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Targeted mapping of ESTs linked to the adult plant resistance gene *Lr46* in wheat using synteny with rice

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Abstract The gene *Lr46* has provided slow-rusting resistance to leaf rust caused by *Puccinia triticina* in wheat (*Triticum aestivum*), which has remained durable for almost 30 years. Using linked markers and wheat deletion stocks, we located *Lr46* in the deletion bin 1BL (0.84–0.89) comprising 5% of the 1BL arm. The distal part of chromosome 1BL of wheat is syntenic to chromosome 5L of rice. Wheat expressed sequence tags (ESTs) mapping in the terminal 15% of chromosome 1BL with significant homology to sequences from the terminal region of chromosome 5L of rice were chosen for sequence-tagged site (STS) primer design and were mapped physically and genetically. In addition, sequences from two rice bacterial artificial chromosome clones covering the targeted syntenic region were used to identify additional linked wheat ESTs. Fourteen new markers potentially linked to *Lr46* were developed; eight were mapped in a segregating population. Markers flanking (2.2 cM proximal and 2.2 cM distal) and cosegregating with *Lr46* were identified. The physical location of *Lr46* was narrowed to a submicroscopic region between the breakpoints of deletion lines 1BL-13 [fraction length (FL)=0.89–1] and 1BL-10 (FL=0.89–3). We are now developing a high-resolution mapping population for the positional cloning of *Lr46*.

Keywords Synteny · Rice · Wheat · Leaf-rust resistance

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Introduction

Leaf rust, or brown rust (caused by *Puccinia triticina* Eriks.), is one of the most damaging diseases of wheat in all wheat-growing regions of the world. There are more than 50 designated leaf-rust resistance genes (McIntosh et al. 2003), most of which condition a hypersensitive reaction and interact with the pathogen in a gene-for-gene fashion (Flor 1942). Virulence in the pathogen population has been selected or has rapidly developed following the deployment of many such resistance genes. Among the alternative approaches to disease control, race non-specific resistance to highly variable pathogens has been observed in many plants. In barley, the *mlo* gene confers resistance to all known isolates of the powdery mildew (*Blumeria graminis* f. sp. *hordei*) pathogen, and it has been durable for more than 20 years (Jørgensen 1992). In this case, the *mlo*-mediated resistance is conditioned by recessive loss-of-function alleles at the resistance locus (Jørgensen 1992; Freialdenhoven et al. 1996; Buschges et al. 1997). In maize, certain haplotypes at the *Rp1* locus provided non-specific, partial adult-plant resistance to both common rust (*Puccinia sorghi*) and southern rust (*Puccinia polysora*, Hu et al. 1997).

In wheat, Singh et al. (1998) reported that the adult plant resistance genes *Lr34* and *Lr46* conferred a slow-rusting type of resistance that is effective against a wide range of different isolates of the pathogen. Because these genes condition a non-hypersensitive type of resistance that has remained effective for over 30 years, they are considered to be durable. In addition, these genes seem to provide resistance to more than one pathogen. Reports indicate ge-

netic association between *Lr34* and *Yr18*, a gene conferring resistance to yellow rust in adult plants (Singh 1992); *Lr34* and tolerance to barley yellow dwarf virus (BYDV; Ayala et al. 2002; Singh 1993); and enhanced resistance to stem rust (Kolmer 1996; Dyck 1993) and slow development of spot blotch (Joshi et al. 2004). Similarly, the yellow-rust resistance gene *Yr29* is either closely linked to, or is the same as, leaf-rust resistance gene *Lr46* (William et al. 2003).

In slow-rusting resistance, there is a delay in the appearance of uredinia on the leaf surface (or an increase in the latent period) observed for resistant lines compared with the susceptible line (Martinez et al. 2001; Rubiales and Niks 1995). In addition, slow-rusting genes are characterized by a reduction in uredinial size and a higher rate of early abortion of haustoria (Martinez et al. 2001; Rubiales and Niks 1995). The accurate scoring of the minor effects of this class of genes in the field and greenhouse is not an easy task because the phenotype is subtle and can be affected by the environment as well as plant developmental stage. The deployment of minor resistance genes in breeding lines and cultivars would be greatly facilitated by the use of linked molecular markers.

The *Lr46/Yr29* locus, conferring resistance to both leaf rust and stripe rust, is located in the terminal portion of the long arm of wheat chromosome 1B. The chromosome location of this gene was determined through an analysis of the substitution lines for the chromosomes of the resistant cultivar Pavon 76 backcrossed into the susceptible spring wheat cultivar Lalbahadur (Singh et al. 1998). Subsequent quantitative trait loci (QTL) analyses have confirmed the location of a minor gene for resistance to leaf rust and stripe rust on the distal portion of chromosome 1BL in a recombinant inbred line population from the cross Avocet× Pavon 76 (William et al. 2003). A QTL for leaf-rust resistance was reported in the same region of 1BL in a doubled haploid (DH) population from the cross of Fukuhokomugi×Oligoculm (Suenaga et al. 2003). Despite extensive mapping efforts, reliable flanking markers closely linked to *Lr46* that are useful for marker-assisted selection (MAS) have not been identified. The chromosomal region also has not been saturated with molecular markers that could facilitate map-based cloning of the gene.

Currently, there are more than 550,000 wheat expressed sequence tag (EST) sequences, with 128,088 unique sequences (http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=wheat), deposited in public databases, providing an excellent resource for mapping genes. A set of wheat deletion lines has been used to locate 7,873 unique ESTs into chromosome bins (Qi et al. 2004; http://wheat.pw.usda.gov/NSF/progress_mapping.html). Although the remaining EST sequences have not been physically or genetically mapped, existing synteny between rice and wheat, as well as other cereals, can be exploited to tentatively position ESTs in silico based on orthology with sequences in the rice genome (La Rota and Sorrells 2004). This approach has been used to enrich chromosome regions of wheat and barley with markers. To saturate a QTL region for resistance to *Fusarium* head blight (FHB) located in chromosome 3BS, rice sequences covering the distal portion of chromosome 1S were used as queries to identify previously unmapped wheat ESTs located in the QTL region. Five sequence-tagged site (STS) markers that flanked the QTL and mapped in the middle of the QTL peak were identified (Liu and Anderson 2003). Comparative mapping between rice and wheat also facilitated the identification of molecular markers for the grain protein content locus *Gpc-6B1* on wheat chromosome 6BS. A small region encompassing the *Gpc-6B1* locus was analysed by restriction fragment length polymorphism (RFLP) mapping wheat ESTs orthologous to sequences from rice chromosome 2S. As a result, five putative genes for grain protein content were identified in a 0.3-cM interval in wheat corresponding to a 64-kb region in rice (Distelfeld et al. 2004).

In barley, this approach was used to identify 11 EST-based markers that were placed into the *Rph16* high-resolution map. Although the rice genomic region did not contain a candidate for the resistance gene, the approach demonstrated the potential of using EST resources for synteny-based marker saturation of a region. However, in some cases, rearrangements have been observed at the microsynteny level between rice and other cereals. In the *Rph16* region, two EST-based barley markers indicated an inversion from the original order in rice (Perovic et al. 2004). Another case of microcolinearity interruption is reported between rice and barley at the *Rph7* locus (Brunner et al. 2003). Saturation of the leaf-rust resistance locus *Rph7* on barley chromosome 3HS was done using ESTs from chromosome 1S in rice. A comparative analysis of the two regions revealed a conservation of five members of the HGA gene family. These genes are clustered in rice, whereas intergenic regions were observed in barley due to the presence of six additional genes that interrupt the microsynteny between the two species (Brunner et al. 2003).

The objective of our study was to use the syntenic relationship between the distal part of chromosome 1BL of wheat and the distal part of chromosome 5L of rice to enrich the *Lr46* chromosomal region with EST-derived markers. We report here the physical location of *Lr46* and tightly linked STS markers.

Materials and methods

Plant material and leaf-rust evaluation

Two mapping populations were developed and evaluated for reaction to leaf rust at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. Wheat cultivar Lalbahadur (Lalb) was used as the susceptible parent. The first population involved a partially backcrossed substitution line [referred to as Lalb(PVN 1B)], where chromosome 1B of cultivar Pavon was substituted into Lalbahadur (Singh et al. 1998) and was used as a resistant parent carrying *Lr46*. Lalbahadur was crossed with five of these chromosome substitution lines, and one F_1 plant from each cross was used for developing ap-

proximately 35 $F_{2:3}$ lines per cross with a total of 184 lines; 101 lines were used for genetic mapping. To obtain F_3 lines, the F_2 plants were space sown, 10–15 cm apart, in the field of the El Batan Research Station, grown under disease-free conditions and harvested individually.

The F_3 lines were evaluated during the 1998–1999 and 1999–2000 crop seasons at Ciudad Obregon, Sonora, Mexico, with *P. triticina* race MCJ/SP (nomenclature based on Singh 1991) as reported by William et al. 2003. Approximately 60 seeds of each line were sown in 75-cm-wide paired-row plots, 1 m in length, with a 20-cm row spacing and a 50-cm pathway between plots. The leaf-rust epidemic was initiated approximately 6 weeks after planting. Spreader rows of highly susceptible cv. Morocco, planted on hills on one side of the plots in the pathway, were sprayed with a suspension of urediniospores in lightweight mineral oil (Soltrol 170, Phillips 66 Co., Bartlesville, OK). The field site used has a favourable environment for disease development. Lines were classified as homozygous resistant (HR), homozygous susceptible (HS) and segregating (Seg). Chi-squared analyses were carried out to compare the observed phenotypic frequencies with the 1:2:1 ratio expected for segregation at a single locus (William et al. 2003).

The second population used in the study involved a partially backcrossed substitution line 'Lalbahadur(Parula 1B)' [referred to as Lalb(PRL 1B)], where chromosome 1B of cultivar Parula was substituted into Lalbahadur. Parula is also known to carry *Lr46* (Singh R., unpublished results). Lalb(PRL 1B) was crossed with Lalbahadur, and two F_1 plants were harvested to develop the recombinant lines. The F_2 plants were space sown, 10–15 cm apart, in the field of EL Batan Research Station, under disease-free conditions and were individually harvested. The population was advanced in the greenhouse by growing four seeds of each F_3 line in a pot and harvesting one plant per line. The same procedure was repeated for the F_4 generation; however, two plants per line were harvested. Eight seeds of each of the F_5 lines were space sown in the field of the El Batan Research Station during the 2003 crop season, and one plant per line was harvested. Leaf-rust evaluation of F_6 lines was carried out during the 2003–2004 crop season at Ciudad Obregon, as described earlier. Lines were classified as HR, Seg and HS. The lines were evaluated twice during the first and second weeks of March. The first evaluation was made when the susceptible lines displayed about 60% rust severity, and the second evaluation was done when they displayed 100% severity based on the modified Cobb Scale (Roelfs et al. 1992; Peterson et al. 1948). Field tests were repeated during the 2004–2005 crop season for seven Lalb×Lalb(PRL 1B) F_6 lines that were thought to be Seg to confirm the phenotypes.

The Wheat Genetics Resource Center at Kansas State University supplied the Chinese Spring (CS) wheat and chromosome-1B-related CS aneuploids used in the study. These included the following: nullisomic 1B–tetrasomic 1A (N1B–T1A), nullisomic 1A–tetrasomic 1B (N1A–T1B) and nullisomic 1D–tetrasomic 1A (N1D–T1A) (Sears 1966); ditelosomic lines 1BL (Dt1BL) and 1BS (Dt1BS) (Sears and Sears 1979); and seven CS deletion lines for the terminal region of the chromosome 1B long arm. The deletion lines are designated by the chromosome arm carrying the deletion and the length of the terminal deletion, expressed as a fraction length (FL) of the whole arm. The deletion lines included were 1BL-3 (FL=0.85), 1BL-4 (FL=0.89), 1BL-5 (FL=0.84), 1BL-8 (FL=0.74), 1BL-10 (FL=0.89), 1BL-13 (FL=0.89) and 1BL-15 (FL=0.82) (Endo and Gill 1996; Qi et al. 2003). Together, the nullisomic–tetrasomic lines and the deletion lines were used for the chromosome and deletion bin mapping of EST-based STS and simple sequence repeat (SSR) markers linked to *Lr46*.

Molecular marker analyses

Tissue was harvested in bulk from 10 to 20 plants of each line of the mapping populations, the parents, CS wheat and the CS aneuploids and deletion lines. Tissue was ground in liquid nitrogen, and genomic DNA was extracted using cetyltrimethylammonium bromide (CTAB) DNA extraction buffer [1.4 M NaCl, 100 mM Tris pH 8.0, 2% CTAB (hexadecyltrimethylammonium bromide), 20 mM ethylenediaminetetraacetic acid (EDTA), 0.5% Na bisulfite and 1% 2-mercaptoethanol], followed by chloroform extraction and alcohol precipitation. Genomic DNA was amplified with SSR and EST-based STS primers. Polymerase chain reaction (PCR) amplifications were performed in 25-µl reactions with 2.5 µl of 10× magnesium-free PCR buffer, 1.5 µl of magnesium chloride (25 mM), 2.0 µl of dNTPs (2.5 mM each dNTP) and 1 µl of each forward and reverse primer (100 pmol/µl) and 100 ng of DNA in a PTC-200 thermal cycler (MJ Research). Primer annealing temperatures varied from 45 to 60°C, depending on the primers. All PCR products were resolved in 2.3% high-resolution agarose (Gene Pure HiRes Agarose, ISC BioExpress) gels with 0.5× Tris–borate EDTA (TBE) buffer and visualized by ethidium bromide staining. After the fragment sizes of the PCR products from EST–STS markers were verified in agarose gels, fragments were separated in single-stranded conformation polymorphism (SSCP) polyacrylamide gels (Martins-Lopes et al. 2001) run at 4 W for 18–20 h. Fragments were visualized by silver staining (Bassam et al. 1991).

The physical locations of wheat SSR markers that were previously reported close to the *Lr46* region, including *Xwmc44* (Suenaga et al. 2003), *Xwms259* and *Xwms140* (William M., personal communication), were determined by evaluating the markers on the CS aneuploid and deletion line stocks using 2.3% high-resolution agarose gel electrophoresis. The data indicated the physical location of *Lr46* and were used for subsequent identification of wheat ESTs likely linked to *Lr46*.

The terminal region of chromosome 1BL of wheat is syntenic to the distal region of chromosome 5L of rice (La Rota and Sorrells 2004) and was targeted for marker enrichment. Forty-nine unique wheat EST sequences that mapped distal to the 1BL-3 (0.85) deletion breakpoint

(http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi) were used to search the rice genome database (http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1) using the BLASTn program (Altschul et al. 1997). Sequences in the target region of the rice genome were also used as queries in BLASTn searches of the wheat EST database (http://tigrblast.tigr.org/tgi/) to identify additional unmapped wheat ESTs that are potentially linked to *Lr46*. Wheat EST sequences with high levels of homology (*E* values less than e^{-15}) to sequences from chromosome 5L of rice were used to design primers for EST-based STS markers. Primer design was done with the software MacVector 6.5.3, and amplicons of 200–600 bp were targeted. All markers were mapped physically using the CS deletion lines. Polymorphic markers that physically mapped in the target region were evaluated on 101 lines of the Lalbahadur/Lalb(PVN 1B) $F_{2:3}$ mapping population. Markers *Xwms259* and *Xwmc44* and the three STS markers most closely linked to *Lr46* were evaluated on 157 lines from the Lalbahadur/Lalb(PRL 1B) $F_{5:6}$ population. Genetic maps were constructed using the program MapMaker for Macintosh V using the Kosambi mapping function to estimate genetic distances with a minimum log of odds (LOD) of 3.0.

Results

The Lalb(PVN 1B) substitution line carrying the slow-rusting resistance gene *Lr46* displayed between 20 and 30% leaf-rust severity during the 1998–1999 and 1999–2000 crop seasons, and the susceptible cultivar Lalbahadur showed 80–100% leaf-rust severity. William et al. (2003) reported that the F_3 lines from the cross Lalbahadur×Lalb (PVN 1B) segregated in a 1:2:1 ratio expected for a single gene.

During the 2003–2004 crop season, the Lalb(PRL 1B) lines displayed between 5 and 10% leaf-rust severity when the first set of notes were taken (60% severity for Lalbahadur) and between 15 and 20% severity when the second evaluation was done, at which time Lalbahadur

Table 1 ESTs or tentative contigs (TC) from which markers were developed, and names of all SSR and EST-based STS markers that were physically mapped in the long arm of chromosome 1B using CS wheat aneuploid stocks and deletion lines

EST/TC[a]	Marker	CS	N1A–T1B	N1D–T1A	N1B–T1D	Dt1BL	Dt1BS	Deletion bin 1BL-3 (0.85)–1BL-13 (0.89–1)	Deletion bin 1BL-13 (0.89–1)–1BL-4 (0.89–2)	Deletion bin 1BL-4 (0.89–2)–1BL-10 (0.89–3)	Deletion bin 1BL-10 (0.89–3–1.00)
	WMC44	+	+	+	−	+	−	−	+	+	+
	WMS259	+	+	+	−	+	−	−	−	−	+
	WMS140	+	+	+	−	+	−	−	−	−	−
BE426661	STS1BL1[b]	+	+	+	−	+	−	−	+	+	+
BE518048	STS1BL2[b]	+	+	+	−	+	−	−	−	+	+
CA599442	STS1BL3	+	+	+	−	+	−	−	−	+	+
TC261755	STS1BL4	+	+	+	−	+	−	−	−	+	+
BQ578325	STS1BL5	+	+	+	−	+	−	−	−	+	+
TC243680	STS1BL6	+	+	+	−	+	−	−	−	+	+
CA497457	STS1BL7	+	+	+	−	+	−	−	−	+	+
CD881510	STS1BL8	+	+	+	−	+	−	−	−	+	+
CA679220	STS1BL9[b]	+	+	+	−	+	−	−	−	+	+
BG313575	STS1BL10	+	+	+	−	+	−	−	−	−	+
CD884701	STS1BL11	+	+	+	−	+	−	−	−	−	+
CA670512	STS1BL12	+	+	+	−	+	−	−	−	−	+
BJ249227	STS1BL13	+	+	+	−	+	−	−	−	−	+
BM135646	STS1BL14	+	+	+	−	+	−	−	−	−	+
BE515715	STS1BL15	+	+	+	−	+	−	−	−	−	+
TC237978	STS1BL16	+	+	+	−	+	−	−	−	−	+
TC265717	STS1BL17[b]	+	+	+	−	+	−	−	−	−	+
TC255004	STS1BL18[b]	+	+	+	−	+	−	−	−	−	+
BE604058	STS1BL19[b]	+	+	+	−	+	−	−	−	−	−
BF474878	STS1BL20[b]	+	+	+	−	+	−	−	−	−	−
BG274687	STS1BL21[b,c]	−	−	−	−	−	−	−	−	−	−
BG262882	STS1BL22[b,c]	−	−	−	−	−	−	−	−	−	−

EST/TCs in bold were previously mapped by the NSF project
[a]Designations of ESTs (GenBank) and TCs [The Institute for Genomic Research (TIGR)] as of August of 2005
[b]Polymorphic STS markers in the mapping populations
[c]STS markers that could not deletion map with the CS aneuploids and deletion lines

displayed 100% leaf rust. These clear differences allowed an accurate characterization of the lines into three categories: HR, Seg and HS. The F_6 lines from the cross Lalbahadur×Lalb(PRL 1B) segregated 68 HR, 15 Seg and 87 HS and conformed to the expected ratio, 0.46875, 0.0625 and 0.46875, respectively, for the segregation of a single gene (χ^2=4.19, P<0.05).

SSR mapping

Three SSR markers, *Xwmc44*, *Xwms259* and *Xwms140*, previously associated with *Lr46* in other mapping populations (Suenaga et al. 2003; William M., personal communication) were evaluated in the $F_{2:3}$ mapping population from the cross Lalbahadur×Lalb(PVN 1B), where *Lr46* was evaluated as a single gene. Although the SSR markers flanked the resistance gene, none was closely linked. Marker *Xwmc44* was 12.5 cM proximal to *Lr46*, *Xwms259* was 11.0 cM distal to *Lr46*, and *Xwms140* was 30.8 cM distal (map not shown).

The two closest SSR markers flanking *Lr46* physically mapped in the terminal region of wheat chromosome 1BL. Fragments of appropriate size were amplified with the corresponding SSR primer pairs from the genomic DNA of CS, Dt1BL, N1A–T1B and N1D–T1A. The corresponding fragments were not amplified from the DNA of Dt1BS and N1B–T1D, which confirmed the presence of these makers in the long arm of chromosome 1B. Primer pair WMS140 amplified a 200-bp fragment from the genomic DNA of CS and Dt1BL, which was not amplified from the seven deletion lines used in this study, locating *Xwms140* distal to the breakpoints of deletion lines 1BL-4, 1BL-10 and 1BL-13 with an FL of 0.89. The 250-bp fragment amplified by primer pair WMC44 in deletion lines 1BL-10, 1BL-4 and 1BL-13 was absent in deletion lines 1BL-3, 1BL-5, 1BL-8 and 1BL-15, placing this marker between FL=0.85 and FL=0.89. The 120-bp fragment amplified by primer pair WMS259 was present in the deletion line 1BL-10 and absent in all other deletion lines. This indicates that *Xwms259* is present in a submicroscopic deletion bin proximal to the breakpoint for line 1BL-10 and distal to the breakpoints for 1BL-4 and 1BL-13. The physical mapping of the SSRs flanking *Lr46* in 4% of the chromosome arm between the breakpoints for deletion lines 1BL-3 and 1BL-10 led us to focus on this region for subsequent analysis.

Physical and genetic mapping of wheat ESTs

Out of 47 unique wheat ESTs previously mapped distal to the deletion bin 1BL3 (0.85–1.00) (Peng et al. 2004), eight had a significant homology to sequences on the terminal region of rice chromosome 5 (from 27.45 to 28.89 Mb), six had no obvious orthologous sequences in rice, and the remaining 33 had significant hits elsewhere in the rice genome, including other regions of chromosome 5. Eight wheat ESTs whose only significant orthologous rice sequence was in the terminal region of chromosome 5 were selected for primer design (Table 1).

Four of the EST-based primer pairs (STS1BL1, ST S1BL2, STS1BL19 and STS1BL20) amplified fragments

Table 2 EST or TC from which markers were developed, primer sequences, annealing temperatures and physical location of markers linked to *Lr46*

EST/TC[a]	Primer sequence	Fragment size[b] (bp)	Annealing temperature (°C)	Deletion bin
BE426661	STS1BL1F 5'-GGCACATCCCTTTGTTGCTCAG-3' STS1BL1R 5'-GCTTTTTCATAGGCATCAGGCG-3'	800	60	1BL-13 (0.89–1)–1BL-4 (0.89–2)
BE518048	STS1BL2F 5'-TGACATTTGGAGCATTGGGTG-3' STS1BL2R 5'-TGATTGGCTGACAG GATGGTTC-3'	600	55	1BL-13 (0.89–1)–1BL-4 (0.89–2)
CA679220	STS1BL9F 5'-CACCGTCATTGTGTCCATC-3' STS1BL9R 5'-TGTTCCCACAAGTTCCAAC-3'	400	45	1BL-13 (0.89–1)–1BL-4 (0.89–2)
TC265717	STS1BL17F 5'-GGAGTCACGCTGCTTC TATTGTTAC-3' STS1BL17R 5'-TTATGCTGAATG GAGCCTCGGG-3'	500	60	1BL-4 (0.89–2)–1BL-10 (0.89–3)
TC255004	STS1BL18F 5'-CGGTATGTGATTTTCTGGGAG-3' STS1BL18R 5'-CAAACGGTTCCTTCTTGAG-3'	800	60	1BL-4 (0.89–2)–1BL-10 (0.89–3)
BE604058	STS1BL19F 5'-CGAGGAGACGATGAAGAAAG-3' STS1BL19R 5'-TGTATCTTCGCAGGTAGTCG-3'	200	60	1BL-13 (0.89–3–1.00)
BG262882	STS1BL22F 5'-CGTAACTTTTCCCGCT CATCTTTC-3' STS1BL22R 5'TTTTCCCTTCTCCGCCACAG-3'	600	60	Could not deletion map
BF474878	STS1BL20F 5'-CTCTCTGGCTGCTATTGG GATG-3' STS1BL20R 5'-CGGTTAGTT CACCTGTGCTTGC-3'	300	60	1BL-13 (0.89–3–1.00)
BG274687	STS1BL21F 5'-AAGACATCTGCCGTGACCTC-3' STS1BL21R 5'-TTGGTGTTGTAGTCGGTCCC-3'	150	60	Could not deletion map

[a]Designations of ESTs (GenBank) and TCs (TIGR) as of August of 2005
[b]Estimation of the fragment size was observed on 2.3% high-resolution agarose gels

that could be deletion mapped in the distal 15% of the long arm of chromosome 1B, consistent with the previous assignment of the ESTs based on RFLP analysis (Peng et al. 2004). Most primers amplified multiple fragments, some of which were assigned to chromosomes 1A and 1D by analysis of the nulli-tetra lines. The use of three deletion lines with breakpoints distal to the 1BL-3 (0.85) breakpoint allowed the more precise placement of these markers on the terminal region of the chromosome. Markers *XSTS1BL1*, *XSTS1BL2*, *XSTS1BL19* and *XSTS1BL20* (Table 2) amplified DNA fragments of corresponding size in CS, Dt1BL, N1A–T1B and N1D–T1A and not from N1B–T1A and Dt1BS (Table 1), indicating that these markers are located on chromosome 1BL. Primer pairs STS1BL19 and STS1 BL20 did not amplify the corresponding DNA fragments in the deletion lines used in the study, placing the markers terminal to the 0.89 breakpoint (Table 1). Primer pair STS1BL1 amplified fragments, which were absent from deletion line 1BL-3, from the DNA of CS and deletion lines 1BL-10, 1BL-4 and 1BL-13, placing this marker in deletion bin 0.85–0.89 (Table 1). Primer pair STS1BL2 amplified a fragment from the DNA of CS, 1BL-10 and 1BL-4 and that was absent in 1BL-3 and 1BL-13. Deletion line 1BL-13 was thus determined to have a larger terminal deletion than do lines 1BL-4 and 1BL-10 (Table 1). For the five remaining primer pairs, amplification of identical fragments from the three genomes of wheat made it impossible to deletion map the markers. Although we were not able to differentiate the three genomes with these PCR-based markers, the wheat National Science Foundation (NSF) project mapped the ESTs in the 1BL-3 (0.85–1.00) deletion bin by RFLP analysis (http://wheat.pw.usda.gov/cgi-bin/graingenes). Our PCR primers targeted relatively small amplicons and likely did not encompass the polymorphisms between the A, B and D genomes that were detected by RFLP analysis.

All of the STS primers that amplified fragments consistently from CS were evaluated on the parents of the mapping populations. All four of the STS markers that were deletion mapped were polymorphic between Lalbahadur and Lalb(PVN 1B) and were evaluated on the 101 $F_{2:3}$ single-chromosome recombinant lines. Although markers *XSTS1BL21* and *XSTS1BL22* were not deletion mapped (Table 1), they were polymorphic between the parents of the mapping population and were therefore placed on the genetic map. Markers *XSTS1BL1* and *XSTS1BL2* (Table 2) were located between SSR marker *Xwmc44* and *Lr46* on the genetic linkage map (Fig. 1). Markers *XSTS1BL19* and *XSTS1BL20* were located distal to *Lr46* on the linkage map, along with markers *XST*

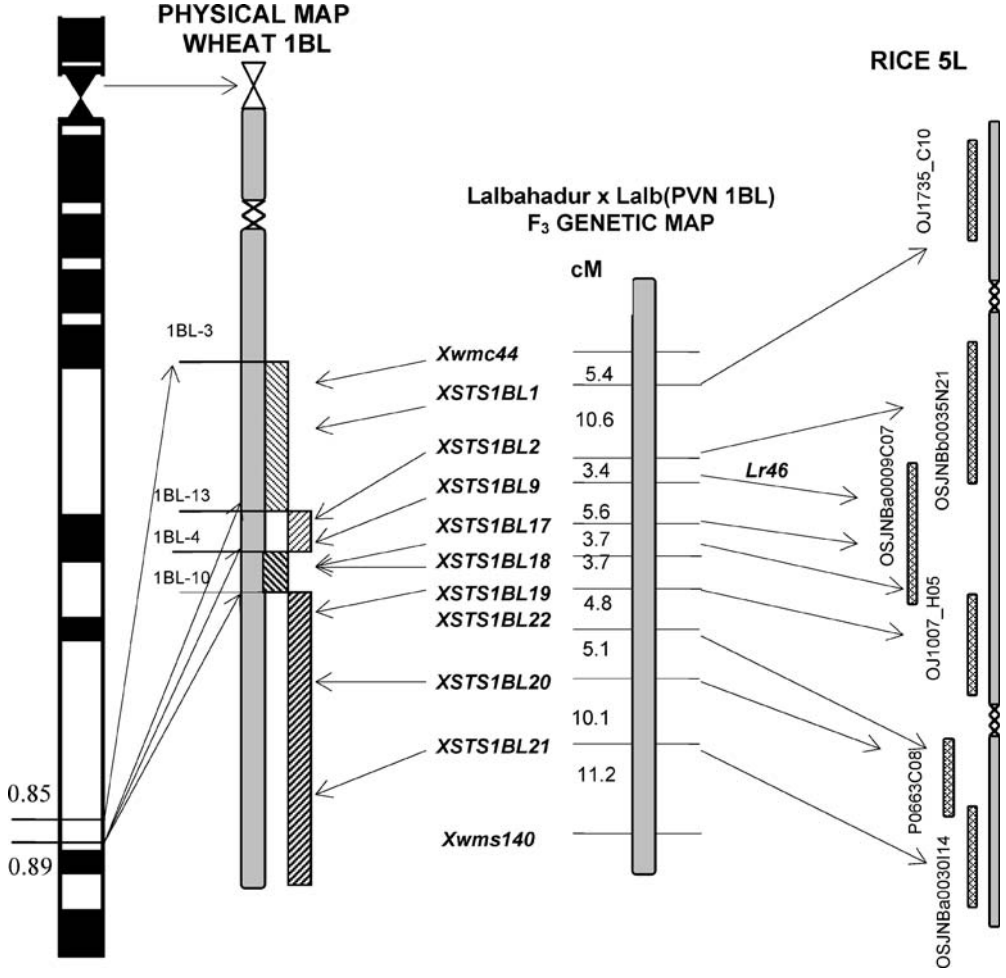

Fig. 1 Comparison of the physical and genetic maps of the wheat chromosome 1BL region with the physical map of the terminal region of rice chromosome 5L

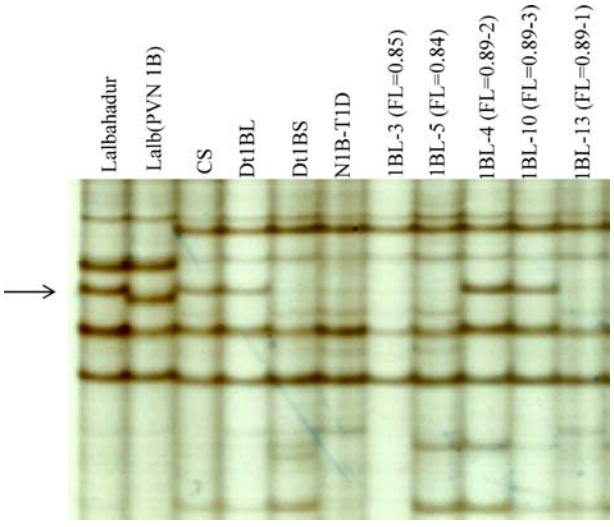

Fig. 2 An SSCP gel pattern of the deletion mapping of the locus *XSTS1BL9*. Amplified fragments are observed in CS and Dt1BL, deletion lines 1BL-4 and 1BL-10, but not in Dt1BS, N1B–T1D and deletion lines 1BL-3, 1BL-5 and 1BL-13. Polymorphism of the B genome fragment was observed between Lalbahadur (S) and Lalb (PVN 1B), as indicated by the *arrow*

S1BL21 and *XSTS1BL22* (Fig. 1). The dominant SSR marker *Xwms259* could not be placed at an LOD greater than 3.0 on the new genetic map derived from the Lalbahadur and Lalb(PVN 1B) F_3 families. However, the combined results of physical and genetic mapping indicated that *Lr46* was located between markers *XSTS1BL2* and *Xwms259*, placing the gene in the submicroscopic region between the deletion breakpoints for 1BL-13 (0.89) and 1BL-10 (0.89).

The order of markers in the map of the $F_{2:3}$ population was consistent with the physical order based on the deletion mapping and the order of orthologous rice sequences on chromosome 5L (Fig. 1), indicating that the synteny of wheat and rice in the region had been maintained and the comparative mapping approach was viable. Thus, a target region in rice consisting of three overlapping rice bacterial artificial chromosome (BAC) clones, OSJNBb0035N21 (28.71–28.80 Mb), OSJNBa0009C07 (28.80–28.95 Mb) and OJ1007_H05 (28.95–29.03 Mb), was identified. Sequences with homology to wheat STS markers closest to *Lr46*, *XSTS1BL2* and *XSTS1BL19*, were present on OSJNBb0035N21 and OJ1007_H05, respectively. Since marker *XSTS1BL2* was more closely linked to *Lr46* (3.4 cm) than was *XSTS1BL19* (12.8 cm), the two more proximal rice BAC clones (OSJNBb0035N21 and OSJNBa0009C07) were chosen for further analysis. The quest for more unmapped wheat ESTs in the region was done by homology searches of each of the predicted open reading frames in the rice BAC clones to the wheat gene index database. As a result, 14 additional STS markers designed from wheat ESTs that were not previously deletion mapped were physically mapped in the submicroscopic region between the deletion breakpoints for lines 1BL-13 (0.89) and 1BL-10 (0.89) (Table 1). Seven primer pairs (STS1BL3 to STS1BL9) amplified fragments in deletion lines 1BL-10 and 1BL-4 that were not amplified from lines 1BL-13 and 1BL-3 (Fig. 2). Nine primer pairs (STS1BL10 to STS1BL16) amplified fragments from deletion line 1BL-10 but no corresponding fragments from 1BL-13 or 1BL-4. The physical order of the EST-derived markers in wheat was in agreement with the order of the orthologous sequences on the rice BAC clones.

Three of the 14 additional markers, *XSTS1BL9*, *XSTS1BL17* and *XSTS1BL18* (Table 2), were polymorphic between the parents of the mapping populations and were added to the genetic map. Markers *XSTS1BL17* and *XSTS1BL18* were located 5.6 and 9.3 cM distal, respectively, to the resistance gene. No recombination was observed between marker *XSTS1BL9* and *Lr46* in this population.

One hundred and fifty-seven $F_{5:6}$ lines from the cross between Lalbahadur and Lalb(PRL 1B) were evaluated with the three markers most closely linked to *Lr46*, along with the SSR markers *Xwmc44* and *Xwms259*. In this population, marker *XSTS1BL2* mapped 2.2 cM proximal to the adult plant resistance gene *Lr46* (Fig. 3). Marker *XSTS1BL17* was located 2.2 cM distal to *Lr46*, and no recombination was found between marker *STS1BL9* and *Lr46* (Fig. 3). The SSR markers *Xwmc44* and *Xwms259* were located 8.0 cM proximal and 6.2 cM distal, respectively, to the resistance gene. Recombination distances observed in the recombinant inbred line (RIL) population from the cross between Lalbahadur and the Parula chromosome 1B substitution line were somewhat less than those observed for the $F_{2:3}$ population from the cross between Lalbahadur and Lalb(PVN 1B). However, the order of the markers was conserved across the two mapping populations.

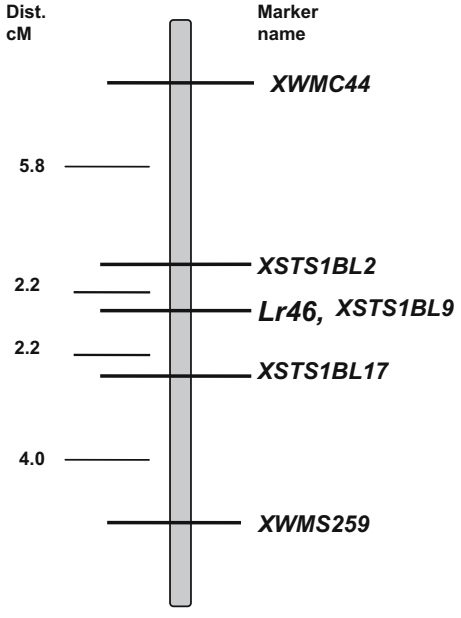

Fig. 3 Genetic map of the *Lr46* gene region with flanking EST-based STS markers and previously mapped SSRs based on the $F_{5:6}$ mapping population of the cross Lalbahadur×Lalb(PRL 1B)

Table 3 Wheat ESTs reported in the *Lr46* region between deletion lines 1BL-13 (0.89–1) and 1BL-10 (0.89–3) and the rice orthologous genes with functions and physical location in the distal region of 5L

Wheat EST/TC[a]	Rice gene (OSA1 genes)	Predicted rice sequence	Physical location (Mb)
BE518048[b]	*11682.m04869*	Protein kinase domain, putative	28.728
None	*11682.m04870*	Serine carboxypeptidase	28.735
None	*11682.m04871*	Serine carboxypeptidase	28.739
None	*11682.m04872*	Hypothetical protein	28.740
TC272729	*11682.m04873*	Serine carboxypeptidase	28.742
TC243193	*11682.m04874*	Retrotransposon protein, putative	28.748
TC270024, TC235575	*11682.m04875*	hAT family dimerisation domain	28.760
TC270024	*11682.m04876*	Similar to CaMK1	28.762
TC255382	*11682.m04877*	Probable transposase	28.767
None	*11682.m04878*	Retrotransposon protein, putative	28.772
TC259065	*11682.m04879*	Similar to At1g15240	28.777
None	*11682.m04880*	Hypothetical protein	28.785
None	*11682.m04881*	Expressed protein	28.790
None	*11682.m04882*	Hypothetical protein	28.797
None	*11682.m04883*	WRKY DNA-binding domain, putative	28.803
TC262006	*11682.m04884*	Late embryogenesis abundant protein	28.808
None	*11682.m04885*	Hypothetical protein	28.810
None	*11682.m04886*	Hypothetical protein	28.816
None	*11682.m04887*	MuDR family transposase, putative	28.823
TC260317, TC243680	*11682.m04888*	ATPase, AAA family, putative	28.832
TC260317, TC243680	*11682.m04889*	ATPase, AAA family, putative	28.832
None	*11682.m04890*	Hypothetical protein	28.845
CA679220[b],CD881510	*11682.m04891*	C-4 dicarboxilate transporter	28.858
None	*11682.m04892*	Similar to L-ZIP+NBS+LRR protein	28.868
None	*11682.m04893*	Hypothetical protein	28.871
None	*11682.m04894*	Hypothetical protein	28.876
TC237978, TC237979	*11682.m04895*	Protein kinase domain, putative	28.879
None	*11682.m04896*	Hypothetical protein	28.889
None	*11682.m04897*	Protein kinase domain, putative	28.893
TC265717[b], TC245975	*11682.m04898*	Mitochondrial solute carrier protein homologue	28.896

[a]Designations of ESTs (GenBank) and TCs (TIGR) as of August of 2005
[b]Closest EST-based STS markers placed in the linkage maps

Characterization of the 1BL-13, 1BL-4 and 1BL-10 deletion lines

Endo and Gill (1996) reported that deletion lines 1BL-13, 1BL-4 and 1BL-10 had the same breakpoint at FL=0.89. EST-based STS markers enabled us to separate these deletion lines into three different size deletions. According to our results, deletion line 1BL-10 has retained the largest portion of the original 1B long arm, 1BL-4 has a slightly larger deletion, and 1BL-13 has a larger deletion than 1BL-4 and 1BL-10 do. With the STS markers described above, we were able to separate these deletion lines into submicroscopic deletion bins: 1BL-13 to 1BL-4, 1BL-4 to 1BL-10 and 1BL-10 to the end of the chromosome 1BL (Fig. 1). We propose to rename these deletion lines as 1BL-13 (0.89–1), 1BL-4 (0.89–2) and 1BL-10 (0.89–3).

The syntenic rice region corresponding to the submicroscopic deletion bin between 1BL-13 (0.89–1) and 1BL-10 (0.89–3) of wheat spans 168 kb of rice sequence with 30 predicted genes (http://www.tigr.org). These genes, their predicted function and their physical location in the rice genome are listed in Table 3. A rice gene with similarity to an NBS+LRR encoding gene (*11682.m4892*) is predicted between sequences with homology to wheat ESTs BE 518048 (*XSTS1BL2*) and TC127200 (*XSTS1BL17*). Other predicted rice genes present between orthologs of BE518048 (*XSTS1BL2*) and TC265717 (*XSTS1BL17*) include three predicted serine carboxipeptidase genes, three protein kinases, including a calcium-dependent protein kinase, a transcription factor and two ATPase genes (Table 3).

Discussion

Previous QTL mapping of the *Lr46* resistance gene identified two amplified fragment length polymorphism

(AFLP) markers (*Pst*AAG*Mse*CTA-1 and *Pst*AAG*Mse*CGA-1) that explained a large proportion of the phenotypic variation for leaf- and stripe-rust severity in a RIL population from the cross Avocet×Pavon 76. The two linked fragments located on 1BL explained 49 and 45%, respectively, of the phenotypic variation for leaf-rust severity and 31 and 24%, respectively, of the variation for yellow-rust severity (William et al. 2003). In another report, a QTL for leaf-rust resistance was found in chromosome 1BL, where the SSR marker *Xwmc44* explained 25.5% of total variation for leaf-rust severity (Suenaga et al. 2003). Both reports showed the presence of this QTL at the distal region of chromosome 1BL. This information provided us with a valuable starting point for our research using chromosome 1B recombinant chromosome substitution line populations to analyse the minor leaf-rust resistance gene *Lr46* as a Mendelian character. Because a single resistance gene was segregating in the recombinant chromosome substitution line populations used in this study, lines could be classified according to Mendelian ratios, and we were able to determine recombination distances between *Lr46* and DNA markers. Although one published and two unpublished SSR markers had been associated with leaf and stripe-rust resistance on chromosome 1BL (William M., personal communication), in our study, none was closely linked to *Lr46*. Using cytological-based physical mapping of the SSR markers flanking *Lr46*, we were initially able to narrow the probable physical location of the gene down to a region comprising approximately 4% of the chromosome arm [deletion bin 1BL (0.85–0.89)].

Knowing the approximate physical location of *Lr46*, we leveraged information from the NSF-funded project to deletion map wheat ESTs. Although 49 wheat ESTs had been deletion mapped in the distal 15% of the chromosome arm 1BL of wheat, we focused on mapping nine ESTs having high homology with sequences from the terminal region of rice chromosome 5L. In this manner, the order of markers in wheat could be compared with the order of homologous sequences in rice. This first round of mapping resulted in the identification of markers flanking *Lr46* that narrowed the syntenic region of rice to three overlapping BAC clones. Using rice sequences from two of these clones to search the wheat gene database resulted in the development of 14 additional markers that could be physically mapped to two submicroscopic deletion bins consisting of less than 1% of the chromosome arm. Placement of polymorphic markers in this region on the genetic linkage map identified two markers flanking *Lr46*, *XSTS1BL2* and *XSTS1BL17*, and physically located *Lr46* to a submicroscopic deletion bin between breakpoints 1BL-13 (0.89–1) and 1BL-10 (0.89–3). Although six EST-based markers in this deletion bin were not polymorphic in the mapping population, they are very likely closely linked to *Lr46*. CS deletion lines 1BL-13 and 1BL-4 and 1BL-10 are being used to evaluate all additional markers potentially linked to *Lr46*.

The data in this study confirm the synteny reported for 1BL of wheat and 5L of rice (La Rota and Sorrells 2004) and the effectiveness of the rice genome as a base for organizing wheat EST sequences for a targeted region of interest. Liu and Anderson (2003) were also able to leverage the synteny of chromosome 3BS of wheat and 1S of rice to enrich the markers near the QTL for resistance to FHB (Liu and Anderson 2003). Comparative RFLP analysis of chromosome 1AS of wheat and 5S of rice identified orthologous sequences between these two species, but there was a reported mosaic of gene conservation on the chromosome arm (Guyot et al. 2004). Thus far, we have not observed any rearrangements of gene order in our region of interest on the long arm of wheat 1B and rice 5 and consider this a region of high homology. However, we do not know if a gene orthologous to *Lr46* exists in rice.

The region of 1BL where *Lr46* has been mapped may be a gene-rich region and a region of high recombination. Erayman et al. (2004) reported that the 1BL-5 (0.85)–1BL-10 (0.89) deletion bin (4% of the 1BL arm) was a gene-rich region of 14 Mb and approximately 280 kb/cm. We placed 16 ESTs belonging to seven tentative contigs and three singletons in less than 1% of the 1BL chromosome arm with at least 12.7 cm of recombination in our mapping populations. We have estimated that the 1BL-13 (0.89–1)–1BL-10 (0.89–3) deletion bin corresponds to 4.7 Mb or 1 cm/343 kb based on data from Gill et al. (1991); thus, the flanking markers identified in this study are physically close to the *Lr46* gene. The syntenic region in rice has high gene density, with more than 30 predicted genes in 168 kb of sequence or about one gene for every 5.6 kb (1.9 cm of genetic length or 1 cm/105 kb; http://www.tigr.org). The gene-rich regions of the wheat genome are suitable for the isolation of genes, and based on this research, we are optimistic that map-based cloning of *Lr46* is feasible.

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