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Molecular markers for wheat leaf rust resistance gene *Lr41*

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Abstract Leaf rust, caused by *Puccinia triticina* Eriks., is an important foliar disease of common wheat (*Triticum aestivum* L.) worldwide. Pyramiding several major rust-resistance genes into one adapted cultivar is one strategy for obtaining more durable resistance. Molecular markers linked to these genes are essential tools for gene pyramiding. The rust-resistance gene *Lr41* from *T. tauschii* has been introgressed into chromosome 2D of several wheat cultivars that are currently under commercial production. To discover molecular markers closely linked to *Lr41*, a set of near-isogenic lines (NILs) of the hard winter wheat cultivar Century were developed through backcrossing. A population of 95 $BC_3F_{2:6}$ NILs were evaluated for leaf rust resistance at both seedling and adult plant stages and analyzed with simple sequence repeat (SSR) markers using bulked segregant analysis. Four markers closely linked to *Lr41* were identified on chromosome 2DS; the closest marker, *Xbarc124*, was about 1 cM from *Lr41*. Physical mapping using Chinese Spring nullitetrasomic and ditelosomic genetic stocks confirmed that markers linked to *Lr41* were on chromosome arm 2DS. Marker analysis in a diverse set of wheat germplasm indicated that primers BARC124, GWM210, and GDM35 amplified polymorphic bands between most resistant and susceptible accessions and can be used for marker-assisted selection in breeding programs.

Keywords *Lr39* · Near-isogenic lines · *Triticum aestivum* · *Puccinia triticina*

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Introduction

Leaf rust caused by *Puccinia triticina* Eriks. can cause yield losses up to 40% in susceptible wheat cultivars (Knott 1989) and is one of the most important diseases of common wheat (*Triticum aestivum* L.) worldwide (Kolmer 1996). Breeding for leaf rust resistance in wheat is challenging because resistance can be completely overcome by a shift in predominant pathogen races in a rust population. Successful control of rust epidemics using genetic resistance has two dimensions: monitoring dynamic changes of rust pathogen populations to identify new virulent races, and deploying resistance genes to defeat the new pathogen race. However, another approach to deter rapid changes in pathogenicity within the leaf rust population is

using cultivars with multiple genes resistant to different pathogen races. Successful examples are spring wheat cultivars with combinations of three resistance genes that withstood virulence changes in *P. triticina* over extended periods of time (Kolmer et al. 2008).

Currently, more than 60 leaf rust-resistance genes have been identified from wheat and its relatives. Many leaf rust-resistance genes have been identified in the wild wheat relative *T. tauschii* including *Lr21* (1DS), *Lr22a* (2DS), *Lr32* (3D), *Lr39*/*Lr41* (2DS), *Lr42* (1D) (Cox et al. 1994; Gill et al. 1991; Kerber 1987; Hiebert et al. 2007; Huang and Gill 2001; Huang et al. 2003; Rowland and Kerber 1974; Raupp et al. 2001). Recombination between the corresponding chromosomes of *T. tauschii* and the D genome of *T. aestivum* occurs at a level similar to that within the cultivated hexaploid species (Fritz et al. 1995). This lessens the challenge of gene introgression from *T. tauschii* with minimal linkage drag, and as such many disease-resistance genes from *T. tauschii* have been transferred into common wheat.

Pyramiding resistance genes in new cultivars may greatly increase durability of wheat resistance to leaf rust. However, pyramiding several resistance genes into a single genetic background using traditional phenotypic analysis requires time-intensive evaluation of a large breeding population exposed to several different races. Thus, molecular markers linked to these resistance genes, either race-specific or non-race-specific, would be essential tools for successfully and rapidly pyramiding new combinations of resistance genes through marker-assisted selection (MAS) in breeding programs.

Lr41 was originally mapped on chromosome 1DS in an early cytogenetic study (Cox et al. 1994) and relocated on 2DS through molecular mapping (Singh et al. 2004). Marker *Xgdm35* was reported to be closely linked with *Lr41* (Singh et al. 2004). This marker has been used in MAS for *Lr41* in hard winter wheat breeding programs in the southern Great Plains of the USA in the past 2 years. Unfortunately, a low frequency of the resistance marker allele in hard winter wheat germplasm made it an ineffective marker for MAS. Therefore, better markers are needed for this gene to be deployed in hard winter wheat through MAS. Objectives of this study were to (1) confirm the chromosome location of the resistance gene *Lr41* using molecular markers and a near-isogenic line (NIL) population, (2) identify molecular markers closely linked to *Lr41* to facilitate effective deployment of the gene in breeding programs, and (3) genotype diverse wheat cultivars and breeding lines for polymorphism at the marker loci.

Materials and methods

Plant materials and rust evaluation

Wheat breeding line KS93U62 was developed by backcrossing an *Lr*41-containing *T. tauschii* accession TA2460 to cultivar Century (Cox et al. 1994; Martin et al. 2003) and then crossed to OK92G205 and OK92G206, two-Century-backcross-derived NILs for the presence or absence of awns, both without the *Lr41* resistance gene (Carver et al. 1993). The corresponding F_2 population was artificially inoculated with *P. triticina* in a greenhouse at Kansas State University, Manhattan, KS, USA to identify plants with *Lr41* resistance (Martin et al. 2003). More than 200 resistant F_2 seedlings were selected from each cross, and their $F_{2:3}$ progeny were further evaluated to identify non-segregating families derived from F_2 plants homozygous for the *Lr41* allele for leaf rust resistance. Selected $F_{2:4}$ and $F_{2:5}$ families were further evaluated for adult-plant resistance in the field in Oklahoma in 1998 and 1999. According to their leaf rust reactions in the presence of natural field infection, 95 $F_{2:6}$ NILs were selected and used in this experiment.

To check seed purity from field-grown plants and to verify resistance of selected NILs, all 95 $F_{2:6}$ NILs were evaluated twice for adult-plant resistance in March (spring) and November (fall) 2007 and for seedling resistance in spring 2008 in a greenhouse at Kansas State University. In the 2007 greenhouse experiments, all NILs were inoculated at early anthesis with isolate PRTUS25 (race MDB, avirulence/virulence formula: 2a, 2c, 3 ka, 9, 11, 16, 17, 18, 19, 26, 30, 39, 41/1, 3, 10, 24), which is avirulent to *Lr41* and virulent to *Lr24* in Century, and a few lines with heterogeneous rust reactions were observed. Plants of those lines from the spring 2007 experiment were harvested separately as a seed source for the fall 2007 and spring 2008 greenhouse experiments. Leaf rust symptoms on leaves of adult plants were scored as resistant and susceptible

compared with both parents two weeks after inoculation. The experiments used a randomized complete block design with two replicates and five plants per replicate. In the spring 2008 seedling resistance test, six plants per NIL were planted in a tray with soil mix (Hummert International, Earth City, MO, USA) and grown in a growth chamber maintained at 20°C at Kansas State University. Seedlings were inoculated at the two-leaf stage with rust isolates PRTUS25 and PRTUS35 (race TNR, avirulence/virulence formula: 16, 17, 19, 26/1, 2a, 2c, 3, 3 ka, 9, 10, 11, 24, 30, 39, 41), which is virulent to both *Lr24* and *Lr41*. Inoculated seedlings were kept in a moist chamber at 20°C with 100% humidity for 12 h. Before scoring for disease, plants were kept in a growth chamber for 10 days at 20°C with 12 h light. Seedling infection types (IT) were determined according to McIntosh et al. (1995).

Chinese spring nullitetrasomic and ditelosomic genetic stocks, Nulli-1D/Tetra-1B (abbreviated as N1D-T1B), N2D-T2A, N2D-T2B, Ditelo1DS (abbreviated as DT1DS), DT1DL, and DT2DL, were used to determine the physical location of *Lr41*. The new markers developed from this study were further surveyed for polymorphism using 73 accessions from different classes and origins. Among them, OK Bullet, Thunderbolt, AP03T6115, Postrock, TX01V5719, Overley, Fuller, Bullet06ERU, and three sister selections of OK Bullet—OK02522W, OK05737W, and OK05741W—were expected to carry *Lr41*.

Marker analysis

Seedlings from the fall 2007 experiment were used as the plant source for DNA isolation. Leaf tissue was collected in 1.1 ml strip tubes and dried in a freeze–drier (Thermo Fisher, Waltham, MA, USA) for 2 days. Tubes containing a 3.2-mm stainless bead and dried tissue were shaken in a Mixer Mill (Retsch GmbH, Rheinische Strasse 36, Germany) at 25 times s^{-1} for 5 min. Genomic DNA was extracted from parents and NILs using the cetyltrimethyl ammonium bromide (CTAB) protocol (Saghai-Maroof et al. 1984). PCR amplifications were performed in a Tetrad Peltier DNA Engine (Bio-Rad Lab, Hercules, CA, USA) with a total volume of 12 µl containing 1.2 µl of 10× NH_4 buffer (Bioline, Taunton, MA, USA), 2.5 mM of $MgCl_2$, 200 µM of each dNTP, 50 nM of forward tailed primer, 250 nM of reverse primer and 200 nM of M13 fluorescent-dye labeled primer, 0.6 U of *Taq* DNA polymerase, and 100 ng template DNA. A touchdown program modified from Ma et al. (2005) was used for PCR. The reaction was incubated at 95°C for 5 min then continued for 5 cycles of 1 min of denaturing at 96°C, 5 min of annealing at 68°C with a decrease of 2°C in each subsequent cycle, and 1 min of extension at 72°C. For another 5 cycles, the annealing temperature started at 58°C for 2 min with a decrease of 2°C for each subsequent cycle. Reactions went through an additional 25 cycles of 1 min at 96°C, 1 min at 50°C, and 1 min at 72°C with a final extension at 72°C for 5 min. PCR products were analyzed on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Bulked segregant analysis was used for screening polymorphic simple sequence repeat (SSR) markers associated with *Lr41*. Equal amounts of DNA were pooled from five *Lr41*-resistant and five *Lr41*-susceptible NILs. Sixty SSR markers from chromosome 1D and 55 markers from 2D (Röder et al. 1998; Somers et al. 2004) were selected for screening the parents and two bulks. Polymorphic markers between the parents and bulks were further analyzed on all NILs for linkage relationship.

Data analysis

Data collected from the ABI DNA Analyzer were further processed using GeneMarker version 1.5 (SoftGenetics LLC, State College, PA, USA) and rechecked twice manually for accuracy. Genetic linkage among SSR makers and the leaf rust locus was determined by Joinmap 3.0 (Van Ooijen and Voorrips 2001) using the Kosambi mapping function (Kosambi 1944) with an LOD threshold of 3.0.

Results

Reactions of NILs and parents to leaf rust infection

Adult and seedling plants of NILs and parents showed expected reactions to leaf rust infection when inoculated with the isolate PRTUS25, which is avirulent to *Lr41*, at two different growth stages. *Lr41*-resistant NILs produced small, hypersensitive, necrotic or chlorotic flecks, whereas leaves of

Table 1 Infection types exhibited by seedling and adult plants of lines containing *Lr* genes derived from *T. tauschii* and susceptible control lines inoculated with two *P. triticina* cultures

Name	PRTUS25	PRTUS35	PRTUS25[b]
KS93U62(*Lr41*)	0;[a]	3	Resistant
OK92G205	3	3	Susceptible
OK92G206	3+	3	Susceptible
TAM 110	3	3	Susceptible

[a] The seedling infection types are: 0—no uredinia or other microscopic sign of infection, ;—no uredinia but small hypersensitive necrotic or chlorotic flecks present, 3—medium sized uredinia with or without chlorosis, +—uredinia somewhat larger than average

[b] The adult plant reaction when it was inoculated with PRTUS25

susceptible NILs were covered with medium-sized uredinia (Table 1). Seedlings of NILs were also inoculated with PRTUS35, an isolate virulent to *Lr41*, to verify that resistance in the NILs was due to *Lr41*, and not other genes. KS93U62 and the check cultivar TAM 110 were susceptible to PRTUS35 (Table 1), and all NILs showed highly susceptible symptoms with IT of 3 to 3+ (data not shown). Nine heterogeneous NILs were identified based on their IT from the first experiment. Both genotypes (*Lr41* and non-*Lr41* types) from these nine lines were harvested separately and evaluated for rust resistance in the second and third experiments. Consistent results were obtained for all nine lines among three experiments. These results indicated that rust ratings for NILs at different stages under different conditions were consistent and leaf rust data were appropriate for gene mapping.

Linked markers to *Lr41*

When 115 SSR markers from chromosome 1D and 2D were screened on parents and bulks, four SSR primers (BARC124, GWM210, GDM35, and CFD36) showed polymorphism between parents and between bulks. For example, primer BARC124 amplified 261-bp fragments in KS93U62 and the resistant bulk (Fig. 1a, c) and 271-bp fragments in the susceptible parents (OK92G205 and OK92G206) and susceptible bulk (Fig. 1b, d). These markers were further used to analyze the 95 NILs. Linkage analysis using the four markers and rust data identified *Xbarc124* as the marker linked most closely, at 1 cM apart, to *Lr41* (Fig. 2b). Three other markers were also close to *Lr41*: *Xgwm210*, *Xgdm35* and *Xcfd36* were 1.6, 2.8, and 4.1 cM proximal to *Lr41*, respectively. A distal flanking marker for *Lr41* was not identified. Comparative analysis with published maps (Singh et al. 2004; Somers et al. 2004; Sourdille et al. 2004) suggested that *Lr41* was near the telomere of chromosome 2DS (Fig. 2).

Marker *Xgdm35* was selected to verify the physical location of *Lr41*. Primer GDM35 amplified a clear band in N1D-T1B, DT1DS, and DT1DL, indicating that the marker was not on 1D (Fig. 3). However, primer GDM35 did not amplify any band in N2D-T2A, N2D-T2B, and DT2DL, indicating that the marker was on the short arm of chromosome 2D. This result provided further evidence to support that *Lr41* was on chromosome 2DS, not 1DS as originally reported.

Because all four markers were closely linked to *Lr41*, they all have potential to be used in MAS. To evaluate the polymorphism of those markers in diverse wheat germplasm, 73 additional wheat cultivars or breeding lines from different wheat classes and several countries were analyzed with these markers (Table 2). Each primer amplified at least one fragment in each of 73 wheat accessions (Table 2). For most markers, many resistant accessions amplified the banding patterns of KS93U62 that harbors *Lr41* while most susceptible accessions amplified different banding patterns from the ones of KS93U62.

Primer BARC124 amplified five fragments among 73 accessions, but only the 261-bp fragment was associated with the *Lr41* allele from *T. tauschii*, and all others were different from that amplified in KS93U62. Most accessions amplified a single fragment, but eight accessions amplified two fragments. Among five accessions amplifying the 261-bp fragment of KS93U62, only two hard winter wheat accessions, Thunderbolt and Tx01V5719, were expected to have *Lr41*. The other three accessions, IL94-1909 from IL, USA, and 117.92 and Sumai 3 from China, did not carry the *Lr41* gene based on available information and amplified two fragments: one 261-bp fragment plus another fragment from wheat.

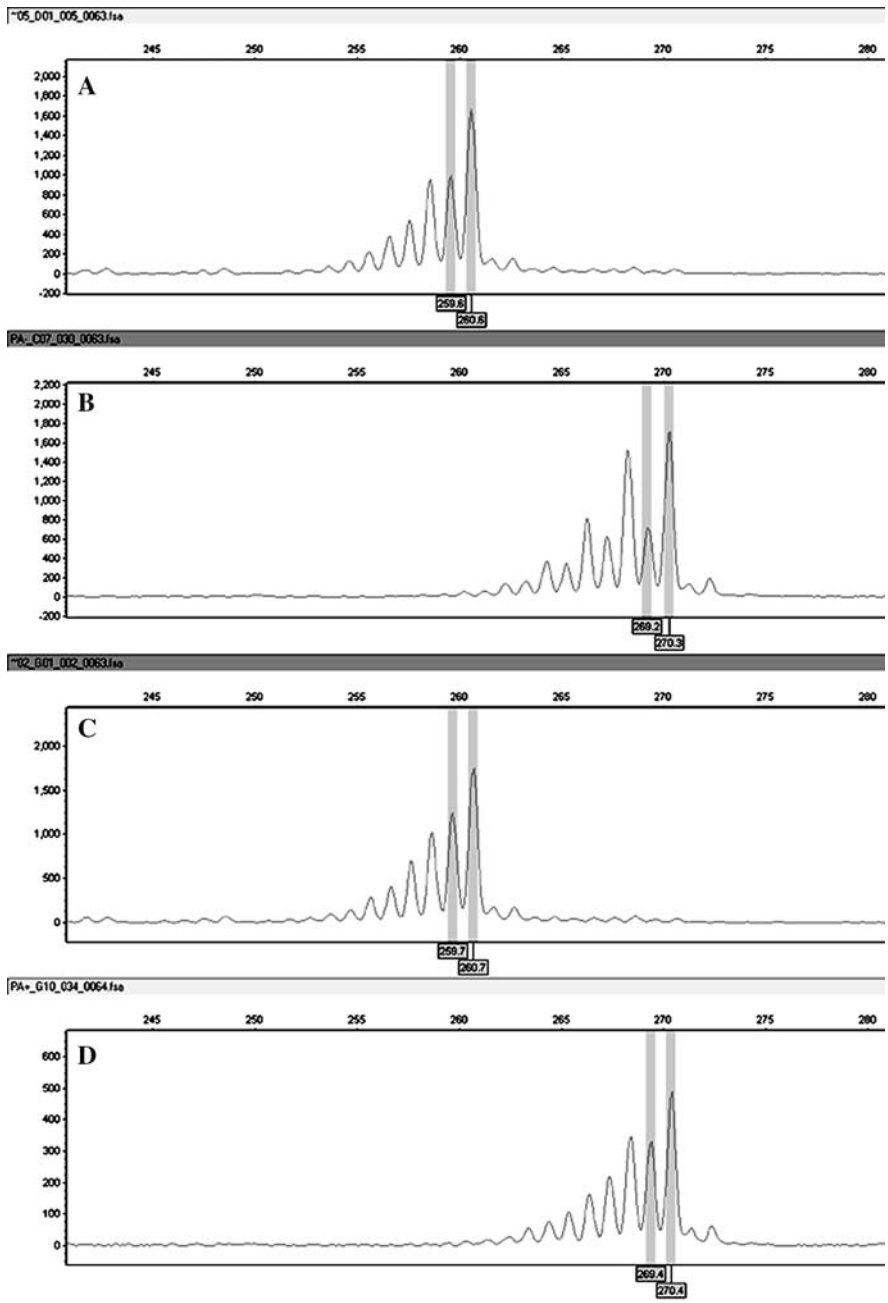

Fig. 1 ABI electrophorograms of SSR marker *Xbarc124* on chromosome 2DS showing polymorphism among KS93U62 (**a** *Lr41*), OK92G206 (**b** susceptible parent), resistant bulk (**c**), and susceptible bulk (**d**)

Primer GWM210 amplified three different banding patterns in 73 accessions, 182/206, 182/184, and 182/184/206 bp. Because the 182-bp fragment was monomorphic across all accessions and KS93U62 amplified 182- and 206-bp fragments, not the 184-bp fragment, the 184-bp fragment was considered a non-KS93U62 marker allele. Excluding the monomorphic 182-bp fragment, marker allele *Xgwm210* (206-bp fragment) was shown in all 11 hard winter wheat accessions that carry *Lr41* and five other accessions without *Lr41*. Among accessions carrying the 206-bp allele without *Lr41*, three were hard winter wheat lines from Oklahoma (OK05903C, OK04525, and OK05830) and two were spring wheat accessions from China (Sumai 3) and Japan (Shinchunaga) (Table 2). An additional 11 accessions that amplified

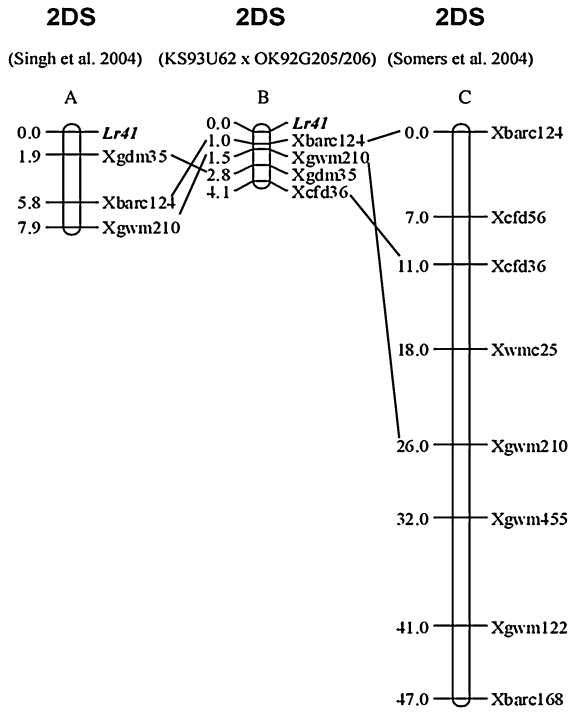

Fig. 2 Alignment of the current *Lr41* genetic map with two other maps obtained from Somers et al. (2004) and Singh et al. (2004). **a** WGRC10/TAM 107, **b** KS93U62/OK92U205 or OK92U206, **c** Consensus map derived from four populations which are Synthetic/Opata, RL4452/AC Domain, Wuhan/Maringa and Superb/BW278. The centromere is toward bottom of the figure

the 206-bp fragment also amplified the 184-bp fragment; they all were susceptible accessions.

Primer GDM35 amplified 18 alleles in 73 accessions, and KS93U62 amplified a 182-bp fragment. Thunderbolt was the only cultivar with *Lr41* that amplified the 182-bp fragment. However, the 182-bp fragment appeared in 21 accessions that do not carry *Lr41*. All 21 accessions with the 182-bp fragment had an additional fragment of varied sizes. Among them, 18 were soft winter wheat lines from the USA and three were soft spring wheat accessions from China. Primer CFD36 amplified five fragments in 73 accessions. The 213-bp fragment amplified in KS93U62 appeared in most soft wheat accessions.

Discussion

Because of rapid changes in predominant rust pathogen races in nature, single-gene resistance in a cultivar may become ineffective soon after it is released. Stacking two or more genes in one cultivar can enhance durability and the level of rust resistance. For example, a gene combination *Lr9* and *Lr24* provided relatively long-lasting resistance (McVey and Long 1993). Also, the combination of seedling resistance gene(s) with an adult-plant resistance gene such as *Lr34* expressed a high level of resistance

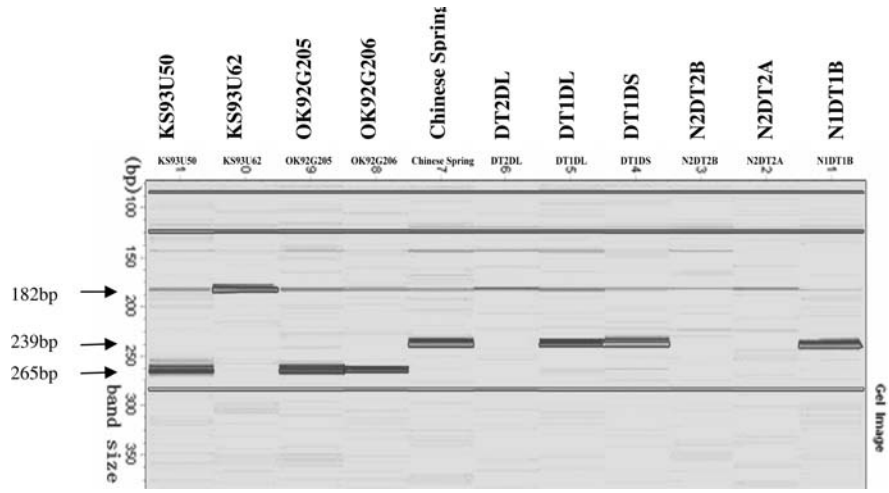

Fig. 3 ABI gel image displaying the fragments amplified by Gwm35 (2DS) found in KS93U50 (carrying *Lr42*), KS93U62 (carrying *Lr41*), OK92G205/206 (susceptible parents), Chinese Spring, DT2DL, DT1DL, DT1DS, N2D-T2B, N2D-T2A, N1D-T1B. The fragment associated with *Lr41* is 182-bp, and the fragment amplified in Chinese Spring is 239-bp. KS93U50 and two susceptible parents have the fragment of 265 bp. Four lines across the image are DNA size standards

Table 2 Pedigrees and marker haplotypes of wheat cultivars and breeding lines and their reactions to leaf rust isolate PRTUS25

Name	Pedigree	Class	Origin	*Lr41*[a]	Size of amplified fragment (bp)			
					Xbarc124	*Xgwm210*	*Xgdm35*	*Xcfd36*
AP03T6115	Karl//Mit/Lancota/3/U1254-4-9-8-V32	HWW	USA	R	271	182/206	265	215
Bullet06ERU	KS96WGRC39/Jagger	HWW	USA	R, R′	271	182/206	265	215
Fuller	Bulk selection	HWW	USA	R′	271	182/206	249	215
KS93U62	Century*3/TA2460	HWW	USA	R	261	182/206	182	213
OK Bullet	KS96WGRC39/Jagger	HWW	USA	R, R′	271	182/206	265	215
OK02522W	KS96WGRC39/Jagger	HWW	USA	R	271	182/206	265	215
OK05737W	KS96WGRC39/Jagger	HWW	USA	R	271	182/206	229	215
OK05741W	KS96WGRC39/Jagger	HWW	USA	R	271	182/206	229	215
Overley	(TAM-107*3/TA 2460)/Heyne ‘S’//Jagger	HWW	USA	R, R′	271	182/206	249	215
Postrock	Ogallala/KSU94U261//Jagger	HWW	USA	R, R′	271	182/206	265	215
Thunderbolt	Abilene/KS90WGRC10	HWW	USA	R	261	182/206	182	215
Tx01V5719	U1254-4-7-3/OGALLALA	HWW	USA	R	261	182/206	261	215
111.92	FengKang15/Cooperacion Nanihue	SWW	Argentina	S	266	182/184	252	213
113.92	FengKang15/Cooperacion Nanihue	SWW	Argentina	S	266	182/184/206	252	213
117.92	FengKang15/Cooperacion Nanihue	SWW	Argentina	S	261/266	182/184/206	182/252	213
38M.A.	Barleta 4d/Chino	SWW	Argentina	S	266	182/184	274	213
BacUp	Nuy Bay/Pioneer2375// Marshall	HSW	USA	S	271	182/184	252	213
Cardinal	Logan*2/3/Va63-5-12/Logan// Blueboy	SWW	USA	S	266	182/184/206	182/239	213
Centerfield	(TXGH12588-105*4/FS4)/ 2*2174	HWW	USA	S	266	182/184	229/265	215
Chinese Spring	Land race	SSW	China	S	266	182/184/206	239	215
Chisholm	Sturdy sib/Nicoma	HWW	USA	S	266/271	182/184	261	215
Coop-Capoildo	Land race	SWW	Argentina	S	266	182/184	252	213
Deliver	(Yantar/2*Chisholm)/Karl	HWW	USA	S	266/271	182/184	229	215
Duster	W0405D/NE78488//W7469C/ TX81V6187	HWW	USA	S	281	182/184	252	215
Encruzilhada	Fortaleza/Kenya Farmer	SWW	Brazil	S	266	182/184	233	235
Endurance	HBY756A/Siouxland//2180	HWW	USA	S	271	182/184	256	215
Ernie	Pike/3/Stoddard/Blueboy// Stoddard/D1707	SWW	USA	S	266	182/184	182/233	213/235
Expert	Extrem/Mexico4040//Neuhof1/ 3/Extrem/HP35719	SWW	Austria	S	266	182/184/206	260	213
Extrem	Record/Br. Herrachweiten	SWW	Austria	S	266	182/184/206	260	213
Foster	Ky83-60/Tyler//KY83-75	SWW	USA	S	266	182/184	182/233	213/235
Freedom	GR876/OH217	SWW	USA	S	266	182/184/206	182/239	213
Guymon	Intrada/Platte	HWW	USA	S	266	182/184	229	215
IL93-2283	IL84-3511/IL84-3348	SWW	USA	S	266	182/184/206	233	213/235
IL94-1549	Auburn/Ark38-1/Arther/ Blueboy	SWW	USA	S	266	182/184	182/239	213

Table 2 continued

Name	Pedigree	Class	Origin	*Lr41*[a]	Size of amplified fragment (bp)			
					Xbarc124	*Xgwm210*	*Xgdm35*	*Xcfd36*
IL94-1909	Fillmore/Amigo//Tyler/Howell	SWW	USA	S	250/261	182/184	182/255	213/219
IL94-2426	Fillmore/Amigo//Tyler/Howell	SWW	USA	S	266	182/184	182/233	213/235
IL94-6280	IL87-3721/Cardinal//P808801-4-2-4-107	SWW	USA	S	271	182/184/206	245	215
IL95-1966	IL87-2834-1/IL87-6512//IL87-1968-1	SWW	USA	S	266	182/184	182/233	235
IL95-2066	IL88-7890/P7924H1-20-2-74	SWW	USA	S	266	182/184	182/239	213
IL95-2909	Freedom//IL84-2191-1/IL84-4046	SWW	USA	S	266	182/184	182/239	213
IL9634-24851	IL90-6364//IL90-9646/Ning 7840	SWW	USA	S	266	182/184	182/233	213/235
Karat	Extrem/Betosfeje1	SWW	Austria	S	266	182/184/206	260	213
Kaskaskia	IL77-2933/IL77-3956//Pike/Caldwell	SWW	USA	S	266	182/184	182/256	213/219
KS93U50	Century*3/TA2450	HWW	USA	S	250	182/184	233/265	213
Livius	Karat/Lentia	SWW	Austria	S	266	182/184/206	256	213
MO-94-193	MO 11728/Becker	SWW	USA	S	266	182/184	239	213
MO94-312	Pioneer brand 2551/Caldwell	SWW	USA	S	266	182/184	182/239	213
NTDHP	Land race from Jiangsu	SSW	China	S	271	182/184/206	239	213/219
OH552	Pur71761A4-31-5-33/MD55-286-21	SWW	USA	S	266	182/184	182/256	213
OH569	Pur 71761A4-31-5-33/MO 55-286-21	SWW	USA	S	266	182/184	182/239	213
OK03716W	Oro Blanco/OK92403 F4:11	HWW	USA	S	271	182/184	252/261	215
OK03825-5403-5	Custer*3/94M81	HWW	USA	S	271	182/184	261	215
OK04525	FFR525W/Hickok//	HWW	USA	S	281	182/206	245/261	215/235
OK05830	OK93617/Jagger	HWW	USA	S	271	182/206	229	215
OK05903C	TXGH12588-120*4/FS4//2174/3/Jagger	HWW	USA	S	271	182/206	229	215
OK05905C	TXGH12588-105*4/FS4//2174/3/Jagger	HWW	USA	S	266	182/184	229	215
OK92G205	Century*5/McNair1003	HWW	USA	S	271	182/184	233/265	213/235
OK92G206	Century*5/McNair1003	HWW	USA	S	271	182/184/206	233/265	213/235
P93D1-10-2	851423/INW9853	SWW	USA	S	266	182/184	182/239	213
PA8769-158	Titan/Caldwell	SWW	USA	S	266	182/184	182/239	213
Par-55	Unknown	SWW	China	S	266	182/184	256	219
PB2555	Coker68-16/MoW 7140//Pioneer brand W521	SWW	USA	S	266	182/184/206	245	215
PC-2	Lira's'//AU/UP301	SWW	China	S	266	182/184	239	213
Perlo	Extrem/Betosfeje1	SWW	Austria	S	250/266	182/184/206	256	213
Poncheau	Sel. from land race	SWW	France	S	266	182/184	250	215
Pontiac	Magnum/Auburn	SWW	USA	S	266	182/184	239	213
Roane	VA71-54-147/Coker68-15//IN65309C1-18-2-3-2	SWW	USA	S	266	182/184	182/229	217
Sanshukomugi	Land race from Mie	SSW	Japan	S	271	182/184/206	239	213
Shinchunaga	Land race from Mie	SSW	Japan	S	271	182/206	256	213/219

Table 2 continued

Name	Pedigree	Class	Origin	*Lr41*[a]	Size of amplified fragment (bp)			
					Xbarc124	*Xgwm210*	*Xgdm35*	*Xcfd36*
Shirasaya 1	Land race from Mie	SSW	Japan	S	266	182/184/206	256	213/219
Spartakus	Perlo/Extrem/Betosfeje1	SWW	Austria	S	266	182/184/206	256	215
Sumai3	Funo/Taiwan Wheat	SSW	China	S	261/266	182/206	256	213/219
Sumai49	N7922/Ning7840	SSW	China	S	266	182/184	182/256	213/219
Vilela-Sol	Land race	SWW	Argentina	S	266/271	182/184	252	213
Wangshuibai	Land race from Jiangsu	SSW	China	S	266	182/184/206	245	215
Xianmai1	Ardito/Tevere//Wannian2	SSW	China	S	266	182/184	182/265	213

[a] R refers to lines that are expected to carry *Lr41* based on pedigree and infection type of isolate PRTUS25; R′ refers to lines that are expected to carry *Lr41* based on leaf rust differential test results (http://www.ars.usda.gov/main/site_main.htm?modecode=36-40-05-00); S refers lines that are not expected to carry *Lr41* based on pedigree and/or infection type

(Kolmer 2003) and durable rust resistance that has proven to be effective over time (Kolmer et al. 2008). Therefore, pyramiding *Lr41* with other durable adult-plant resistance genes such as *Lr34* or *Lr46* or some broadly effective resistance gene such as *Lr21* could be an effective strategy for minimizing losses caused by rust epidemics.

Knowledge of chromosome locations of genes is essential for using gene pyramiding to develop cultivars with multiple-resistance genes in breeding programs. In an early cytogenetic study, *Lr41* was located on the short arm of chromosome 1D (Cox et al. 1994). More recently, molecular mapping relocated the gene on 2DS, and established the close linkage, or possibly allelism with *Lr39* (Singh et al. 2004). In the present study, using ditelosomic and nullitetrasomic genetic stocks and markers linked to *Lr41*, we physically confirmed *Lr41* on chromosome 2DS. Marker *Xgdm35*, rather than the more closely linked marker *Xbarc124*, was selected to verify the physical location of *Lr41* in this study because *Xbarc124* is not chromosome specific and thus amplified loci on other chromosomes of Chinese Spring besides 2DS (http://wheat.pw.usda.gov/). Confirming the physical location of *Lr41* provides useful information, allowing breeders to make decisions about what other genes can be combined to enhance durable resistance in a cultivar.

In this study, four markers were mapped within 4.1 cM from *Lr41*. A distal flanking marker was not found, even when all published SSR markers from 2DS (Somers et al. 2004) were screened between parents. Slight differences in marker order were observed between linkage groups constructed in this study and those reported in previous studies (Singh et al. 2004; Somers et al. 2004). In the map by Singh et al. (2004), *Xgdm35* was the closest marker to *Lr41*; in our study *Xbarc124* was the closest marker to *Lr41*, which agrees with Somers et al. (2004). However, *Xcfd56* was located between *Xbarc124* and *Xgwm210* in the Somers et al. (2004) map but was the furthest marker from *Lr41* in our study. The slight differences in marker order and marker interval among three maps could be due to differences in mapping population and population type used for map construction. In this study, we used a backcross population; other studies used a recombinant inbred line population (Somers et al. 2004) or F_2 population (Singh et al. 2004). Nevertheless, the four markers are very closely linked to *Lr41* (<8 cM) based on maps from this study and Singh et al. (2004). Therefore, markers identified in this study would be good candidates for use in MAS, assuming that sufficient polymorphism exits between parents used in breeding programs.

The actual utility of markers developed from linkage mapping in MAS will depend on the frequency of the resistance marker allele in resistant parents and the level of polymorphism between resistant and adapted parents. To indirectly assess the usefulness of these markers for *Lr41* in future MAS, markers linked to *Lr41* were analyzed in a collection of 73 accessions from Argentina, Brazil, USA, Austria, France, China, and Japan. Among the four markers, *Xbarc124* resided the closest to *Lr41*. Of 11 cultivars thought to carry *Lr41*, only two (TX01V5719 and Thunderbolt)

amplified a single 261-bp allele of *Xbarc124* as present in KS93U62, the source of *Lr41* in our NIL population. Thunderbolt was derived from a cross with KS90WGRC10 (*Lr41* donor), and TX01V5719 was derived from U1254, which likely inherited *Lr41*. Other resistant cultivars were selected from crosses using KS90WGRC39's derivative as the *Lr41* donor, but it is possible that the marker allele associated with *Lr41* was lost during its early transfer into new germplasm. Only three soft wheat accessions amplified the KS93U62 marker allele, but they do not carry *Lr41*. These accessions were IL94-1909 from IL, USA and 117.92 and Sumai 3 from China and also amplified an additional fragment of either 250 or 266 bp.

Results indicate that the polymorphic level is high between resistant and susceptible accessions, albeit at a low frequency of the *Xbarc124* allele in the accessions carrying *Lr41*. Obviously, the 261-bp fragment can be a useful marker for *Lr41* if TX01V5719 and Thunderbolt are specifically used as the *Lr41* donor parents in populations undergoing MAS. If other parents listed in Table 2 are used as an *Lr41* donor, the 261-bp allele cannot be detected; in this case, *Xbarc124* is not recommended for MAS.

GWM210 amplified two fragments in KS93U62: a monomorphic 182-bp fragment that appeared in all 73 accessions and a 206-bp fragment that appeared in all *Lr41*-containing accessions and a few of non-*Lr41*-containing accessions. Meanwhile, almost all accessions without *Lr41* amplified a 184-bp fragment except three Oklahoma lines, therefore the 184-bp fragment can be regarded as the marker allele that was associated with susceptible genotypes. In the case of the susceptible parent, OK92G206, which amplified both the 184- and 206-bp fragments, the 206-bp fragment was not associated with *Lr41* resistance. Therefore if GWM210 amplifies the 206-bp fragment without accompanying of the 184-bp fragment in an accession, this accession most likely carries *Lr41*. A total of 16 accessions, including all 11 US hard winter wheat accessions that carry *Lr41*, amplified the 206-bp fragment without the 184-bp fragment. The remaining five accessions that did not have *Lr41* include three Oklahoma hard winter wheat lines and two soft wheat cultivars from China and Japan. Thus, *Xgwm210* is a highly polymorphic marker among wheat accessions evaluated and can be a good marker for introgression of *Lr41* into elite wheat backgrounds.

For primer GDM35, only Thunderbolt amplified a single 182-bp fragment as in KS93U62. All other accessions that carry *Lr41* did not amplify the 182-bp fragment. Although 21 other accessions also amplified the 182-bp fragment, these accessions amplified an additional fragment of varied sizes. These 21 accessions included 18 soft red winter wheat cultivars from the USA and three soft red spring wheat accessions from China. The specificity of marker *Xgdm35* to the resistant cultivar, Thunderbolt, indicates that *Xgdm35* can be effective for MAS only if Thunderbolt is used as the *Lr41* donor. If other resistant parents are used, one of three fragments—229-, 249-, and 265-bp—can be used as target marker alleles for GWM35.

Primer CFD36 amplified a 213-bp fragment in KS93U62. The 213-bp fragment was not amplified in any *Lr41*-containing accessions. Instead, a 215-bp fragment, which was not polymorphic with the remaining hard winter wheat accessions without *Lr41*, was identified in all 11 resistant accessions. In contrast, the 213-bp fragment was amplified in most soft wheat accessions without *Lr41*. Therefore, *Xcfd36* is not a usable marker for MAS.

In summary, *Lr41* was physically located on the distal end of chromosome 2DS. Four markers were identified tightly linked to *Lr41*. Among them, marker *Xgwm210* appears to have the greatest utility for MAS because of its high frequency in *Lr41*-containing accessions and high polymorphism in a large set of germplasm collection. However, this marker should be used with caution to predict the presence of *Lr41* in a natural population for its possibility of false positive. Fragments that are associated with both resistant (206 bp) and susceptible (184 bp) genotypes should be evaluated. The lines that carry *Lr41* most likely amplify the 206-bp fragment without the 184-bp fragment. For MAS, it can be scored as a codominant marker if both fragments are polymorphic between parents; or it can be scored as a dominant marker if only 184-bp fragment is polymorphic between parents. Markers *Xbarc124* and *Xgdm35* can also be used for MAS if *Lr41* donor parents have the marker alleles of KS93U62. A survey of allelic frequency in resistant accessions and polymorphism level in a germplasm collection or association mapping can ensure that a marker linked to a resistance gene can be widely used in MAS and may provide valuable supplemental

information to facilitate quick deployment of genes in breeding programs.

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