Theor Appl Genet (2001) 103:1007–1013

L. Huang · B.S. Gill

An RGA – like marker detects all known *Lr21* leaf rust resistance gene family members in *Aegilops tauschii* and wheat

Received: 22 December 2000 / Accepted: 12 February 2001

Abstract Leaf rust is one of the most important diseases of wheat worldwide, particularly in the Great Plains region of the USA. One long-term strategy for the control of this disease may be through durable genetic resistance by gene pyramiding. An important step in this strategy is identifying molecular markers linked to different leaf rust-resistance genes. Here we report the molecular tagging of a leaf rust-resistance gene that may have the potential for durable resistance through further genetic manipulation and gene pyramiding. *Lr39* was previously designated for a leaf rust-resistance gene introgressed from *Aegilops tauschii* accession TA1675 into the common wheat germplasm WGRC2. *Lr40* was designated for a gene derived from *Ae. tauschii* accession TA1649 and is present in germplasm WGRC7. These genes are now believed to be allelic to *Lr21*, which was transferred to wheat from a different accession of *Ae. tauschii*. Molecular mapping of *Lr39* and *Lr40* indicates that both genes come from TA1649. WGRC2 and WRGC7 also have a similar infection type against rust culture PRTUS6. We suggest the designation of the gene in WGRC2 should be changed to *Lr40*. RFLP marker KSUD14 (locus *Xksud14*) was found 0.2-cM proximal to *Lr40* in a WGRC2/Wichita F_2 population (218 individuals), and co-segregated with the gene in a WGRC7/Wichita F_2 population (165 individuals). A PCR-based molecular marker developed from the sequence-tagged-site (STS) of *Xksud14* was mapped to the same locus as the RFLP marker KSUD14 in both populations. KSUD14 has the structure of a resistance gene analog (RGA) including kinase2a and kinase3 domains similar to the *Cre3* gene of wheat and the rust resistance gene *Rp1-D* of maize. When the PCR products amplified from KSU14 STS were cleaved with restriction enzyme *Msp*I, an 885-bp fragment was found in WGRC2, WGRC7, the *Lr21* near-isogenic line, and eight accessions of *Ae. tauschii* shown to have resistance gene alleles at the *Lr21* locus. The KSUD14 PCR-based assay provides an excellent marker for *Lr40* and *Lr21* in diverse wheat breeding and wild *Ae. tauschii* populations.

Keywords Leaf rust resistance · Molecular marker · *Aegilops tauschii* · Sequence-tagged-site

Communicated by H.C. Becker

L. Huang · B.S. Gill (✉)
Wheat Genetics Resources Center, Department of Plant Pathology, Throckmorton Plant Science Center, Kansas State University, Manhattan, KS 66506-5502, USA
e-mail: bsg@ksu.edu

Introduction

Leaf rust, also called brown rust, is caused by the fungal pathogen *Puccinia triticina*, and is the most-widespread disease of wheat (Kolmer 1996). The fungus produces small elliptical orange-red pustules on the upper leaf surface, causes premature defoliation that results in shriveled kernels, and may reduce grain yield by as much as 40% (Knott 1989). Utilization of genetic resistance is the most economical and environment-friendly strategy for disease control.

To-date, more than 50 leaf rust-resistance genes have been identified (for a review see McIntosh et al. 1995). Nineteen *Lr* genes have been tagged by molecular/cytological markers (for a review see Langridge and Chalmers 1998; Seyfarth et al. 1999; Seah et al. 2000; Spielmeyer et al. 2000). However, tightly linked PCR-based markers have been developed only for *Lr*1 (Feuillet *et al*. 1995), *Lr10* (Feuillet et al. 1997), *Lr25* and *Lr29* (Procunier et al. 1995), *Lr28* (Naik et al. 1998), *Lr35* (Seyfarth et al. 1999) and *Lr37* (Seah et al. 2000). Molecular markers, particularly PCR-based markers, allow for large-scale genotypic selection of individuals in breeding populations. These markers may be used to pyramid different leaf rust-resistance genes. Moreover, molecular markers tightly linked to a gene also are the starting points for positional cloning of the gene (Martin et al. 1993; Song et al. 1995).

In our laboratory, we have focused on the mapping of leaf rust-resistance genes transferred from *Aegilops taus-*

chii into wheat. To-date, six named genes (chromosome and/or arm location in brackets) *Lr21* (1DS), *Lr22a* (2DS), *Lr32* (3D), *Lr41* (1D), *Lr42* (1DS) and *Lr43* (7DS) (reviewed in Cox et al. 1994, see Hussien et al. 1997 for *Lr43*), and two other genes *Lr39* and *Lr40* whose designations are under consideration, have been described. In allelism tests, *Lr41* segregated independently of all other genes. *Lr42* was linked to *Lr21* on 1DS arm with a recombination value of 0.286±0.23. Cox et al. (1994) found that *Lr39* and *Lr40* were allelic to *Lr21* although *Lr39* was mapped previously on 2DS (Raupp et al. 1991). Six *Ae. tauschii* accessions (not including the donor accessions for *Lr21* and *Lr40*) were found to have a leaf rust-resistance gene allelic to the *Lr21* locus (for details see Materials and methods section). The objective of this study was to identify molecular markers linked to leaf rust-resistance gene *Lr40* and to further clarify its relationship with *Lr21*.

Materials and methods

Plant material

The information and pedigrees of the plant materials used in this study are listed in Table 1. The pedigrees of the resistant germplasm and the origin of donor accessions are listed. Wichita, released in 1944, is a hard red winter wheat cultivar bred for the wheat-growing areas of the Great Plains and is susceptible to leaf rust. Two F_2 populations derived from crosses of WGRC2/Wichita (218 individuals) and WGRC7/Wichita (165 individuals) were used for molecular mapping. *Lr21* (TC), a Thatcher near-isognic line containing *Lr21*, was obtained from P. Dyck (Agriculture Canada). *Ae. tauschii* accessions TA1691, TA2378, TA2483, TA2495, TA2527 and TA2528 each have a leaf rust-resistance gene that is allelic to *Lr21* (Miller 1991; Cox et al. 1994, and Cox, personal communication). TA2460 and TA2540 are the donor accessions of *Lr41* and *Lr42*, respectively (Cox et al. 1994). Accession TA1704 is susceptible to the leaf rust pathogen.

Table 1 The plant material used in this study

Germplasm	Pedigree/description
KS86WGRC2	*Ae. tauschii* TA1675/3* Wichita
KS89WGRC7	Wichita//*Ae. tauschii* TA1649/2* Wichita
Lr21 (TC)	Thatcher/*Ae. tauschii* TA1599
Cultivar	
Wichita	Hard red winter wheat
Ae. tauschii accession	Origin
TA1649	Iran
TA1675	Turkmenistan
TA1691	Unknown
TA1599	Iran
TA2378	Iran
TA2483	Iran
TA2495	Iran
TA2527	Iran
TA2528	Iran
TA1704	Tajikistan
TA2460	Iran
TA2540	Iran

Plant disease inoculation and scoring

For gene tagging, F_2 and $F_{2:3}$ progeny plants (20 seeds from each bagged F_2 spike) were inoculated with *P. triticina* culture PRTUS 6, which belongs to race PBJ-10 (Long and Kolmer 1989). Inoculations were conducted at the two-leaf stage as described by Browder (1971). WGRC2 and WGRC7 were used as resistant checks and Wichita was the susceptible check. Disease severity was assessed 8–10 days after inoculation according to the scale of Roelfs (1985).

Bulk segregant analysis

For bulk segregant analysis, genomic DNA from the F_2 of 15 homozygous resistant plants (infection type 1), and 15 homozygous susceptible plants (infection type 4), were mixed in equal proportions to constitute the resistant and susceptible bulks. Genomic DNA was isolated after the method of Riede and Anderson (1996).

RFLP analysis

Enzyme digestion, gel-electrophoresis, Southern blotting, probe labeling, and hybridization were performed following the protocols described by Sharp et al. (1988). The probes designated as KSU (*Ae. tauschii* cDNA and genomic DNA) were reported by Gill et al. (1991); the BCD (barley cDNA) probes reported by Heun et al. (1991), were obtained from M. Sorrells (Cornell, USA); and the MWG clones (barley genomic DNA) were provided by A. Graner (IPK, Germany). All probes were prepared by PCR and purified by spin-column chromatography. The mapping data were analyzed with MAPMAKER V2.0 for Macintosh (Lander et al. 1987).

Gliadin gel-electrophoresis

Ten seeds from each F_3 family were used for gliadin gel-electrophoresis analysis. Each seed was cut in half, and the brush-end of the seed was used for analysis. Five half seeds were bulked for one sample. The gliadin proteins were extracted after Morris et al. (1989).

STS analysis

The primers based on the RFLP clone KSUD14 were developed by Talbert et al. (1994). The reaction mixture was 25 µl including 100–350 ng of template DNA, 1 pmol of each primer, 1 unit of Taq DNA polymerase (Promega), 1.25 µl of 50 mM $MgCl_2$, 2.5 µl of 10× buffer, and 2 µl of 2.5 mM dNTPs. PCR was performed in a Perkin-Elmer PCR System 9700: 5 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C each. The last cycle was for 5 min at 72 °C. PCR products were checked in 2.5% agarose gels at 50 V constant voltage for 5 h, or were digested by the 4-base pair cutter *Msp*I in a 15-µl volume containing 1.5 µl of 10× buffer and 10 units of enzyme at 37°C for 3 h, and then separated in 2.2% agarose gels at 35 V constant voltage.

PCR product cloning

The PCR product was cut from the gel and recovered using a Nucleo Trap kit (Clontech, cat# K3070–1). The purified product was cloned using a TOPO XL PCR cloning kit (Invitrogen, cat# K4700–10).

Results

Disease scoring

For the molecular mapping of the genes in WGRC2 and WGRC7, the two segregating F_2 populations and the $F_{2:3}$ families were inoculated with *P. triticinia* culture PRTUS6. WGRC2 and WGRC7 had the same phenotype, characterized by necrotic flecks or small pustules surrounded by necrosis. Resistant plants of the two populations showed infection types ranging from ;1 to ;2+. Heterozygous plants gave an intermediate infection type (;2 to ;2+). Disease scoring of the $F_{2:3}$ families revealed the genotype of the F_2 plants from the two populations (Table 2). The chi-square test indicated that the scoring results fit a 1:2:1 ratio. The data indicate that a single dominant gene controls the leaf rust resistance to culture PRTUS6 in each population.

Bulk segregant analysis

To identify molecular markers closely linked to the rust resistance genes, we used a bulk segregant analysis in the two populations. The bulks were assayed with ten 1DS-specific RFLP markers (KSUD14, KSUE18, BCD1434, BCD98, CDO388, CDO99, CDO580, MWG938, MWG36 and MWG837) using five restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV and *Hin*dIII) (for a total of 50 combinations). Only markers KSUD14 and BCD1434 showed polymorphism between the resistant and susceptible parents and the resistant and susceptible bulks from the two populations. The polymorphic enzyme/probe combinations revealed the same polymorphism patterns between the resistant/susceptible parents and the resistant /susceptible bulks in each population (data not shown). We noted that the fragments linked to the gene in the WGRC2 population are the same as those in the WGRC7 population, which correspond to TA1649 and not TA1675 (Fig. 1). Thus the gene in WGRC2 is from *Ae. tauschii* accession TA1649 and not TA1675. The analysis of the gliadin protein gene (*Gli-D1*) also supported this conclusion (data not shown).

Linkage analysis

To determine linkage relationships, *Gli-D1*, KSUD14 and BCD1434 were mapped in the two F_2 populations segregating for the genes conferring resistance to rust culture PRTUS6 (218 plants in the WGRC2/Wichita, and 165 plants in the WGRC7/Wichita, populations). Among the tested markers, five types of recombination events occurred between *Lr40/Xksud14*, *Xksud14/Gli-D1* and *Gli-D1/Xbcd1434* regions in the WGRC2/Wichita mapping population (Fig. 2a), including a double cross-over in the *Xksud14/Gli-D1* interval. Recombinants were also observed in the same marker intervals except in the *Lr40/Xksud14* interval in the WGRC7/Wichita mapping population (Fig. 2b). Linkage analysis indicated that *XksuD14* is 0.2 cM proximal to *Lr40* in WGRC2 (Fig. 2a), but co-segregated with *Lr40* in WGRC7 (Fig. 2b). *Gli-D1* and BCD1434 were mapped proximally to the rust gene in each population.

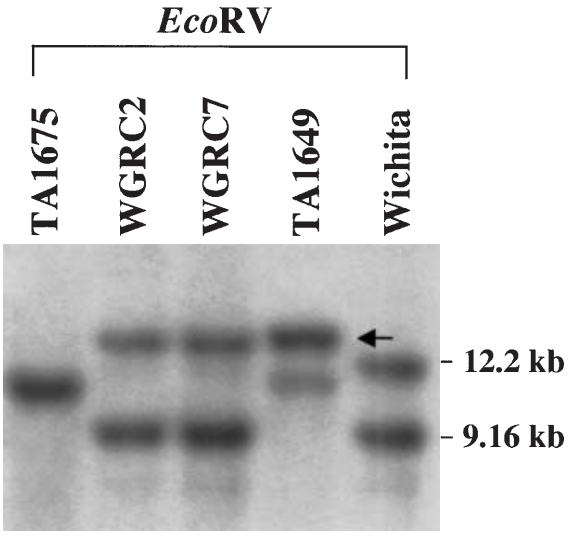

Fig. 1 RFLP polymorphism between WGRC2/Wichita, WGRC7/Wichita and their putative donor accessions of *Ae. tauschii* revealed by KSUD14 with restriction enzyme *Eco*RV. The *arrow* indicates the *Ae. tauschii* specific band in WGRC2, and WGRC7 matches that from TA1649 rather than TA1675

STS analysis

In order to develop a more-efficient marker for screening large populations, an STS marker assay based on the RFLP clone KSUD14 was developed. The PCR product amplified from WGRC2 and WGRC7 was approximately 1.36 kb in length. Two fragments of 1.49-kb and 1.59-kb in size from Wichita were amplified (Fig. 3). The 1.36-kb fragment was completely linked to the gene in the WGRC7/Wichita population and revealed the same re-

Table 2 F_2 segregation of genes in WGRC2/Wichita and WGRC7/Wichita populations

Cross	Genotype (F_2)			χ^2 (1:2:1)	*P* (value)
	RR	Rr	*rr*		
WRGC2/Wichita	58	113	47	1.404	0.5–0.75
WGRC7/Wichita	38	75	52	0.43	0.75–0.9

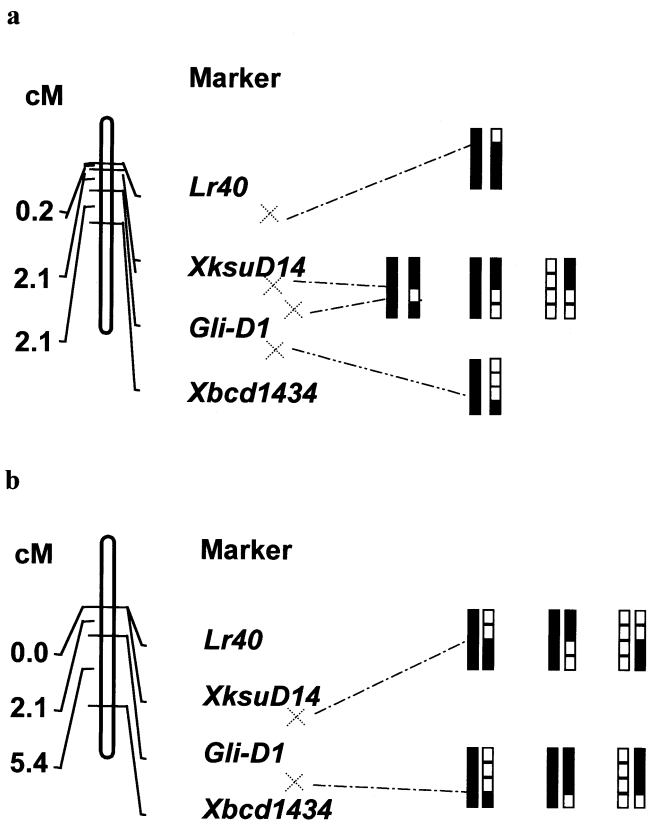

Fig. 2 The position of *Lr40* in the genetic linkage map of the 1D short arm of wheat detected in the WGRC2/Wichita (**a**) and WGRC7/Wichita (**b**) populations. In each map the centromere is towards the bottom end. The recombinant haplotypes detected for the corresponding map regions are shown on the right. The *symbols* between markers represent the number of crossovers detected in that marker interval

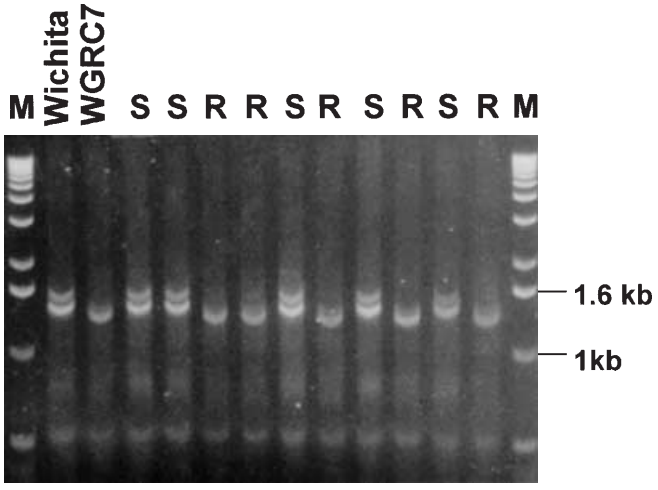

Fig. 3 Gel-electrophoresis patterns revealed by a sequence-tagged-site marker of *XksuD14* in a WGRC7/Wichita population. A 1.36-kb fragment is present in WGRC7 and all the leaf rust-resistant progenies. Two fragments of 1.49 kb and 1.59 kb in size are amplified from Wichita and the susceptible progenies. *M* 1-kb ladder; *R* resistant plant; *S* susceptible plant

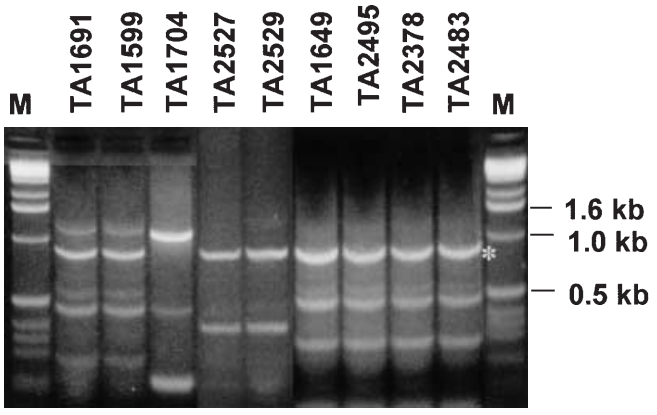

Fig. 4 Gel-electrophoresis patterns of *Ae. tauschii* accessions revealed by a sequence-tagged-site marker of *XksuD14* after digestion with the restriction enzyme *Msp*I. The 885-bp fragment (*asterisk*) is present in all leaf rust-resistant accessions with an *Lr* gene allelic to *Lr21* but is absent in the susceptible accession TA1704, which instead has a 1.0-kb fragment

combinant in the WGRC2/Wichita mapping population as the RFLP marker KSUD14. The STS marker also was used to test 12 *Ae. tauschii* accessions including TA1675 and TA1649. When the products were cut with the 4-base-pair cutter *Msp*I, an 885-bp fragment was detected in WGRC2, WGRC7, *Lr21* (TC) (data not shown), and all the *Ae. tauschii* accessions that have a leaf rust-resistance gene mapped at the *Lr21* locus (Fig. 4). This suggested that the 885-bp fragment may serve as an assay for resistant alleles at the *Lr21* locus. Instead, a 1-kb fragment was observed in Wichita and *Ae. tauschii* accession TA1704, which are both rust susceptible; accession TA1675, which has the rust-resistance *Lr39* gene on 2DS (Raupp et al. 2000); TA2460, the *Lr41* donor accession, and TA2540, the *Lr42* donor accession (data not shown).

Southern hybridization and sequence data of the 1.36-kb fragment

The 1.36-kb fragment amplified from TA1649 was cloned and sequenced (accession number: AF257240). Blast search of the sequence showed that the cloned fragment is a resistance gene analog and encodes kinase2a and kinase3 domains similar to the *Cre3* gene from wheat and the rust resistance gene *Rp1-D* in maize. Southern hybridization probed with the 1.36-kb fragment revealed multiple bands in *Ae. tauschii* accession TA1649 by *Xba*I (Fig. 5). Two of the fragments were transferred into WGRC2 and WGRC7. Nulli-tetrasomic analysis indicated the fragments transferred into WGRC2 and WGRC7 are located in wheat chromosome 1D.

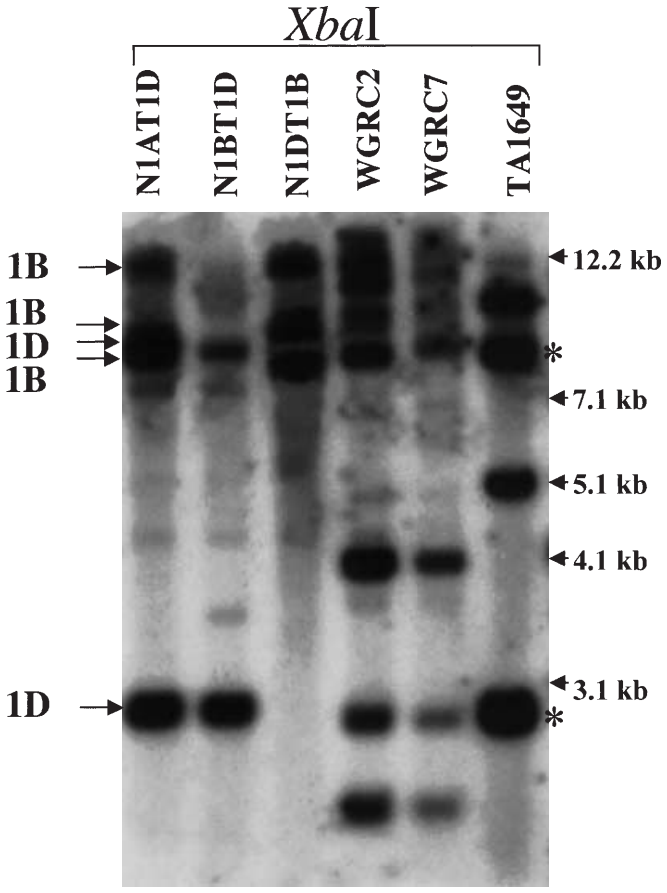

Fig. 5 RFLP patterns probed with the 1.36-kb fragment amplified from *Ae. tauschii* accession TA1649. Of the four bands revealed in TA1649, two were transferred to WGRC2 and WGRC7, and are indicated by an *asterisk* (*). Nulli-tetrasomic analysis revealed the chromosome location of specific fragments (shown on the left). The two fragments transferred from TA1649 to WGRC2 and WGRC7 correspond to the fragments on chromosome 1D

Discussion

Raupp et al. (1991) mapped the gene derived from *Ae. tauschii* accession TA1675 (*Lr39*, present in WGRC2) to chromosome arm 2DS by monosomic and telosomic analysis. Cox et al. (1994) located the gene on 1DS. Our mapping data from the population derived from WGRC2/Wichita support Cox's result. However, our data revealed that the gene in WGRC2 used for this study is derived from TA1649 not TA1675. Thus, the line 88–63 (WGRC2) used by Raupp et al. for monosomic analysis is different from the WGRC2 line used by Cox et al. and our study. Recently, Raupp et al. (2000) further confirmed that *Lr39* gene tracing to *Ae. tauschii* accession TA1675 is present in line 88–63 but not in the released germplasm WGRC2. Furthermore, marker analysis also confirmed that *Lr39* is located on 2D and that it is distinct from all other genes derived from *Ae. tauschii*. Based on their data and the data reported in this paper, we suggest that rust-resistance genes present in WGRC2 and WGRC7 should be tentatively designated *Lr40* pending further allelic studies with *Lr21*, which are now underway.

Previous studies revealed that each of the eight *Ae. tauschii* accessions collected from different locations in Iran have a resistance gene allelic, or tightly linked, to *Lr21* (Miller 1991; Cox et al. 1994). Some of the allelism studies used intercrosses among *Ae. tauschii* accessions with TA1599, the original donor of *Lr21* (Miller 1991). Cox et al. (1994) transferred resistance genes from *Ae. tauschii* accessions TA2495 and TA2470 into wheat, as breeding lines U1866 and U1865, respectively. All 330 F_2 plants from the cross U1865/*Lr21* (TC) gave a resistant reaction. All 883 F_2 plants from the crosses of U1866 with *Lr21* (TC), WGRC2, WGRC7 and 88–307 gave a resistant reaction. 88–307 is a hexaploid wheat line having an *Lr* gene from *Ae. tauschii* accession TA1691. However, in such F_2 populations, a susceptible plant has to result from two recombinant gametes without the resistance allele. If the recombination frequency between the two tested genes is lower than 0.067 (6.7%), it is impossible to resolve if these genes are alleles at a single locus, or different members of a complex locus, using a population of 883 F_2 plants. Further studies of the relationship among the genes that were mapped to the *Lr21* locus are needed. However, the accessions that have a gene mapped to the *Lr21* locus share a common fragment generated by the PCR-based marker KSUD14-STS. This fragment is absent in rust-susceptible accession TA1704 and three other resistance accessions TA1675 (*Lr39*), TA2460 (*Lr41*) and TA2540 (*Lr42*). Therefore, the KSUD14-STS marker assays all alleles or members at the *Lr21* locus.

With one exception, KSUD14 co-segregated with rust resistance in all 382 F_2 plants (764 gametes) from the crosses WGRC2/Wichita and WGRC7/Wichita. KSUD14 is a genomic clone derived from a *Pst*1-digested library of *Ae. tauschii* accession TA1691. The mapping population used for constructing the first linkage map of *Ae. tauschii* was segregating for a rust-resistance gene (designated as "RUST") derived from TA1691 (Gill et al. 1991). The *Lr* gene was mapped to the *Lr21* locus by allelism analysis (Cox et al. 1994). The "RUST" locus was mapped to the distal end of 1DS arm 43 cM from KSUD14 in the *Ae. tauschii* linkage map (Gill et al. 1991). This population was scored again for rust resistance, KSUD14 and *Gli-D1* loci, and we observed seven recombinants (6.25 cM) between KSUD14 and the rust resistance gene in a population of 56 F_2 plants (our unpublished results). These data may indicate higher rates of recombination in *Ae. tauschii* than in wheat. Alternatively, the *Lr21* family members may be dispersed over a large genomic region and various members may show variable rates of recombination with the KSUD14 marker.

The 1.36-kb fragment cloned from TA1649 that detects all *Lr21/Lr40* members carries an open reading frame that may encode 194 amino acids. There are two motifs including kinase2a and kinase3 domains where the amino-acid sequences from the cloned fragment

showed high similarity to the *Cre3* gene from wheat and the rust resistance gene *Rp1-D* from maize. The cloned fragment may be part of a gene that belongs to the NBS-LRR class of plant disease resistance genes.

Why is the 885-bp fragment so highly conserved in diverse alleles at the *Lr21* locus? There are three possible hypotheses to explain these results. The first hypothesis is that *Lr21* and *Lr40* are possibly paralogous rust resistance genes in the *Lr21* complex locus. The common fragment may belong to the same NBS-LRR family member as *Lr21/Lr40*, and may be a conserved sequence in all the members. Multiple fragments amplified from TA1649 and TA1599 with KSUD14-STS (data not shown), and multiple hybridization fragments observed in the *Ae. tauschii* accessions with KSUD14 (Fig. 5), indicated multiple loci of *XksuD14*. Sequence comparison among the fragments amplified with KSUD14-STS from TA1599 (*Lr21* donor), TA1691 and TA1649 showed that these fragments share over 96% similarity (data not shown). This may imply that multiple loci of KSUD14 were derived from a common ancestor. The one recombinant detected by KSUD14 in the WGRC2 mapping population has the same sequence as the *Xksud14* locus in the resistant parent but is susceptible to the pathogen PRTUS6, perhaps due to recombination between the paralogues.

The second hypothesis is that many different families of NBS-LRR are clustered in the *Lr21/Lr40* region. It has been found that RgaYr10, which is an unrelated NBS-LRR resistance-gene analog from KSUD14, identified at least three gene family members on 1DS of *Ae. tauschii*. One of the members cosegregated with *Lr21/Lr40* (Spielmeyer et al. 2000), and cosegregated with KSUD14 in the populations of WGRC2/Wichita and WGRC7/Wichita (our unpublished results). It is possible that KSUD14 represents a different NBS-LRR family member from the *Lr21/Lr40* gene family and the recombination is suppressed in this region among the tested accessions. A similar example is the *Mla* resistance gene region on chromosome 5 (1H) in barley. Three distinct NBS-LRR gene families, cosegregated with *Mla*, are located within a 240-kb interval. Recombination within families was suppressed (Wei et al. 1999).

The third hypothesis is that KSUD14 is a part of a gene required for *Lr21*/*Lr40* to confer resistance to the leaf-rust pathogen. A good example is the *Prf* gene in the *Pto* gene cluster (Salmeron et al. 1996). *Prf* is a member of the leucine-rich repeat class of plant disease resistance genes, and is embedded within the *Pto* kinase gene cluster, 24-kb from the *Pto* gene. Mutational studies revealed that *Prf* and *Pto* are both required for the signalling pathway in tomato that leads to resistance to *Pseudomonas syringae* pv *tomato*.

The comparison of physical maps with their corresponding genetic linkage maps revealed that genes are clustered in high recombination "hot spot" regions. According to the physical map of chromosome 1D, the RFLP marker KSUD14 is present in the very distal "hot spot" of the chromosome 1D short arm (Gill et al. 1996). In such a region of high recombination, a tightly linked marker 0.2 cM away from the gene would be physically close to the gene. Therefore, KSUD14 is not only a very useful marker for gene tagging, but may also provide a good starting point for the positional cloning of the gene.

Acknowledgments A special note of thanks to Duane L. Wilson, Elburn Parker and W. Jon Raupp for their instruction and technical assistance in rust inoculation, scoring and protein gel-electrophoresis of wheat plants. Kansas Agricultural Experiment Station journal article No.01–215-J. Research supported by grants from Kansas Wheat Commission and USDA special grant to the Wheat Genetics Resource Center at Kansas State University.

References

Browder LE (1971) Pathogenic specialization in cereal rust fungi, especially *Puccinia recondita* f. sp. *tritici*: concepts, methods of study, and application. Tech Bull No. 1432, pp 45

Cox TS, Raupp WJ, Gill BS (1994) Leaf rust-resistance genes *Lr41*, *Lr42* and *Lr43* transferred from *Triticum tauschii* to common wheat. Crop Sci 34:339-343

Feuillet C, Messmer M, Schachermayr G, Keller B (1995) Genetic and physical characterization of the *Lr1* leaf rust resistance locus in wheat (Triticum aestivum L.). Mol Gen Genet 248(5): 553–562

Feuillet C, Schachermayr G, Keller B (1997) Molecular cloning of a new receptor-like kinase gene encoded at the *Lr10* disease resistance locus of wheat. Plant J 11:45–52

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34: 362–374

Gill KS, Gill BS, Endo TR, Taylor T (1996) Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics 144:1883–1891

Heun M, Kennedy AE, Anderson JA, Laptian NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Hussien T, Bowden RL, Gill BS, Cox TS (1997) Chromosome location of leaf rust resistance gene *Lr43* from *Aegilops tauschii* in common wheat. Crop Sci 37:1–3

Knott DR (1989) The wheat rusts breeding for resistance. Monographs on Theoretical and Applied Genetics 12, (Springer, Berlin, pp 1–198

Kolmer JA (1996) Genetics of resistance to wheat leaf rust. Annu Rev Phytopathol 34:435–455

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Langridge P, Chalmers K (1998) Techniques for marker development. Proc 9th Int Wheat Genet Symp, Saskatchewan, Canada, Vol 1, pp 107–117

Long DL, Kolmer JA (1989) A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopathology 79:525–529

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436

McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts, an atlas of resistance genes. Kluwer academic publishers, Dordrecht Boston London

Miller DE (1991) Genetic analysis of leaf rust resistance in *Triticum tauschii*, the D-genome progenitor of wheat. Masters thesis, Kansas State University

Morris KLD, Raupp JW, Gill BS (1989) Isolation of H^t genome chromosome additions from polyploid *Elymus trachycaulus* (S^t S^t H^t H^t) into common wheat (*Triticum aestivum*). Genome 33:16–22

Naik S, Gill KS, Prakasa Rao VS, Gupta VS, Tamhankar SA, Pujar S, Gill BS, Ranjekar PK (1998) Identification of a STS marker linked to the *Aegilops* speltoides-derived leaf rust resistance gene *Lr28* in wheat. Theor Appl Genet 97:535–540

Procunier JD, Townley–Smith TF, Fox S, Prashar S, Gray M, Kim WK, Czarnecki E, Dyck PL (1995) PCR-based RAPD/DGGE markers linked to leaf rust resistance genes Lr29 and Lr25 in wheat (*Triticum aestivum* L.) J Genet Breed 49:87–92

Raupp WJ, Gill BS, Cox TS, Wilson DL, Browder LE (1991) Two leaf rust resistance genes derived from *Triticum tauschii* are located on the wheat chromosome arms 1DS and 2DS. Agron Abstr p 113

Raupp WJ, Singh S, Brown-Guedira GL, Gill BS (2000) Cytogenetic and molecular mapping of the leaf rust resistance gene *Lr39* in wheat. Theor Appl Genet 102:347–352

Riede CR, Anderson JA (1996) Linkage of RFLP markers to an aluminum tolerance gene in wheat. Crop Sci 36:905–909

Roelfs AP (1985) Wheat and rye stem rust. In: AP Roelfs, WR Bushnell (eds) The cereal rust. Disease, distribution, epidemiology and control. Academic Press, New York London Orlando, pp 3–37

Salmeron JM, Oldroyd GED, Tommens CMT, Scofield SR, Kim H, Lavelle DT, Dahlbeck D, Staskawicz BJ (1996) Tomato Prf is a member of the leucine-rich repeat class of plant disease resistance genes and lies embedded with the Pto kinase gene cluster. Cell 86:123–133

Seah S, Bariana H, Jahier J, Sivasithamparam K, Lagudah ES (2000) The introgressed segment carrying rust resistance genes Yr17, Lr37 and Sr38 in wheat can be assayed by a cloned disease resistance gene-like sequence. Theor Appl Genet 102:600–605

Seyfarth R, Feuillet C, Schachermayr G, Winzeler M, Keller B (1999) Development of a molecular marker for the adult plant leaf rust resistance gene *Lr35* in wheat. Theor Appl Genet 99:554–560

Sharp PJ, Chao S, Desai S, Gale MD (1988) The isolation, characterization and application in the Triticeae of a set of wheat RFLP probes identifying each homoeologous chromosome arm. Theor Appl Genet 78:342–348

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Gardner J, Wang B, Holsten T, Zhai WX, Zhu LH, Fauquet C, Ronald PC (1995) A receptor kinase-like protein encoded by the rice disease resistance gene *Xa21*. Science 270:1804–1806

Spielmeyer W, Huang L, Bariana H, Laroche A, Gill BS, Lagudah ES (2000) NBS-LRR sequence family is associated with leaf and stripe rust resistance on the end of homoeologous chromosome group 1S of wheat. Theor Appl Genet 101:1139–1144

Talbert LE, Blake NK, Chee PW, Blake TK, and Magyar GM (1994) Evaluation of "sequence-tagged-site" PCR products as molecular markers in wheat. Theor Appl Genet 87:789–794

Wei F, Gobelman-Werner K, Morrol SM, Kurth J, Mao L, Wing R, Leister D, Schulze-Lefert P, Wise RP (1999) The *Mla* (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. Genetics 153:1929–1948