Molecular Mapping of Wheat Leaf Rust Resistance Gene *Lr42*

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ABSTRACT

Leaf rust, caused by *Puccinia triticina* Eriks., is an important foliar disease of wheat (*Triticum aestivum* L.) worldwide. Leaf rust resistance gene *Lr42* from *Aegilops tauschii* Coss. has been used as a source of rust resistance in breeding programs. To identify molecular markers closely linked to *Lr42*, a segregating population of near-isogenic lines contrasting for the presence of *Lr42* was developed in the hard winter wheat cultivar Century background and evaluated for rust infection type at both seedling and adult-plant stages. Simple sequence repeat (SSR) markers were screened using bulked-segregant analysis. Two markers closely linked to *Lr42* were identified on chromosome 1DS. The closest marker, *Xwmc432*, is about 0.8 cM from *Lr42*. Physical mapping of both SSR markers using Chinese Spring nullitetrasomic and ditelosomic genetic stocks confirmed that the markers linked to *Lr42* were on 1DS. Markers for *Lr42* were highly polymorphic between parents and among a diverse set of wheat germplasm collected from several countries, indicating that these markers are useful for marker-assisted selection for *Lr42*.

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Abbreviations: IT, infection type; MAS, marker-assisted selection; NIL, near-isogenic line; PCR, polymerase chain reaction; SSR, simple sequence repeat.

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 wheat (*Triticum aestivum* L.) worldwide (Kolmer, 1996). One of the most effective approaches for minimizing losses due to leaf rust is the use of resistant cultivars. However, race-specific resistance is often a temporary solution because it can be overcome by a shift in the pathogen population. This has prompted a continuous search for new sources of resistance.

More than 50 leaf rust resistance genes have been reported in wheat and its relatives. Many leaf rust resistance genes are derived from wheat wild relative *Aegilops tauschii* Coss., including *Lr21* (located on wheat chromosome 1DS), *Lr22a* (2DS), *Lr32* (3D), *Lr39* (2DS), *Lr41* (2DS), and *Lr42* (1DS) (Rowland and Kerber, 1974; Gill et al., 1991; Kerber, 1987; Cox et al., 1994). It has been reported that recombination between the corresponding chromosomes of *A. 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 allows gene introgression from *A. tauschii* with minimal linkage drag.

Lr42, a race-specific gene introgressed from *A. tauschii,* was located on wheat chromosome 1DS in an earlier genetic study (Cox et al., 1994). Germplasm lines containing *Lr42* have been utilized by several U.S. and international breeding programs (Bacon et al.,

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2006; Singh et al., 2007). Martin et al. (2003) reported the agronomic effects of *Lr42* resistance using near-isogenic lines (NILs) for the gene and concluded that *Lr42* played a significant role in increasing yield, test weight, and kernel size in Oklahoma winter wheat.

One strategy for prolonging the usefulness of race-specific resistance genes is to pyramid or combine multiple resistance genes in one cultivar. Successful examples of this strategy can be found in spring wheat cultivars with a combination of at least three resistance genes that have maintained effective resistance for extended time periods (Kolmer et al., 2008a). However, pyramiding several resistance genes in one cultivar using traditional breeding methods requires time-consuming evaluation of a large breeding population with several different races in repeated experiments. In contrast, molecular markers linked to these resistance genes can simplify pyramiding efforts because selection can be practiced using multiple markers simultaneously. Closely linked molecular markers are essential for marker-assisted selection (MAS) in breeding programs. Markers closely linked to *Lr42* have not been reported. In this study, we used NILs for *Lr42* to confirm the physical location of the gene by using molecular markers in conjunction with ditelosomic and nullitetrasomic wheat genetic stocks, and we identified two closely linked markers to *Lr42* in a population developed from a cross between contrasting NILs.

MATERIALS AND METHODS

Plant Materials and Rust Evaluation

The Century backcross-derived line KS91WGRC11 (Century*3/TA2450, PI 566668) contains *Lr42* derived from *A. tauschii* accession TA2450 (Cox et al., 1994). Line KS93U50, a selection from KS91WGRC11, was crossed to OK92G205 (Century*5/'McNair 1003', PI 561731) and OK92G206 (Century*5/McNair 1003, PI 561733), two additional Century backcross-derived lines that do not contain *Lr42* (Carver et al., 1993). OK92G205 and OK92G206 are two NILs contrasting in presence of awn and the *Lr42* NILs contrasting in presence of awn were originally developed to test effect of awn on the expression of *Lr42* (Martin et al., 2003). The corresponding two F_2 populations, which were considered near-isogenic relative to the *Lr42* locus, were artificially inoculated with PRTUS25 in a greenhouse in Manhattan, KS, to select leaf rust resistant or susceptible lines. Plants were sprayed with a suspension of urediniospores in Soltrol 170 light mineral oil (Phillips Petroleum, Bartlesville, OK) and then incubated overnight in a dew chamber at 20 to 24°C. The F_2 plants were grown in the greenhouse at 20 to 24°C, from which 108 resistant and 51 susceptible plants were selected for further study. The $F_{2:3}$ progenies were evaluated for leaf rust resistance at the adult-plant stage in the field at Stillwater, OK, to confirm nonsegregating families homozygous for either allele at the *Lr42* locus. Forty-five $F_{2:4}$ and $F_{2:5}$ families were further evaluated for adult plant resistance under natural infection conditions in the field in Oklahoma in 1998 and 1999 (Martin et al., 2003). Forty-four $F_{2:6}$ NILs (31 from KS93U50/OK92G205 and 13 from KS93U50/OK92G206) were selected on the basis of their leaf rust reactions and used in this study.

To verify the resistance of selected NILs, all 44 $F_{2:6}$ NILs were evaluated twice for resistance as adults in March (spring) and November (fall) 2007 and for seedling resistance in spring 2008 with different isolates in the growth chamber. In the 2007 greenhouse experiments, plants were grown in Metro-Mix 360 soil mix (Hummert International, Earth City, MO) in 1-L pots. All NILs were inoculated at early anthesis with the isolate PRTUS25 (race MDBJG using North American race nomenclature [Kolmer et al., 2008b; Long and Kolmer, 1989], avirulence/virulence formula: 2a, 2c, 9, 16, 26, 3ka, 11, 17, 30, B, 18, 21, 41, 42/1, 3, 24, 10, 14a, 28). The rust inoculation method was the same as described above. The experiments used a randomized complete block design with two replicates and five plants per replicate. Infection types of leaf rust on the leaves of adult plants were compared with both parents 2 wk after inoculation and scored as either resistant or susceptible (McIntosh et al., 1995). In the spring 2008 seedling test, six plants per NIL were planted in Metro-Mix 360 soil mix. Seedlings were inoculated with rust cultures PRTUS25, PRTUS35 (race TNRJD, avirulence/virulence formula: 16, 26, 17, B, 18, 21, 28, 42/1, 2a, 2c, 3, 9, 24, 3ka, 11, 30, 10, 14a, 41), and PNMRJ (avirulence/virulence formula: 2a, 16, 26, 11, 17, 14a, 21, 42/1, 2c, 3, 9, 24, 3ka, 30, B, 10, 18, 28, 41) at the two-leaf stage. All three isolates are avirulent to *Lr42* but virulent to *Lr24*, which is present in Century (Cox et al., 1994). TAM 110 (PI 595757) was used as the susceptible check. Inoculated seedlings were kept in a dew chamber at 20 ± 1°C with 100% humidity for 12 h and then grown in a growth chamber for 10 d at 20 ± 1°C with 12 h of light. Seedling infection types were scored according to McIntosh et al. (1995).

Chinese Spring nullitetrasomic and ditelosomic genetic stocks, Nullisomic-1D/Tetrasomic-1B (abbreviated as N1D-T1B), N2D-T2A, N2D-T2B, Ditelosomic 1DS (abbreviated as DT1DS), DT1DL, and DT2DL (http://www.k-state.edu/wgrc/Germplasm/Stocks/stocks.html), were used to physically map the markers linked to *Lr42*. An international collection of 85 genetically diverse wheat germplasm lines from Argentina, Brazil, United States, Austria, France, China, and Japan was used to evaluate polymorphism for the new markers developed in this study. Among these lines, AR93005 and Fannin were reported to derive from *A. tauchii* accession TA2450 (Table 1).

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, dried in a freezer drier (Thermo Fisher, Waltham, MA) for 2 d, and ground in a Mixer Mill (Retsch Inc., Newtown, PA) to fine powder by shaking strip tubes with a 3.2-mm stainless steel bead at 25 times s^{-1} for 5 min. Genomic DNA was extracted from parents and NILs by using the cetyltrimethyl ammonium bromide (CTAB) method (Saghai-Maroof et al., 1984). Polymerase chain reaction (PCR) amplifications were performed in a Tetrad Peltier DNA Engine (Bio-Rad Lab, Hercules, CA). A 12-μL PCR mix contained 1.2 μL of 10× NH_4 buffer (Bioline Inc. Taunton, MA), 2.5 mM $MgCl_2$, 200 μM of each dNTP, 100 nM forward tailed primer, 200 nM reverse primer, 100 nM of M13 fluorescent-dye labeled primer, 1 U of *Taq* DNA polymerase, and 50 ng of template DNA. A

Table 1. Haplotypes of the markers linked to the *Lr42* gene in three parents of near-isogenic population and 85 additional wheat accessions collected from the USA and other countries.

Variety	Pedigree	Source	*Xcfd15*†	*Xwmc432*
KS93U50	Century*3/TA2450	USA	220	204/211
OK92G205	Century*5/McNair1003	USA	178	202/217
OK92G206	Century*5/McNair1004	USA	178	202/217
Bullet06ERU	KS96WGRC39/Jagger	USA	178	202/217/236
Centerfield	(TXGH12588-105*4/FS4)/2*2174	USA	178	236
Chisholm	Sturdy sib/Nicoma	USA	178/194	202/217/238
CO02W237	98HW519(93HW91/93HW255)/96HW94	USA	178	202/213
Deliver	(Yantar/2*Chisholm)/Karl	USA	178	238
Duster	W0405D/NE78488//W7469C/TX81V6187	USA	178/200	202/217
Endurance	HBY756A/Siouxland//2180	USA	178	238
Fuller	Bulk selection	USA	178	238
Guymon	Intrada/Platte	USA	178/194	202/217/236
KS93U62	Century*3/TA2460	USA	178	202/217
OK Bullet	KS96WGRC39/Jagger	USA	178	236
OK Rising	KS96WGRC39/Jagger	USA	178	236
OK03716W	Oro Blanco/OK92403	USA	178	204
OK03825-5403-5	Custer*3/94M81	USA	178/194	238
OK04525	FFR525W/Hickok//	USA	178	202/217
OK05737W	KS96WGRC39/Jagger	USA	178	236
OK05741W	KS96WGRC39/Jagger	USA	178	236
OK05830	OK93617/Jagger	USA	178/194	202/208/217
OK05903C	(TXGH12588-120*4/FS4)/2174//Jagger	USA	178/194	202/208/217/236
OK05905C	(TXGH12588-105*4/FS4)/2174//Jagger	USA	178	236
Overley	(TAM-107 *3/TA 2460)/Heyne 'S'//Jagger	USA	178	202/208/217
PostRock	Ogallala/KSU94U261//Jagger	USA	178	202/208/217
Thunderbolt	Abilene/KS90WGRC10	USA	178	236
TX01V5719	U1254-4-7-3/Ogallala	USA	178/194	202/217
AP03T6115	Karl//Mit/Lancota/3/U1254-4-9-8-V32	USA	178	236
AP05T2413	(KS95U522/TX95VA0011)F1/Jagger	USA	178	236
AR93005	Wakefield/KS91WGRC11	USA	178/194/220	204/211
Fannin	TAM 105/3/NE70654/BBY//BOW 'S'/4/Century*3/TA2450	USA	178/194	202/217/238
KS970187-1-10	TAM107*2/TA759//HBC197F-1/3/2145	USA	178/194	202/213
NE02558	Jagger/Alliance	USA	178	202/213
NE05496	KS95HW62-6 (= KS87H325/Rio Blanco)/Hallam	USA	178	202/213
Pete	N40/OK94P455	USA	178/194	238
SD06W117	Alice/SD00W024	USA	178	238
T153	T136/T151	USA	178	238
TX03A0563	X96V107/Ogallala	USA	178	236
TX04M410211	Mason/Jagger//Ogallala	USA	178	236
Bacup	Nuy Bay/Pioneer2375//Marshall	USA	178	202
Cardinal	Logan *2/3/Va63-5-12/Logan//Blueboy	USA	178/194	202/217
Ernie	Pike/3/Stoddard/Blueboy//Stoddard/D1707	USA	178	202/208/217
Foster	Ky83-60/Tyler//KY83-75	USA	178	202
Freedom	GR876/OH217	USA	178	202

touchdown program modified from Ma et al. (2005) was used for the PCR amplifications. The reaction was incubated at 95°C for 5 min then continued for five 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 five cycles, the annealing temperature started at 58°C for 2 min with a decrease of 2°C for each subsequent cycle. The amplification 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. Polymerase chain reaction products were analyzed on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA).

Bulked-segregant analysis (Michelmore et al., 1991) was used to identify polymorphic simple sequence repeat (SSR)

Table 1. Continued.

Variety	Pedigree	Source	*Xcfd15*†	*Xwmc432*
IL93-2283	IL84-3511/IL84-3348	USA	178/200	202
IL94-1549	Auburn/Ark38-1/Arther/Blueboy	USA	178/200	202/217
IL94-1909	Fillmore/Amigo//Tyler/Howell	USA	178	202
IL94-2426	Fillmore/Amigo//Tyler/Howell	USA	178	202
IL94-6280	IL87-3721/Cardinal//P808801-4-2-4-107	USA	178/200	202/217
IL95-1966	IL87-2834-1/IL87-6512//IL87-1968-1	USA	178	202
IL95-2066	IL88-7890/P7924H1-20-2-74	USA	178/200	202
IL95-2909	Freedom//IL84-2191-1/IL84-4046	USA	178	202
IL9634-24851	IL90-6364//IL90-9646/Ning 7840	USA		202
Kaskaskia	IL77-2933/IL77-3956//Pike/Caldwell	USA	178	202
MO-94-193	MO 11728/Becker	USA	178	202
MO94-312	Pioneer brand 2551/Caldwell	USA	178	202/217
OH552	Pur71761A4-31-5-33/MD55-286-21	USA	178/200	202
OH569	Pur 71761A4-31-5-33/MO 55-286-21	USA	178/200	202
P93D1-10-2	851423/INW9853	USA	178	202
PA8769-158	Titan/Caldwell	USA	178/200	202
PB2555	Coker68-16/MoW 7140//Pioneer brand W521	USA	178/200	202/217
Pontiac	Magnum/Auburn	USA	178/194/200	202/238
Roane	VA71-54-147/Coker68-15//IN65309C1-18-2-3-2	USA	178	202
Poncheau	Sel. from land race	France	178	202/208/217
Encruzilhada	Fortaleza/Kenya Farmer	Brazil	178	202
Expert	Extrem/Mexico4040//Neuhof1/3/Extrem/HP35719	Austria	178	202/208/217
Extrem	Record/Br. Herrachweiten	Austria	178	202
Karat	Extrem/Betosfeje1	Austria	178	202/236
Livius	Karat/Lentia	Austria	178	202/217
Perlo	Extrem/Betosfeje1	Austria	178	202
Spartakus	Perlo/Extrem/Betosfeje1	Austria	178	202
111.92	FengKang15/Cooperacion Nanihue	Argentina	178	202/236
113.92	FengKang15/Cooperacion Nanihue	Argentina	178	202/236
117.92	FengKang15/Cooperacion Nanihue	Argentina	178	202/236
38M.A.	Barleta 4d/Chino	Argentina	178/194	202/217
Coop-Capoildo	Landrace	Argentina	178	202/236
Coop-Millan	Unknown	Argentina	178	240
Vilela-Sol	Landrace	Argentina	178	202/236
Chinese Spring	Landrace	China	178	202/211
NTDHP	Landrace from Jiangsu	China	178	202
Par-55	Unknown	China	178	202/236
PC-2	Lira's'//AU/UP301	China	178	202/238
Wangshuibai	Landrace from Jiangsu	China	178	202/217
Xianmai1	Ardito/Tevere//Wannian2	China	178	202/236
Sumai3	Funo/Taiwan Wheat	China	178	202/236
Sumai49	N7922/Ning7840	China	178	202/236
Sanshukomugi	Landrace from Mie	Japan	178	202/238
Shinchunaga	Landrace from Mie	Japan	178	202/238
ShirasayaNo1	Landrace from Mie	Japan	178	202/238

†Size of amplified fragments in base pairs from each wheat accession.

markers associated with *Lr42*. Equal amounts of DNA were pooled separately from five *Lr42*-resistant and five *Lr42*-susceptible NILs. In the original *Lr42* report, *Lr42* was located on 1DS with gene *Lr41* (Cox et al., 1994). However, recent molecular mapping work relocated *Lr41* (Sun et al., 2009, Singh et al., 2004) to 2DS, not 1DS. To further confirm the physical location of *Lr42*, 60 microsatellite markers (SSR) from chromosome 1D and 55 markers from 2D (Somers et al., 2004; Röder et al., 1998) were screened between the parents and between the two bulks. Polymorphic markers between the bulks were further analyzed on all the NILs for linkage analysis.

Data Analysis

The data collected from the ABI DNA analyzer were processed by using GeneMarker version 1.6 (SoftGenetics LLC, State College, PA) and rechecked twice manually for accuracy. Genetic linkage among SSR makers and the leaf rust resistance locus was determined by JoinMap 3.0 (Van Ooijen and Voorrips, 2001) using the Kosambi mapping function (Kosambi, 1944) with a LOD threshold of 3.0.

RESULTS

Reactions of NILs and Genetically Diverse Accessions to Leaf Rust Infection

OK92G205 and OK92G206 showed a fully susceptible reaction when they were inoculated with the isolate PRTUS25 at the adult growth stage. KS93U50 adult plants showed moderate resistance with an infection type (IT) of 2+. Near-isogenic lines ranged from fully susceptible to moderately resistant to leaf rust infection. When the parents were inoculated with PRTUS25 and PRTUS35 at the seedling stage, KS93U50 showed incomplete resistance with ITs of 2C and 2+, respectively, whereas the ITs of two susceptible parents and the check TAM 110 were 3 and 3+ (Table 2). The culture PNMRJ induced ITs of 1 in KS93U50 and 3+ in OK92G205 and OK92G206. The seedling resistance classification of each individual NIL to all three isolates was consistent and agreed with results from the adult plants with three exceptions of susceptible adults that were scored as resistant seedlings. These were interpreted as scoring errors of the adult plants.

Table 2. Infection types evaluated by inoculating three wheat parents and controls contrasting in *Lr42* gene derived from *A. tauschii* with three *Puccinia triticina* isolates at the seedling stage and one isolate at the adult stage.

Name	PRTUS25†	PRTUS35	PNMRJ	PRTUS25‡
KS93U50 (*Lr42*)	2C	2+	1	Moderately resistant
OK92G205	3	3	3+	Susceptible
OK92G206	3+	3	3+	Susceptible
Fannin	0;	3–	2+3–	Not done
AR93005	1;	1;	1;	Not done
TAM 110	3	3	3+	Susceptible

†The seedling infection types are: 0 = no uredinia or other signs of infection; (;) = hypersensitive flecks; 1 = small uredinia surrounded by necrosis; 2 = small to medium uredinia surrounded by necrosis or chlorosis; 3 = medium-sized uredinia with or without chlorosis; + or – = uredinia somewhat larger or smaller than average for the class; C = extra chlorosis. Rating scale from McIntosh et al. (1995).

‡The adult plant reaction when inoculated with leaf rust isolate PRTUS25.

Markers for *Lr42*

When 115 SSR markers from chromosomes 1D and 2D were screened between parents and bulks, two SSR markers on 1DS (*Xcfd15* and *Xwmc432*) showed polymorphism between parents and between bulks. Primer CFD15 amplified a 220-bp fragment in KS93U50 and the resistant bulk (Fig. 1a,c) and a 178-bp fragment in the susceptible parents (OK92G205 and OK92G206) and the susceptible bulk (Fig. 1b,d). Primer WMC432 amplified two fragments, 204 and 211 bp, in KS93U50 as the specific banding pattern associated with *Lr42*. These markers were further used to analyze the 44 NILs. Linkage analysis using the two markers and rust data identified *Xwmc432* as a closely linked marker at 0.8 cM proximal to *Lr42* (Fig. 2a). Marker *Xcfd15* was also close, about 1.6 cM proximal to *Lr42*. A distal flanking marker for *Lr42* was not identified.

To verify the physical location of *Lr42*, the proximal marker *Xwmc432* was analyzed in a set of nullitetrasomic lines. The primer WMC432 amplified two fragments of 204 and 211 bp in N2D-T2A and N2D-T2B but not in N1DT1B and DT1DL, suggesting that marker *Xwmc432* was on chromosome 1D (Fig. 3). Furthermore, appearance of the marker in DT1DS confirmed that *Lr42* is on chromosome 1DS.

To evaluate the potential use of these *Lr42* markers in MAS, polymorphism of these markers was determined in

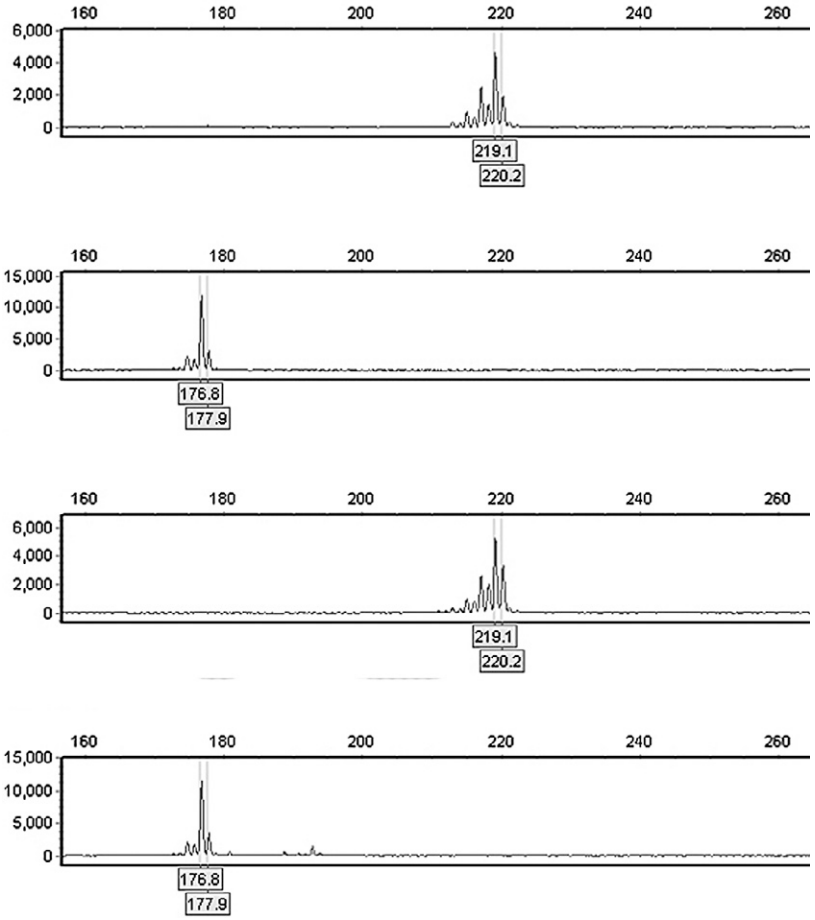

Figure 1. ABI electrophorograms of simple sequence repeat (SSR) marker *Xcfd15* on chromosome 1D showing polymorphism among (a) KS93U50 (*Lr42*), (b) OK92G206 (susceptible parent), (c) resistant bulk, and (d) susceptible bulk. The allele sizes in the figure were rounded to the closest integer.

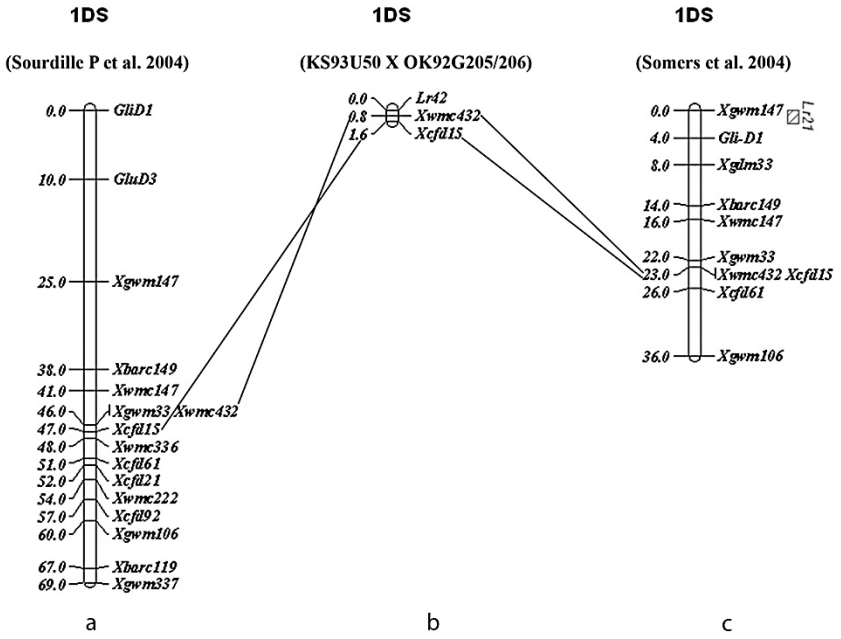

Figure 2. Comparison of the *Lr42* genetic map with a previously reported consensus map (Somers et al. 2004) to show location of *Lr42* in chromosome arm 1DS. (a) The simple sequence marker (SSR) genetic map developed by Sourdille et al. (2004). (b) The map with *Lr42* developed in this study. (c) The consensus map developed by Somers et al. (2004). The centromere is toward the bottom of the map. The region of gene *Lr21* was estimated from Huang et al. (2003).

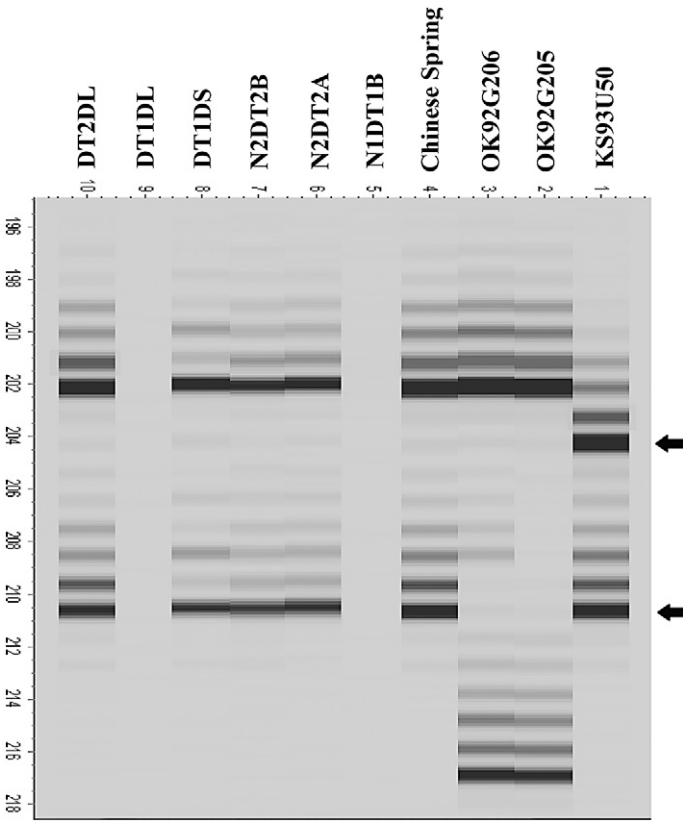

Figure 3. ABI gel image to show the fragments amplified by WMC432 in KS93U50 (*Lr42*-carrier), OK92G205 (susceptible parent), OK92G206 (susceptible parent), Chinese Spring, and Chinese Spring DT2DL, DT1DL, DT1DS, N2D-T2B, N2D-T2A, and N1D-T1B. The fragments associated with *Lr42* are 204 and 211 bp (arrow). The *y* axis shows ladder size.

a diverse set of 85 additional wheat cultivars or breeding lines from different wheat classes and geographic areas (Table 1). WMC432 amplified nine alleles among 85 accessions with a single fragment amplified in 43 accessions and at least two fragments in 42 accessions. A 204/211-bp fragment combination was amplified by WMC432 in KS93U50 and another U.S. line, AR93005. In addition, a single 204-bp fragment without the 211-bp fragment was amplified in a U.S. hard winter wheat, OK03716W. A 211-bp fragment accompanied by an additional 202-bp fragment was amplified in the Chinese landrace, Chinese Spring. Primer CFD15 amplified four fragments across the 85 accessions. The 220-bp fragment was associated with *Lr42*-resistance as seen in KS93U50, whereas the 178-bp fragment was present in both susceptible parents and was not associated with *Lr42*. Among 85 accessions, the 220-bp fragment was only amplified from AR93005.

DISCUSSION

In this study, we determined the location of wheat leaf rust resistance gene *Lr42* from *A. tauschii* through genetic linkage mapping and aneuploid analysis of linked markers. The small number of polymorphic markers identified in this study was expected because the populations were derived from NILs in a Century background. Genetic analysis of the population of 44 NILs showed that both marker loci, *Xwmc432* and *Xcfd15*, were tightly linked to *Lr42*. The chromosome arm containing *Lr42* was confirmed by mapping one of the two markers on 1DS ditelosomic and 1D nullitetrasomic aneuploid stocks (Fig. 3). Based on previously published positions for the linked markers (Somers et al., 2004; Sourdille et al., 2004), *Lr42* is located near the middle of the short arm of chromosome arm 1D (Fig. 2).

In addition to *Lr42*, two other leaf rust resistance genes were reported on 1DS. *Lr21* is located at the distal end of 1DS about 4 cM distal to marker *Gli-D1* (Huang et al., 2003). Cox et al. (1994) estimated a recombination frequency of 0.286 ± 0.023 (approximately 33 cM using the Kosambi function) between *Lr21* and *Lr42* in a compilation of several crossing experiments. The consensus map of Somers et al. (2004) indicates that *Xwmc432* and *Xcfd15* cosegregate and are approximately 20 cM proximal to *Gli-D1*, so the location of *Lr42* may be closer to *Lr21* than expected based on the results of Cox et al. (1994). Due to lack of polymorphism for *Lr21* and associated markers, we were not able to directly estimate the distance in this study. Hiebert et al. (2008) located *Lr60* 8.4 cM distal to *Xbarc149* on 1DS, which should put *Lr60* about 17 cM distal to *Lr42* according to the map of Somers et al. (2004). The location would also be near *Lr21* based on the map of Huang et al.

(2003). In an allelism test, Hiebert et al. (2008) concluded that *Lr60* is 13.5 cM distal to *Lr21*, which would place *Lr60* and *Lr42* approximately 40 cM apart (Huang et al., 2003, Somers et al., 2004) To confirm the relationship between *Lr60* and *Lr42,* an appropriate population needs to be developed to test genetic linkage between *Lr60* and *Lr42*.

Cox et al. (1994) reported that the *Lr42* phenotype varied from a hypersensitive fleck to a mixed reaction of flecks and small sporulating pustules surrounded by necrosis or chlorosis. In the present study, resistant infection types were higher and ranged from 1 (small uredinia surrounded by necrosis) to 2+ (medium uredinia surrounded by necrosis or chlorosis) (Table 2). This difference may be partially attributed to the utilization of different rust cultures, although one culture (PRTUS25) was used in both studies. Culture PNMRJ gave the lowest infection type and clearly separated resistant from susceptible phenotypes in the NIL population. PNMRJ was fully virulent on *Lr24* and was most useful for phenotyping *Lr42*.

Virulence to *Lr42* in the United States was initially reported to be infrequent (Kolmer et al., 2006), but virulence has apparently increased to significant levels in the most recent surveys (Kolmer et al., 2008b). Nevertheless, line KS91WGRC11 containing *Lr42* plus *Lr24* continues to show a moderately resistant reaction in the field in Manhattan, KS, whereas the cultivar Century containing *Lr24* is highly susceptible (D. Wilson, personal communication, 2009) *Lr42* should be used in combinations with other leaf rust resistance genes to maximize its usefulness.

To date, *Lr42* has not been widely deployed in wheat breeding programs. Among 85 accessions, only two have the *Lr42* donor, TA2450, in their pedigrees (Table 1). A soft red winter wheat, AR93005 derived from KSWGRC11, carried the 220-bp fragment for marker *Xcfd15* and the 204/211-bp banding pattern for marker *Xwmc432* as seen in KS93U50 (Table 1). However, *Xwmc432* and *Xcfd15* alleles amplified in hard red winter wheat Fannin did not match those from KS93U50 and the resistant NILs. AR93005 had similar resistance to KS91WGRC11 when inoculated with culture PNMRJ (an avirulent isolate on *Lr42*), while Fannin was more susceptible than KS93U50 (Table 2). These data suggest that AR93005, but not Fannin, carries *Lr42* and that the two SSR markers, *Xcfd15* and *Xwmc432,* predict the presence of *Lr42* derived from KSWGRC11.

Using MAS to assemble gene combinations with *Lr42* requires closely linked or flanking markers and sufficient marker polymorphism in the parental lines. The two new markers are mapped within 2 cM of *Lr42* and the 220-bp fragment for marker *Xcfd15* and the 204/211-bp banding pattern for marker *Xwmc432* are good predictors for *Lr42* therefore are adequate for MAS. After screening 85 genetically diverse accessions from different regions, we suggest that the polymorphism for both markers is suitable for MAS in a broad range of germplasm.

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