtggtccg
41960				
IWB	BobWhite_rep_c50349_139	gaccgaggtggagctcga	gaccgaggtggagctcgg	atccgactgaagcccacg
4875				
aA1 primer labeled with FAM: 5' GAAGGTGACCAAGTTCATGCT 3'				
bA2 primer labeled with VIC: 5' GAAGGTCGGAGTCAACGGATT 3'				

Linkage map construction and data analysis

Chi squared ($\chi 2$) analysis was performed on the data collected from the rust screening tests to confirm the goodness-of-fit of observed ratios to theoretical expectations. Linkage maps were constructed using Join Map 4.0 [19], the Kosambi mapping function [20] was used to estimate map distance, and linkage groups were constructed based upon a minimum logarithm of odds (LOD) threshold value of 3.0. MapChart 2.2 [21] was used to draw linkage maps.

Results

The Lr18 host differential RL6009 (IT=12;)/Jamestown (IT=12;) population showed no segregation in reaction to P. triticina race TNRJJ, e.g., all progeny were resistant (IT=12;). This indicates that both RL6009 and Jamestown contain the resistance gene Lr18, and Jamestown has a single dominant resistance gene. Progeny derived from populations Jamestown/MCIA Venus $\chi^2_{3:1}$=0.04, p=0.84), Jamestown/VA10W-21 ($\chi^2_{3:1}$ =2.47, p=0.12), RL6009/VA10W-21 ($\chi^2_{3:1}$=2.19, p=0.14), Jamestown/P0537A1-12 ($\chi^2_{3:1}$=0.79, p=0.37), and VA08MAS-369/Jamestown ($\chi^2_{3:1}$=2.32, p=0.13) segregated in reaction to TNRJJ and fit a 3:1 single gene segregation pattern, thus validating that P0537A1-12, VA08MAS-369, VA10W-21, and MCIA Venus lack Lr18.Progeny (F2) derived from populations RL6009/VA09W-110 ($\chi^2_{3:1}$=2.47, p=0.12) and RL6009/VA10W-119 ($\chi^2_{3:1}$=1.18, p=0.28), also segregated and fit a single gene segregation pattern indicating that VA09W-110 and VA10W-119 do not possess Lr18. The RL6009/Shirley population segregated 180 resistant to 15 susceptible plants and fit a 15:1 segregation pattern ($\chi^2_{15:1}$ =0.69, p=0.40) indicating that two genes were segregating. Shirley is known to possess gene Lr26 residing on the 1BL. 1RS translocation, which also confers resistance to race TNRJJ [8]. These data indicate that Shirley does not possess Lr18 or another gene for seedling resistance to TNRJJ.

In the Pioneer 25R47/Jamestown population Lr18 mapped to the distal end of chromosome 5BL using the 90K SNP array data. A set of 24 SNP markers (Supplemental Table S2) residing near Lr18 in the Pioneer 25R47/Jamestown population mapped to within 10 cM and flanked Lr18 in the Jamestown/VA10W-21 and RL6009/VA10W-21 populations. Three markers were polymorphic among the Jamestown/Pioneer 25R47, Jamestown/VA10W-21, and RL6009/VA10W-21 populations (Table 2). The SNP maker IWB41960 mapped 3 to 5 cM proximal to Lr18 in all three populations.

To validate the results and in an effort to trace back the lineage of Lr18 marker IWB41960 was used to screen the cultivars in Table 1 along with a phenotypic screening using TNRJJ. The results concluded that Lr18 can be traced back to the cultivar GA1123 and that the phenotypic response of 12; corresponded with presence of Lr18 using marker IWB41960 and Jamestown as the positive control. Marker IWB41960 was also run on multiple parental lines in the Virginia Tech small grains breeding program (Table 3) to illustrate the distribution of Lr18 throughout the SRW germplasm.

Table 3. *Soft red winter wheat cultivars used as parental lines in the virginia tech small grains breeding program.*

Line	IWB41960a	2017 TNRJJ	2016 TNRJJ	2015 TNRJJ	2014 TNRJJ
Hilliard	Present	12;	23;	23;	1;
VA11W-108PA	Present	12-;	2;		
VA11W-106	Present	12-;	23;	23;	;1=
VA11W-279	Present	0;	0;	0;	0;
VA11W-313	Present	1;	3;	23-;	;1

VA12W-72	Present	;1-	23;	2;	1;
VA12W-68	Present	;1-	23;	23;	1-;
VA13W-174	Present	23/0;	0;/3	23/0;	0;1;
VA14W-29	Present	12;	3	12;	12;
VA14W-28	Present	;1	23;	1;	;1-
VA14W-32	Present	12;	23-;	12;	1;
VA09MAS3-34-2-1	Present	1;	23	23	;12
VA07MAS1-7047-1-1-4-2	Present	;1-	12;	1-;	;1=
VA09MAS1-12-5-1	Present	2;	23	3	23;
VA09MAS1-12-5-1-1	Present	1-;	3;	3-	
VA09MAS1-12-5-1-3	Present	1-;	23;	23;	
VA09MAS1-12-8-4	Present	;1-	23;	12;	;1
VA09MAS6-122-7-1	Present	12-;	23	2;	12;
VA09MAS6-122-7-1-1	Present	12-;	12;	12;	
VA09MAS6-122-7-1-4	Present	12-;	12;	12;	
VA14FHB-22	Present	3	3	3	23;
VA14FHB-29	Present	3	3-	3	3
VA15W-94	Present	3/Tr0;	3	3	
VA15W-101	Present	23;	3	3	
DH11SRW061-16	Absent	3	3	3	
DH11SRW065-23	Present	3	3	3	
DH11SRW065-26	Present	0;	0;	0;	
DH12SRW057-006	Present	23;	3	3-	
Pioneer Brand 26R59	Absent	2;	3;		
SY Viper	Present	0;	0;		
NC13-20332	Present	12-;	2;		
NC13-21213	Present	12-;	3-;		
GA07169-14LE24	Present		3		
GA07353-14E19	Present	0;	12;		
GAJT 141-14E45	Present	1-;	12;		
TX EL2	Absent	1-;	2;		
TN1604	Present	1-;/Tr3	23;		
15 MW 133	Present	0;		1-;	;1-
MDC07026-F2-19-13-1	Present			12;	;1-
L11541	Present		23;	1;	12-;
MD272-8-4-14-8	Present	0;	0;	0;	
MD07W478-14-6	Present		3;	23;	
CROPLAN 8550	Absent	23-;	3		
OH09-207-68	Present		3		

X08C-1077-11-18-3	Absent		;1=		
VA09MAS8-34-5-2	Absent	;1/Tr3	0;Tr23	0;/Tr3	;1=
VA09MAS2-131-6-2	Absent	;1=	0;	0;	0;
VA09MAS2-131-6-2-4	Present	0;	0;	;1=	
VA15W-63	Absent	0;	0;	0;	
DH11SRW069-70	Present	;1-	;1=	0;	
DH12SRW056-058	Absent	23-;	3	3-	
VA13W-38	Present	;1/Tr3	;12	;12	;1=
VA12W-31	Present	;1-	1-;	1;	;1-
VA12FHB-8	Absent	3	3	3	3
DH11SRW070-14	Absent	12;	2;	23;C	;1-
VA08MAS1-188-6-4-1	Present	0;	0;	;1=	0;
Shirley	Absent	0;	;1=	0;	0;
KWS 074	Present		3		
P0762A1-2-8	Absent	3/Tr;1-	3/;1-	12;	
P04620A1-1-7-4-17	Absent		3		

a Indicates the presence or absence of Lr18

Discussion

Data from greenhouse seedling tests conducted at the Cereal Disease Lab and Virginia Tech, previously indicated that Jamestown possesses Lr18. The other leaf rust resistance genes reported to reside on chromosome 5B, Lr52 and LrK1, were mapped to the short arm [22,23], while the current study mapped gene Lr18 to the distal end of 5BL of which is in agreement with previous studies conducted using C-banded chromosomes [6]. It also is unlikely that the source of resistance in Jamestown is Lr52 as this gene provides higher levels of resistance than Lr18 in seedling tests [22]. Gene Lr18 does not provide effective resistance in the field when used as the sole source of leaf rust resistance [7], but has contributed to effective resistance when combined with other complementary resistance genes. As Jamestown possesses adult plant leaf rust resistance at higher temperatures [8], it is conceivable that Lr18 is working in an epistatic or additive manner with QLr.vt-5B.1 and QLr.vt.5B.2 which were identified in Jamestown [24].

Gene Lr18 was linked (within 5 cM) to SNP marker IWB41960 in the F5:9 population of Pioneer 25R47/ Jamestown, and in the F2 populations Jamestown/VA10W-21 and RL6009/VA10W-21. Allelism tests validated that Jamestown possesses Lr18, while Shirley, P0537A1-12, VA08MAS-369, VA10W-21, VA09W-110, VA10W-119, and MCIA Venus lack Lr18. Marker IWB41960 validated results of the allelism tests using Jamestown as the positive control. Marker IWB41960 was also run on multiple parental lines in the Virginia Tech small grains breeding program and indicated that Lr18 is widely distributed throughout the germplasm (Table 3) and, thus, is still contributing to overall leaf rust resistance in current cultivars. Therefore, it is likely that selection for leaf rust resistance per se has maintained Lr18 in breeding programs since it was first introduced into SRW wheat germplasm more than 50 years ago. The likely source of resistance from the SRW wheat germplasm is GA1123 (Table 1) which was released in 1961 and contains T. timopheevi in its pedigree.

The DNA markers identified as being linked to Lr18 in this study are useful for understanding the relationship with other leaf rust resistance genes. In this study IWB41960 proved diagnostic for identifying Lr18 in both Jamestown and RL6009. However, while this marker is diagnostic for Lr18 it is not perfect for marker assisted genotyping or selection because linkage between Lr18 and the closest marker is not extremely tight (3.1 cM), and there are no distal flanking markers tightly linked to Lr18. This is evident in Table 3 as a few susceptible lines (IT=3) were identified as possessing Lr18, although this could also be due to suppressor genes which have been identified for leaf rust seedling resistance genes such as Lr23 [25]. Future work on Lr18 should include the cloning or further identification of more tightly linked markers via fine mapping that would allow for more reliable selection in breeding programs.

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