NOTE

Molecular Markers for Leaf Rust Resistance Gene *Lr42* in Wheat

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ABSTRACT

Wheat leaf rust, caused by *Puccinia triticina* Eriks., is an important wheat (*Triticum aestivum* L.) foliar disease worldwide. Growing cultivars incorporating genetic resistance is one of the most effective approaches for disease control. Leaf rust resistance gene *Lr42* was identified from a wheat relative, *Aegilops tauschii* Coss. (goatgrass), and has been transferred into hard winter wheat. A previous study identified two markers closely linked to the gene on the short arm of chromosome 1D (1DS) using a near-isogenic population, but flanking markers for *Lr42* were not found. In this study, a new mapping population was developed from a cross between 'KS93U50' (a *Lr42* carrier) and a susceptible parent, 'Morocco'. An F_2 population was analyzed with all simple sequence repeat (SSR) markers available from chromosome 1D and F_2 plants and F_3 families were evaluated for seedling resistance to isolate PNMRJ, a rust isolate avirulent to *Lr42*. The F_2 and F_3 rust data showed that *Lr42* was recessive. Seven markers formed the linkage group on chromosome 1DS. The *Lr42* region was flanked by two SSR markers, *Xwmc432* and *Xgdm33*, at 18 cM apart. The results confirmed that *Lr42* is positioned on the distal end of chromosome 1DS. The flanking markers for *Lr42* should be useful for map-based cloning and marker-assisted pyramiding of *Lr42* with other leaf rust resistance genes.

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

Leaf rust of wheat, caused by *Puccinia triticina* Eriks., is one of the most important wheat foliar diseases worldwide (Kolmer, 1996). Severe leaf rust epidemics can cause yield losses up to 40% (Knott, 1989). Growing genetically resistant cultivars is one of the most effective strategies to minimize yield losses due to the disease. To date, at least 71 genes for resistance to leaf rust have been cataloged in wheat (Singh et al., 2012). Of these, about half were introgressed from related species. Many of them were from *Aegilops tauschii* [syn. Triticum tauschii (coss.) Schmalh], the D genome donor of common wheat, including *Lr21* (chromosome 1DS), *Lr22a* (chromosome 2DS), *Lr32* (chromosome 3DS), *Lr39* (chromosome 2DS), and *Lr42* (chromosome 1DS) (Rowland and Kerber, 1974; Kerber, 1987; Gill et al., 1991; Cox et al., 1994).

Most *Lr* genes confer race-specific seedling resistance and are vulnerable to defeat by new virulent races. Greater durability of resistance could be achieved through combinations of race-specific genes or by using race-nonspecific resistance genes, such as *Lr34* and *Lr46* (Kolmer et al., 2008a, 2008b). However, such genes provide low levels of resistance when deployed singly (Zhang et al., 2008). A third option is to combine both race-specific and race-nonspecific resistance.

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Combining race-specific and race-nonspecific resistance genes in a single cultivar could significantly improve both durability and the level of resistance (Kolmer et al., 2008a; Zhang et al., 2008). For instance, in spring wheat, the combination of *Lr16*, *Lr23* (race-specific resistance genes), and *Lr34* (a race-nonspecific resistance gene) has provided effective and durable resistance for many years (Kolmer et al., 2008a, 2008b). Genes *Lr34* and *Lr13* were demonstrated to enhance the level of resistance synergistically when in combination with other leaf rust resistance genes (Germán and Kolmer, 1992; Kolmer, 1992). Two recent papers also demonstrated the ability of quantitative race-nonspecific resistance to extend the usefulness of qualitative race-specific resistance genes (Brun et al., 2010; Pailloix et al., 2009).

Stacking of several leaf rust resistance genes using phenotypic selection is difficult. A variety of rust races are often not available that could distinguish the presence or absence of different genes. If some genes in the stack confer high resistance, they can mask the phenotypic expression of other genes. Molecular markers can make it possible to stack several genes simultaneously in a single genetic background; therefore, identifying closely linked markers for each resistance gene is essential for successful use of these genes in breeding.

Lr42 is a gene conferring rust resistance at both seedling and adult plant stages and remains effective against all leaf rust races reported to date (J. Kolmer, personal communication). Lines containing *Lr42* have been used as a parent in some breeding programs with success (Bacon et al., 2006; Singh et al., 2007). Previous work located *Lr42* on chromosome 1DS (Cox et al., 1994) and found that *Lr42* also played a significant role in increasing wheat yield and kernel size, apparently due to the resistance (Martin et al., 2003). Using molecular mapping, Czembor et al. (2008) located *Lr42* to a different chromosome (3D) using Diversity Arrays Technology markers and reported that the gene was dominant. Sun et al. (2010) further mapped *Lr42* to the distal end of chromosome 1DS using simple sequence repeat (SSR) markers and the same near-isogenic population developed by Martin et al. (2003) and identified two closely linked markers. Therefore, additional mapping work using different populations is necessary to validate the chromosome location of *Lr42*. The current study examined a population derived from the cross KS93U50 × Morocco to (i) confirm the gene location in a new population, (ii) study the inheritance pattern of the gene, and (iii) identify flanking markers linked to the gene for marker-assisted selection (MAS).

MATERIALS AND METHODS

Plant Materials and Rust Inoculation

The mapping population was developed by crossing KS93U50 and Morocco. KS93U50 is a hard red winter wheat accession containing *Lr42* selected from 'KS91WGRC11' (PI 566668; pedigree 'Century'★ 3/*Ae. tauschii* TA2450) and provided by the Wheat Genetic and Genomic Resources Center, Kansas State University, Manhattan, KS. Morocco is an old soft white spring cultivar originally collected from North Africa and frequently used as a susceptible check for leaf, stem, and stripe rust.

A total of 371 F_2 plants derived from the cross KS93U50 × Morocco was inoculated with rust race PNMRJ. The isolate PNMRJ is avirulent to *Lr42* but virulent *Lr24* and several cultivars tested (Sun et al., 2010). All F_2 plants and both parents were planted in 72-cell plastic flats and inoculated with PNMRJ at the two-leaf stage. Before inoculation, rust spores from a liquid N_2 tank were heat shocked in a water bath at 42°C for 5 min to break dormancy. The spores were suspended in Soltrol 170 light oil (Chevron Phillips Chemical Company) and misted uniformly over the seedling leaves using a pressure sprayer. After inoculation, the plants were incubated in a moist chamber at 20°C for 24 h before being moved to a greenhouse bench for rust establishment. The plants were grown at 20°C ±3°C supplemented with 10 h daylight. The rust infection type (IT) on a 1 to 4 scale as described previously (Stakman et al., 1962; Roelfs and Martens, 1988) was recorded at 10 d after inoculation and rechecked 2 d later for confirmation. After rust data were collected, all F_2 plants were transplanted to 13 by 13 cm plastic pots for seed increase. Seeds from 361 F_2 plants (10 F_2 plants did not set any seed) were harvested individually and planted in 72-cell plastic growth trays for rust evaluation. Six seeds per $F_{2:3}$ family were planted and the experiments had two replications. Six seeds per parent also were planted in each tray as control. Rust inoculation and disease scoring for the parents and $F_{2:3}$ families were the same as for the F_2 population. Based on parent reactions to PNMRJ, plants with IT ≤ 2 were classified as resistant and IT ≥ 2^+ as susceptible for segregation analysis. The segregation ratio from $F_{2:3}$ families was used to determine the genotypes of F_2 plants and the combined data from both generations were used to determine inheritance pattern.

Marker Genotyping and Data Analysis

After rust evaluation, newly developed disease-free wheat leaf tissue was collected in 1.1 mL 96-deepwell plates for DNA isolation. Tissue samples were immediately dried in a freeze-dryer (ThermoSavant) for 48 h and ground to fine powder using a Mixer Mill (MM300; Rotsch) by shaking the tubes at 25 times s^{-1} for 5 min with a 3.2-mm stainless steel bead in each well. Genomic DNA was extracted using a modified cetyltrimethyl ammonium bromide method (Sun et al., 2009). Because *Lr42* was previously located on chromosome 1D (Cox et al., 1994), 27 SSR primers on chromosome 1D (Somers et al., 2004; Song et al., 2005; Röder et al., 1998) were selected to screen the parents for polymorphism. Polymerase chain reaction (PCR) amplifications were performed in a Tetrad Peltier DNA Engine (Bio-Rad Lab). A 12 μL PCR mixture contained 1.2 μL of 10x PCR buffer (Bioline), 2.5 mM of $MgCl_2$, 200 μM of each deoxyribonucleotide triphosphate, 50 nM of forward M13-tailed primer, 250 nM of reverse primer and 200 nM of M13 fluorescent-dye-labeled primer, 0.6 U of *Taq* DNA polymerase, and about 50 ng template DNA. A touchdown PCR program was used for PCR amplification. In brief, the reaction was incubated at 95°C for 5 min and then continued for five cycles of 1 min at 96°C, 5 min at 68°C with a decrease of 2°C in each subsequent cycle, and 1 min 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

Table 1. Segregation of *Lr42* in F_2 and $F_{2:3}$ populations derived from a cross of KS93U50 × Morocco when inoculated with *Puccinia triticina* Eriks. isolate PNMR at seedling stage.

Generation	No. of lines	Observed ratio†	Expected ratio†	χ^2	*P*-value
F_2	371	89:282	1:3	0.202	0.653
F_3	361	86:167:113	1:2:1	6.781	0.034

†F_2 ratio is resistant:susceptible; F_3 ratio is resistant:segregating:susceptible.

subsequent cycle. Reactions then 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 3730xl DNA Analyzer (Applied Biosystems). Data were analyzed using GeneMarker software version 1.6 (SoftGenetics, 2011) and manually checked twice to remove ambiguous data.

A linkage map was constructed using Kosambi (1944) function and MultiPoint package (Mester et al., 2003). A logarithm of odds threshold of 3 was used to identify linkage groups.

RESULTS

Ten days after inoculation with PNMRJ, the susceptible parent Morocco showed severe rust symptoms with IT ≥ 2^+ while most of plants of the resistant parent, KS93U50, only showed tiny pustules surrounded by necrosis and with IT ≤ 2. In F_2 and F_3 populations, IT ranged from 1 to 4 for different genotypes. Because most of the plants of the susceptible parent had IT ≥ 3 with only a few plants that had IT = 2^+, plants with IT ≥ 2^+ were classified as susceptible genotypes and plants with IT ≤ 2 were classified as resistant genotypes. Of 371 F_2 plants evaluated 282 were susceptible and 89 were resistant, fitting a 3:1 ratio (χ^2 = 0.202, *P* = 0.653), indicating that *Lr42* behaved as recessive in the KS93U50 × Morocco population. The $F_{2:3}$ family segregation fit a 1 resistant:2 segregating:1 susceptible ratio (χ^2 = 6.781, *P* = 0.034), with slight skewing toward susceptible families (Table 1). In each segregating family, most of the plants were susceptible, which confirmed that *Lr42* is recessive.

Of the 27 SSR markers available on chromosome 1D, 11 proved polymorphic between two parents. Eight markers were mapped to one linkage group and three others were unlinked. Two of the eight markers, *Xbarc229* and *Xwmc336*, showed significant segregation distortion and were removed from the linkage group. The map with six markers covered 68.5 cM on chromosome 1D (Fig. 1). *Lr42* was mapped between *Xwmc432* and *Xgdm33* at 4 cM from *Xwmc432*. A slight segregation distortion was observed for the markers in the region of *Lr42* (Table 2).

Of the primers for these markers, Gdm33 amplified a fragment of 177 bp in KS93U50 and a fragment of 145 bp in Morocco. Primer Wmc432 amplified two fragments in each of the two parents, 204 and 212 bp from KS93U50 and 203 and 218 bp from Morocco, but only the larger fragments were segregating in the population and mapped in the *Lr42* region.

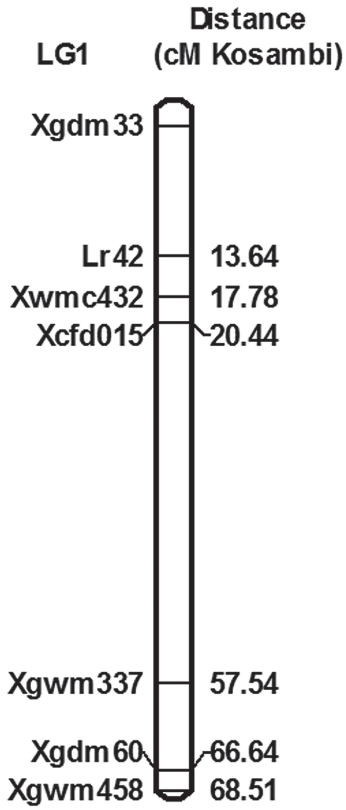

Figure 1. A genetic linkage map developed from the F_2 population of KS93U50 × Morocco showing *Lr42* on the short arm of the chromosome 1D of wheat.

Table 2. Segregation of marker alleles located on chromosome 1D.

Marker	No. of lines	Observed ratio†	Expected ratio†	χ^2	*P*-value
Xgdm33	359	76:174:109	1:2:1	6.404	0.041
Xwmc432	360	83:167:110	1:2:1	5.928	0.052
Xcfd0015	364	84:169:111	1:2:1	5.863	0.053
Xgwm337	355	80:188:87	1:2:1	1.518	0.468
Xgdm60	359	89:183:87	1:2:1	0.159	0.924
Xgwm458	357	93:173:91	1:2:1	0.361	0.835

†Ratio is for the marker alleles associated with *Lr42* alleles for homozygous resistance, heterozygous and homozygous susceptibility.

DISCUSSION

In this study, most F_2 plants were susceptible and segregation indicated a single recessive gene for resistance. Segregation in the F_3 population was consistent with the hypothesis that resistance to rust isolate PNMRJ due to *Lr42* in KS93U50 was recessive. This result disagrees with previous reports that *Lr42* was dominant (Czembor et al., 2008) or partially dominant (Cox et al., 1994). The discrepancy could result from different isolates and genetic materials used in different studies. Kolmer and Dyck (1994) demonstrated that expression of resistance genes could range from complete dominance to complete recessiveness and the expression of resistance and avirulence genes in wheat leaf rust system is highly dependent on the genotypes of the host lines and pathogen isolates used. In this

study, KS93U50 was used as the source of *Lr42* that was a selection from KS91WGRC11 while KS91WGRC11 was used in the other studies (Czembor et al., 2008; Cox et al., 1994). Pathogen inocula used in these studies might also be different. In this study, several local isolates were evaluated using a set of parents and near-isogenic lines and we found that PNMR could clearly differentiate *Lr42* from various susceptible genotypes (Sun et al., 2010). Therefore, it was the isolate of choice used in a previous study. However, for direct resistance evaluation in segregating breeding materials, this isolate may not be ideal because phenotypic differences are not sufficient to distinguish heterozygous from homozygous susceptible plants.

The closest marker to *Lr42* is *Xwmc432*, which confirmed the previous report (Sun et al., 2010). In the previous study, a backcross-derived homozygous near-isogenic population was used and disease scores were treated as binary data. In this study, F_2 was used for mapping. The genetic distance between *Lr42* and X*wmc432* is about 3 cM longer in this study than in the previous study. In this study, F_3 rust data agreed with F_2 in general, but heterozygous status of some F_2 plants could not be detected due to a limited number of F_3 plants per family evaluated for rust resistance, which might contribute to expanded genetic distance between *Lr42* and *Xwmc432*. In addition, we identified one new linked marker, *Xgdm33*, distal to *Lr42*. *Xwmc432* and *Xgdm33* defined *Lr42* in a 17.8 cM region. Therefore, they can be used as flanking markers for further fine mapping in the region to facilitate map-based cloning of the gene.

Two markers developed in this study are good candidate markers for MAS of *Lr42*. Primer Wmc432 amplified two fragments in both parents, but only the 212 bp fragment from KS93U50 and the 218 bp fragment from Morocco associated with *Lr42*, as in a previous study (Sun et al., 2010). This marker should be the first choice for MAS. Because allele size differences between the two parents were relatively large for *Xwmc432* and *Xgwm33*, they can be analyzed in an agarose gel in breeding programs. A high concentration agarose gel is recommended when using *Xwmc432*.

In this study, four markers closely linked to *Lr42* were positioned on chromosome 1DS based on the previously reported linkage maps (Somers et al., 2004; Song et al., 2005), which disagrees with Czembor et al. (2008). However, our results are in agreement with the previous report by Sun et al. (2010) despite different types of populations (near-isogenic versus F_2 populations) and susceptible parents used in the two studies. The discrepancy among different studies could be due to difference in resistant parents used in different studies as discussed previously. Therefore, if the markers developed in this study are to be used for screening *Lr42* gene, it is advised that KS93U50 be used as the source of resistance.

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