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PCR Markers for *Triticum speltoides* Leaf Rust Resistance Gene *Lr51* and Their Use to Develop Isogenic Hard Red Spring Wheat Lines

M. Helguera, L. Vanzetti, M. Soria, I. A. Khan, J. Kolmer, and J. Dubcovsky*

ABSTRACT

New leaf rust resistance genes are needed in wheat (*Triticum aestivum* L.) to provide additional sources of resistance to the highly variable and dynamic leaf rust pathogen *Puccinia triticina* Eriks. Leaf rust resistance gene *Lr51*, located within a segment of *Triticum speltoides* Taush chromosome 1S translocated to the long arm of chromosome 1B of bread wheat, is resistant to the current predominant races of leaf rust in the USA. The objectives of this study were to determine the genetic length of the translocated 1S segment, develop a PCR marker for *Lr51*, and use this marker to generate isogenic lines for this gene. Characterization of two translocation lines (F-7-3 and F-7-12) with 10 molecular markers indicated that F-7-3 has an interstitial *T. speltoides* chromosome segment of 14 to 32 cM long including loci *XAga7* and *Xmwg710*, whereas line F-7-12 has a complex series of translocations among chromosomes of homeologous group 1. On the basis of the DNA sequence of the A, B, D, and S alleles of the *XAga7* locus, we designed a cleavage amplified polymorphic sequence (CAPS) marker for the S genome allele. Primers S30-13L and AGA7-759R preferentially amplified the *XAga7* 1S (819 bp) and 1B alleles (783 bp). These amplification products can be separated in agarose gels after digestion with *Pst*I or *Bam*HI restriction enzymes. This CAPS marker was validated in a collection of 32 common wheat cultivars and was used to develop three pairs of hard red spring isogenic lines from the donor parent F-7-3. These isogenic lines will be valuable for future assessment of the effect of this chromosome introgression on agronomic performance and end-use quality.

Leaf rust (caused by *Puccinia triticina* Eriks.) is one of the most common and widespread diseases of wheat worldwide; therefore, incorporating genetic resistance to this pathogen into adapted germplasm is a major goal of most wheat breeding programs. In the USA, yield losses due to leaf rust occur annually throughout the soft red winter wheat region of the eastern states, the hard red winter wheat region of the southern and mid Great Plains states, and the northern spring wheat area of the upper Midwest. Yield losses of 10% or more occur when heavy rust infections defoliate flag leaves during grain filling (Chester, 1946).

Utilizing disease resistance genes minimizes the need for the application of costly fungicides, thus reducing environmental contamination risks and decreasing production costs. Approximately 50 leaf rust resistance genes from wheat and wheat relatives have been cataloged (McIntosh et al., 2003), and molecular markers are available for many of them (Procunier et al., 1995; Gold et al., 1999; Helguera et al., 2000; Huang and Gill, 2001; Helguera et al. (2003), http://maswheat.ucdavis.edu/; verified 18 November 2004). Unfortunately, many of the race specific genes are no longer effective against new virulent races of this pathogen. Wild relatives of wheat may be a useful source of additional resistance genes to counter balance the continuous evolution of leaf rust populations.

Triticum speltoides Taush ($2n = 14$, S genome) is an attractive source of high levels of resistance to leaf, stem, and stripe rust of wheat (Dvorak, 1977), and leaf rust resistance genes *Lr28*, *Lr35*, *Lr36*, and *Lr47* were derived from this species (McIntosh et al., 2003). Crosses between this species and hexaploid wheat (*T. aestivum* L. $2n = 42$, ABD genomes), followed by selection of resistant progeny, frequently resulted in translocations between the *T. speltoides* and common wheat chromosomes since many *T. speltoides* genotypes have the ability to promote homeologous chromosome pairing when hybridized with wheat (Dvorak, 1977).

The wheat breeding line 'Neepawa'*6/*Triticum speltoides* F-7 was selected from the cross *T. aestivum* cv. Neepawa × *T. speltoides* accession F (Dvorak, 1977). A 3-to-1 segregation ratio of resistant to susceptible plants in the F_2 population indicated that a single gene determined this source of resistance. Monosomic and ditelosomic analysis provided evidence that the resistance gene was transferred to chromosome arm 1BL (41% recombination with centromere) (Dvorak and Knott, 1980). This gene, temporarily named *LrF7*, has been designated *Lr51* (B. McIntosh, personal communication).

In tests for resistance to *P. triticina* race 5, plants homozygous for *Lr51* were highly resistant with hypersensitive flecks, whereas heterozygous plants showed slightly lower levels of resistance with small pustules surrounded by necrosis and chlorosis, indicating incomplete dominance (Dvorak, 1977). The Neepawa*6/*Triticum speltoides* F-7 line exhibited very low infection types with the seven leaf rust races tested (Dvorak and Knott, 1980).

In spite of its high levels of resistance to predominant leaf rust races, *Lr51* has not been widely deployed in breeding programs, probably because of negative genetic effects associated with the presence of large *T. speltoides* chromosome segments and/or additional homeologous translocations in other wheat chromosomes. The objectives of this study were to (i) determine the length of the *T. speltoides* translocations in distinct *Lr51*

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Abbreviations: BC, backcross; CAPS, cleaved amplified polymorphic sequence; CS, Chinese Spring; HRS, hard red spring; MAS, marker assisted selection; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

lines, (ii) explore the presence of additional structural changes induced by *T. speltoides*, (iii) develop a set of PCR markers for efficient selection of *Lr51* among segregating progeny, and (iv) develop hard red spring (HRS) *Lr51* isogenic lines for use in evaluating the effect of this alien chromosome introgression on agronomic performance and bread-making quality.

MATERIALS AND METHODS

Seeds from the two lines derived from Neepawa*6/*Triticum speltoides* F-7 line (Dvorak, 1977; Dvorak and Knott, 1980), F-7-3, and F-7-12 were kindly provided by Dr. J. Dvorak (University of California, Davis). He also provided seeds of the original recurrent parent Neepawa, Chinese Spring nullisomic-tetrasomic lines N1AT1B, N1BT1D, and N1DT1A (Sears, 1954), *T. speltoides* (accessions # S3362, S3343), *T. tauschii* (Cosson) Schmalh. (D genome, accession DV148), and *T. urartu* Tum. (A genome, accession G3221).

Recurrent HRS cultivars Express (WestBred), Kern (University of California), and breeding line UC1037 (University of California) were crossed with the Neepawa*6/*Triticum speltoides* F-7-3 line. The F_1s were backcrossed six times with the respective recurrent parents, and in each generation, two individuals heterozygous for *Lr51* were selected by marker-assisted selection (MAS). Finally, BC_6 plants heterozygous for *Lr51* were self-pollinated and homozygous plants were selected among BC_6F_2 plants using the molecular marker described below. After six backcrosses, selected plants are expected to be more than 99% identical to the recurrent parent.

Finally, a diverse set of 32 wheat cultivars and breeding lines (mainly from USA and Argentina) was analyzed to validate the *Lr51* CAPS marker (Table 1).

Tests for Resistance to *P. triticina*

Lines homozygous for *Lr51* and sister lines homozygous for the complementary 1BL segment without the resistance gene were selected from BC_6F_2 plants for each of the three recurrent backcross populations (Kern, Express, and UC-1037). The homozygous 1BL plants from the BC_6F_2 populations were preferred as a negative control over the recurrent parent to rule out possible residual heterozygosity in genes affecting the resistance reaction.

Four to six BC_6F_3 plants from one or two independent homozygous BC_6F_2 plants per genotype were evaluated for seedling resistance to leaf rust at the Cereal Disease Laboratory (St. Paul, MN), as previously described (Kolmer et al., 2003). Plants were grown in a greenhouse set at 18 to 21°C with 8 h of metal halide supplemental lighting at 400 to 450 μmol m^{-2} s^{-1} at bench level. Seedlings were inoculated with leaf rust races THBJ (avirulence/virulence formula 9, 24, 3ka, 11, 17, 30, 18/1, 2a, 2c, 3, 16, 26, 10) and MCDS (avirulence/virulence formula 1, 3, 26, 17, 10/2a, 2c, 9, 16, 24, 3ka, 11, 30, 18), which are common races in the USA (Kolmer et al., 2003). A preliminary screening of the recurrent parents and the *Lr51* donor line indicated that race THBJ was virulent on Express but avirulent on *Lr51* donor line. Race MCDS was virulent on Kern and UC1037 and was avirulent on the *Lr51* donor line. Seedlings were inoculated at 7 to 8 d after planting, when the primary leaves were fully extended. For each race, an oil-spore mixture was atomized onto the seedling plants. The plants were allowed to dry, and were then placed in a dew chamber for 18 h, with no light. After incubation, the plants were placed in a greenhouse set at 18 to 21°C with supplemental metal halide lighting. Infection types were scored 12 d after inoculation by the scale in Long and Kolmer (1989).

DNA Extraction and RFLP Procedures

A large-scale DNA isolation procedure (Dvorak et al., 1988) was used for the restriction fragment length polymorphism analysis (RFLP) and to optimize the amplification conditions for PCR markers. A fast, small-scale DNA isolation procedure more appropriate for MAS (Wining and Langridge, 1991) was used to test BC and BC-F_2 populations. Procedures for Southern blots and hybridization were as described previously (Dubcovsky et al., 1994). A total of 10 low copy probes previously mapped on chromosome $1A^m$ from *T. monococcum* (Dubcovsky et al., 1996) were used to characterize the *T. speltoides* segment carrying the leaf rust resistance gene *Lr51* (Fig. 1).

Cloning and Sequence Analysis

Genomic DNAs from Neepawa, Neepawa*6/*T. speltoides* F-7-3 and F-7-12 lines, Chinese Spring nullisomic-tetrasomic lines of group 1 chromosomes, *T. speltoides* accessions S3343, and S3362, *T. tauschii* accession DV148 and *T. urartu* accession G3221 were used as template for PCR-amplification using primers AGA7-342F and AGA7-759-R (Table 2). Purified PCR products were cloned into pCR4-TOPO vector (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's protocols. Recombinant clones were purified and sequenced using an ABI377 automatic sequencer. Program Primer3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi; verified

Table 1. Origin and accession numbers of hexaploid cultivars and breeding lines used to validate the CAPS marker for *Lr51*.

Name	Origin	Accession†	Name	Origin	Accession†
'Buck Manantial'	Argentina	PI 344455	'Sonora 64'	Mexico	CItr 13930
'Coop. Millan'	Argentina	NA‡	'Avalon'	UK	PI 446910
'Klein Pegaso'	Argentina	NA	'Brooks'	USA	NA (CA)
'Klein Rendidor'	Argentina	PI 351622	'Cavalier'	USA	NA (CA)
'Prointa Gaucho'	Argentina	NA	'Hyak'	USA	PI 511674
'Prointa Granar'	Argentina	NA	'Kern'	USA	NA (CA)
'Prointa Milenium'	Argentina	NA	'Len'	USA	CItr 17790
'Prointa Oasis'	Argentina	NA	'Penawawa'	USA	PI 495916
'Avocet'	Australia	PI 464644	'Tadinia'	USA	PI 494096
'Sunfield'	Australia	NA	'Yecora Rojo'	USA	CItr 17414
'Columbus'	Canada	PI 496258	'Yolo'	USA	CItr 17961
'Manitou'	Canada	CItr 13775	UC896	USA	Breeding line CA
'Neepawa'	Canada	CItr 15073	UC1041	USA	Breeding line CA
'Chinese Spring'	China	CItr 14108	UC1107	USA	Breeding line CA
'Fugimi Komugi'	China	NA	UC1110	USA	Breeding line CA
'Pavon 76'	Mexico	PI 519847	ND683	USA	Breeding line ND

† Accession numbers were obtained from http://www.ars-grin.gov/npgs/acc/acc_queries.html; verified 18 November 2004.
‡ NA = not available.

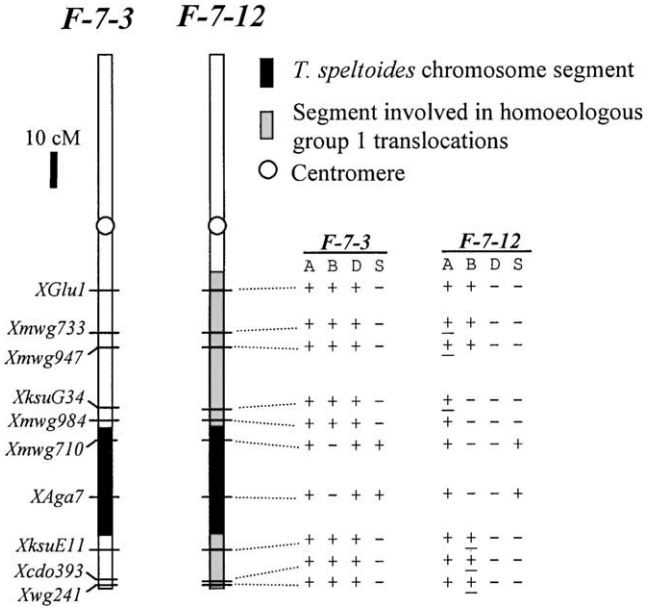

Fig. 1. Inferred RFLP map of the translocated chromosomes in Neepawa F-7-3 and F-7-12. The *T. speltoides* segment is indicated in black and regions involved in additional translocations among chromosomes from homeologous group 1 are indicated in gray. The + and – signs in the right panels indicate the presence or absence of RFLP fragments from the A, B, D, and S genomes. Underlined ± signs indicate a relatively higher hybridization intensity. The order and distances among markers were inferred from a *T. monococcum* map including the same markers (Dubcovsky et al., 1996).

18 November 2004) was used for primer design. Identity values for the best-fit alignments between sequences were obtained by means of the computer program Blast 2 Sequences Version 2.2.6 (Tatusova and Madden, 1999).

PCR Procedures

The PCR reactions were performed with thermocyclers PerkinElmer (Foster City, CA) GeneAmp PCR system 9700 and MJ Research (Waltham, MA) PTC100. PCR reactions contained 100 ng of wheat genomic DNA, 1× *Taq* polymerase buffer (Promega Corp., Madison, WI), 1.0 U *Taq* DNA polymerase (Promega), 0.2 μ*M* of each primer, and 200 μ*M* of each dNTP in a final volume of 25 μL per PCR reaction. Primer names, function, sequences, $MgCl_2$ concentrations, and PCR cycling conditions are summarized in Table 2.

For the CAPS markers, 10 μL of the PCR amplification products were digested with restriction enzymes *Pst*I or *Bam*HI (Promega) by adding 5 units of enzyme to the PCR product. Samples were separated by electrophoresis in 2% (w/v) agarose gel and visualized by means of ethidium bromide and UV light.

RESULTS AND DISCUSSION

Infection Types to *Puccinia triticina*

The presence of *Lr51* in F-7-3 improved resistance to leaf rust isolates BBDL, MCDS, MDRL, MBRL, SBDB, TFGQ, and THBJ relative to the recurrent parent Neepawa (data not shown). Seedlings of the recurrent parent Express had high infection types with race THBJ, whereas Kern and UC1037 had high infection types with race MCDS (Table 3). All the plants homozygous for the *T. speltoides* chromosome segment carrying the *Lr51* gene derived from backcrosses with Express, UC1037, and Kern, were homogeneous for very low infection types (hypersensitive flecks), similar to the Neepawa*6/Triticum speltoides F-7-3 line, the 1S#F7L translocation donor. As expected, all sister line plants lacking the *T. speltoides* segment were homozygous susceptible, with high infection types similar to those observed in the original parental lines (Table 3). These results demonstrate that the *Lr51* gene was successfully transferred into these three recurrent parents by MAS and that *Lr51* was effective in the three genetic backgrounds tested in this study.

Molecular Characterization of the *T. speltoides* Chromosome Segment

Molecular characterization of translocation lines Neepawa F-7-3 and F-7-12 with 10 molecular markers revealed that different chromosome rearrangements exist in these lines (Fig. 1). Neepawa F-7-3 had RFLP not present in the recurrent parent Neepawa for loci *XAga7* and *Xmwg710* (Fig. 1). The corresponding 1B fragments for these two loci were missing, confirming that the *T. speltoides* segment was translocated to wheat chromosome 1B (Dvorak and Knott, 1980). The other eight molecular markers from the long arm of homeologous group 1 did not detect polymorphism between Neepawa and Neepawa F-7-3. The *T. speltoides* translocation does not include the high-molecular weight glutenin *Glu-B1* locus, which has a significant impact on bread-making quality.

Since the original *T. speltoides* F7 accession was not available, it was not possible to determine if the absence of polymorphisms in the markers flanking *XAga7* and *Xmwg710* was originated in the absence of the *T. speltoides* segment or in the lack of polymorphism between the S and B genomes. The first hypothesis is more likely, since *T. speltoides* is an outcrossing species with very high levels of polymorphism (Dvorak et al., 1998). Previous studies that compared the alleles present in *T. speltoides* populations with those present in the B genome, found that almost all RFLP loci were polymorphic using

Table 2. Primer used for (1) initial cloning of the *XAga7* A, B, D and S genome alleles and (2) B and S allele specific CAPS primers.

	Name	Sequence (5′–3′)	PCR conditions
1	AGA7-342F	GCT TCA ACA GTG GCA TCA ACA AG	annealing temperature: 60°C.
	AGA7-759R	TGG CTG CTC AGA AAA CTG GAC C	40 cycles†. 3 m*M* $MgCl_2$.
2	S30-13L	GCA TCA ACA AGA TAT TCG TTA TGA CC	annealing temperature: 52°C.
	AGA7-759R	TGG CTG CTC AGA AAA CTG GAC C	40 cycles†. 1.5 m*M* $MgCl_2$.

† Each cycle includes a 45 s denaturation step at 94°C, a 45 s annealing step at 60°C or 52°C, and a 60 s extension step at 72°C. Both cycles include a final extension step at 72°C for 10 min.

Table 3. Infection types of BC_6F_2 lines homozygous for the presence of the *T. speltoides* chromosome 1S translocation or for the non translocated *T. aestivum* chromosome 1B. All lines were tested for RFLP markers *XAga7* and *Xmwg710*: S = *T. speltoides* chromosome 1S allele, B = *T. aestivum* chromosome 1B allele. At least 4 plants from each line were inoculated with *Puccinia triticina* races THBJ or MCDS.

Cultivar/BC_6F_2 Line	*XAga7* allele	*Xmwg710* allele	Predicted *Lr51*	Rust race	Infection types†
Neepawa	B	B	Absent	THBJ	33+
Neepawa-F-7-3	S	S	Present	THBJ	;
Express	B	B	Absent	THBJ	2+3
Express 00696/69	B	B	Absent	THBJ	33+
Express 00696/54	B	B	Absent	THBJ	33+
Express-*Lr51* 00696/65	S	S	Present	THBJ	;
Express-*Lr51* 00696/57	S	S	Present	THBJ	;
Neepawa	B	B	Absent	MCDS	33+
Neepawa-F-7-3	S	S	Present	MCDS	;
UC1037	B	B	Absent	MCDS	33+
UC1037 00696/40	B	B	Absent	MCDS	33+
UC1037-*Lr51* 00696/47	S	S	Present	MCDS	;
Kern	B	B	Absent	MCDS	33+
Kern 00696/3	B	B	Absent	MCDS	33+
Kern 00696/4	B	B	Absent	MCDS	33+
Kern-*Lr51* 00696/8	S	S	Present	MCDS	;
Kern-*Lr51* 00696/11	S	S	Present	MCDS	;

† '0' = Immune; ';' = necrotic flecks; '1' = necrotic flecks and small uredinia; '2' = small uredinia with chlorosis; '3' = moderate size pustules without chlorosis or necrosis; '4' = Large uredinia without necrosis or chlorosis. Symbols "–" and "+" denote smaller or larger uredinia. The most common infection is listed first. For example, 33^+ indicates infection types of 3 and 3+.

only one restriction enzyme (J. Dvorak, personal communication). Since none of the four restriction enzymes used in this study (*Eco*RI, *Bam*HI, *Hin*dIII, and *Xba*I) detected polymorphisms for the markers flanking *XAga7* and *Xmwg710*, we assumed this indicated that the *T. speltoides* segment did not extend to those flanking markers. On the basis of this assumption, the minimum and maximum estimates for the genetic length of the interstitial 1S chromosome segment were 14 cM (*XAga7* and *Xmwg710*) and 32 cM (*Xmwg984* and *XksuE11*), respectively (Fig. 1). These genetic distances were based on a *T. monococcum* map (Dubcovsky et al., 1996) that included all of the markers used in this study.

Translocations in F-7-12 were more complex than in F-7-3. Although *T. speltoides* bands for the *XAga7* and *Xmwg710* loci were found in both lines, the missing bands from the B genome extended to loci *Xmwg984* and *XksuG34* in F-7-12 (Fig. 1). In addition, D genome fragments were missing for all of the markers, B genome fragments showed double hybridization intensity for markers distal to the *XAga7* locus, and A genome fragments showed double hybridization intensity for markers proximal to *Xmwg710.* The double intensity restriction fragments suggest the presence of duplicated chromosome segments. A translocation of the *T. speltoides* segment and its distal 1B chromosome segment to chromosome 1A, followed by a second translocation distal to *XGlu-1* to chromosome 1D would explain the absence of D genome loci at both sides of *T. speltoides* segment and the duplicated 1A chromosome segment in the proximal region and for chromosome 1B in the distal region (Fig. 1).

The presence of complex translocations among the three homeologous group 1 chromosomes in F-7-12 could explain the limited use of this line in breeding programs. The chromosome duplications would generate multivalents during meiosis, reducing fertility and generating off type plants. In addition the large deletion in chromosome 1D may have a negative effect on agronomic or end-use quality characteristics. On the basis of these results, we decided to introgress *Lr51* from the Neepawa F-7-3 line, which does not have the complex homeologous translocations found in F-7-12.

Selection of the RFLP Probe to Be Converted into a PCR Marker

Of the two RFLP loci detected within the *T. speltoides* chromosome segment, *XAga7* has a more simple hybridization pattern compared with *Xmwg710* and, therefore, was selected for conversion into a PCR marker. The *XAga7* locus was detected by probe *AGA7*, a 1798-bp cDNA coding for the large subunit of the wheat endosperm ADP-glucose pyrophosphorylase gene (*AGP2*, GenBank # X14350.1, Olive et al., 1989). *AGP2* homeologous genes are located on the long arms of chromosomes 1A, 1B and 1D, approximately 80 cM away from the centromere (Ainsworth et al., 1995).

The interstitial *T. speltoides* chromosome segment is not expected to recombine with the 1BL chromosome segment in the presence of the *Ph* gene and the absence of the rest of the *T. speltoides* chromosomes (Dvorak, 1977). Therefore, a single marker located within this segment should be sufficient to transfer the *Lr51* gene into breeding lines. We tested the homozygous BC_6F_2 lines from the three recurrent parents with markers for both *XAga7* and *Xmwg710* loci and found no recombination between these markers or between these two markers and the *Lr51* gene (Table 3). These results indicate that there was no recombination among these loci during the six generations of backcrossing and one generation of self-pollination in any of the five BC_6F_2 homozygous *Lr51* lines tested (Table 3). This is equivalent to detecting complete linkage in a backcross or double haploid segregating population of 40 plants.

Sequence Analysis of the *XAga7* locus

Two PCR fragments of approximately 790 and 830 bp were amplified with primers AGA7-342F and AGA7-759R (Table 2) from Neepawa, but only the 830-bp

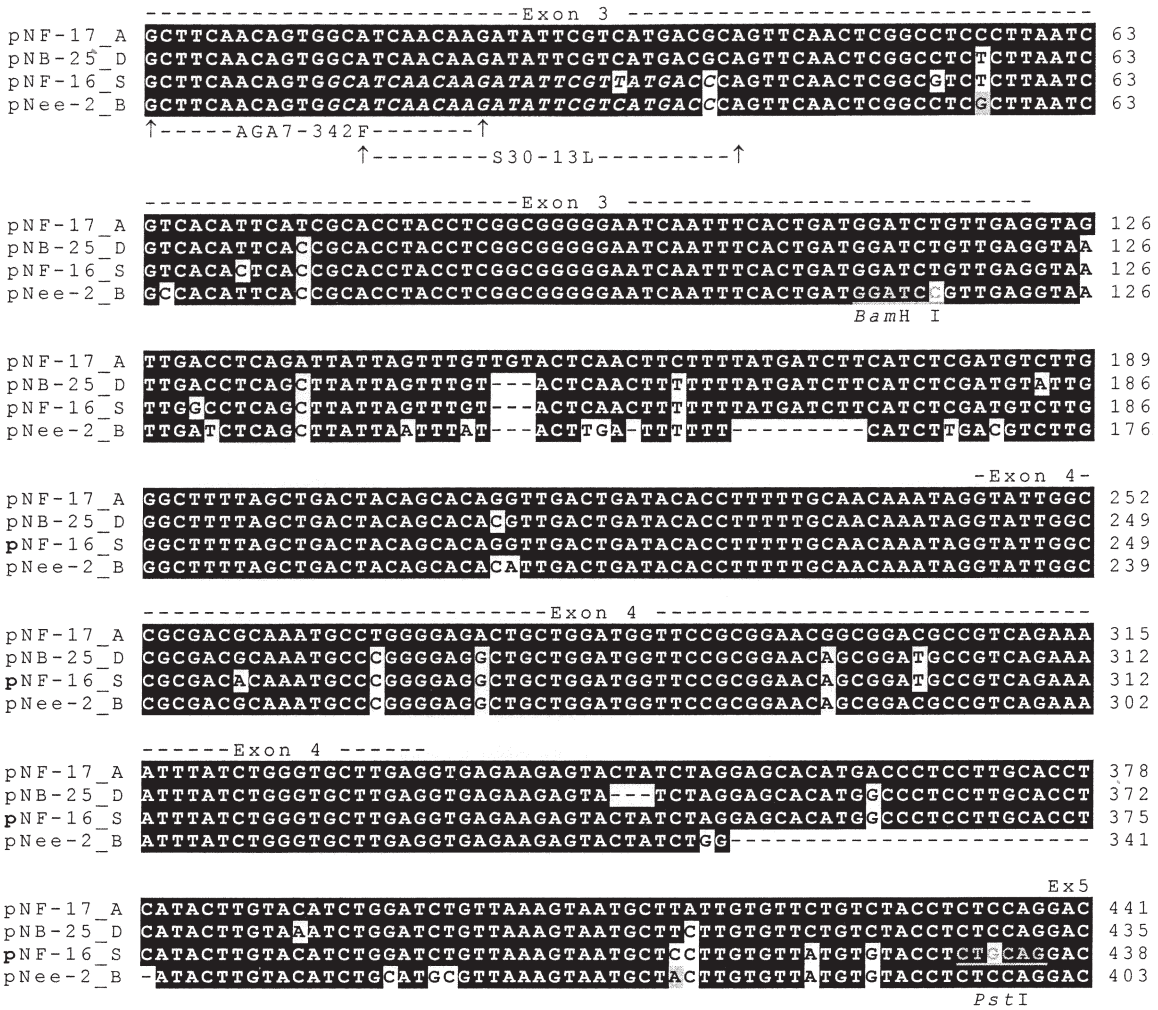

Fig. 2. Best-fit alignment of partial nucleotide sequences from wheat clones from the A (pNF7-17), D (pNB-25), S (pNF7-16), and B (pNee-2) genomes. Gaps were introduced to maximize nucleotide alignment and are indicated with dashes. Locations of PCR primers AGA7-342F and S30-13L are indicated with arrows and sequences from the primers are italicized. *Bam*HI and *Pst*I restriction sites used to develop the CAPS marker are underlined with letters in gray color. Exons are numbered from the first transcribed exon and indicated above the sequences.

fragment was present in Neepawa-F-7-3, suggesting that the 790-bp fragment was amplified from the B genome. This hypothesis was confirmed by nullitetrasomic analysis: the 790-bp fragment was amplified in lines N1AT1B, N1DT1A but it was absent in N1BT1D (data not shown). The 830-bp fragment did not disappear in any of the three nullitetrasomic lines indicating that is likely a mixture of products from the *XAga7-A* and *XAga7-D* loci. The 830-bp fragment also was detected in PCR amplifications from DNAs of diploid *T. urartu, T. tauschii*, and *T. speltoides* accessions S3343 and S3362.

The 790-bp amplification product was cloned from Neepawa and Chinese Spring (CS) (B genome) and the 830-bp band was cloned from Neepawa-F-7-3 (expected mixture of A, D, and S products) and from the three diploid species (separate A, D, and S products). The three 790-bp B-genome clones (795 bp based on sequence) were almost identical, and only the sequence from the Neepawa B-genome clone (pNee-2, GenBank AY589012) is presented in Fig. 2.

The sequences from CS 830-bp clone pNB-25 (GenBank AY589010) and pT-41 from *T. tauschii* were identical and represented the *XAga7* 1D allele (Fig. 2). Two 830-bp clones from Neepawa-F-7-3, pNF16 (GenBank AY589011) and pNF17 (GenBank AY589009) were assigned to the other two genomes. The sequence from clone pNF17 showed higher identity with the sequence from *T. urartu* clone pU35 (97%) than with those from *T. speltoides* clones pS29 and pS30 (95%) indicating that it was from the A genome. The sequence from clone pNF16 showed the highest identity values with the *T. speltoides* clones (97.5%) indicating that it was the sequence from the *XAga7*1S allele donated to Neepawa-F-7-3.

Development of a Cleavage Amplified Polymorphic Sequence Marker

Primers S30-13L and AGA7-759R (Table 2) amplified preferentially alleles from the S (pNF7-16) and B (pNee-2) genomes, respectively (Fig. 2). The AGA7-759R primer is not genome-specific, but the 3' end of the S30-13L primer was selected to match a cytosine (position 38, Fig. 2) that differentiates the S and B genome alleles from the A (pNF7-17) and D (pNB-25) genome alleles. An additional polymorphism, a thymine

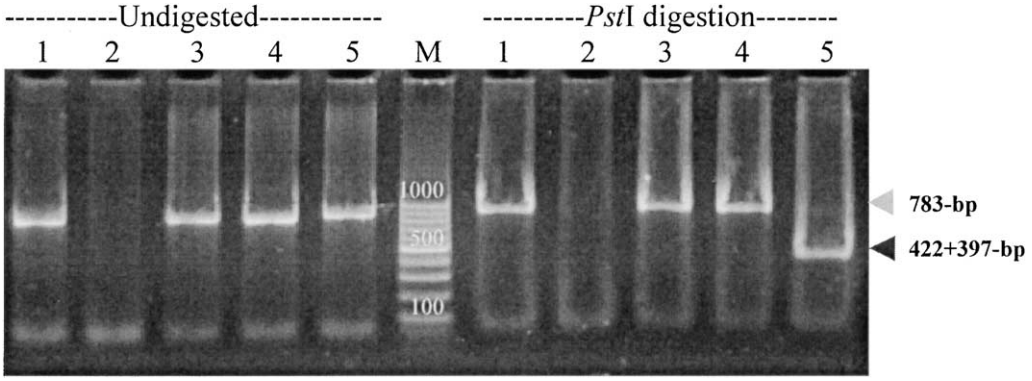

Fig. 3. Undigested and *Pst*I digested PCR amplification products with CAPS primers S30-13L and AGA7-759R. 1-3) Chinese Spring nullisomic-tetrasomic lines. 1) N1AT1B, 2) N1BT1D, 3) N1DT1A, 4) Neepawa, 5) Neepawa*6/Triticum speltoides F-7-3. The gray arrowhead indicates the 780-bp product band from the B genome. The black arrowhead indicates the *Pst*I digested product from the S genome. "M" indicates the molecular markers in bp (100-bp ladder, Biodynamics Corp., Seattle, WA).

at position 32, differentiates the S allele from the alleles in the other three genomes. The presence of this difference alone did not interfere with the amplification of the B genome allele, probably because of the internal position of the polymorphisms within the primer. PCR amplification of nullitetrasomic DNAs with these primers produced a 783-bp fragment (B-allele) in lines N1AT1B and N1DT1A but no PCR product in N1BT1D, demonstrating that these primers do not amplify the A or D genome alleles under the PCR conditions used in this study (Fig. 3, Lanes 1, 2, and 3).

The amplification products from primers S30-13L/AGA7-759R from the B (783 bp) and S (819 bp) alleles can be separated by polyacrylamide gels (14%, w/v). These two amplification products also can be separated in agarose gels (2%) after digestion with *Pst*I or *Bam*HI (Fig. 3 and 4). The S genome amplification product has a unique *Pst*I restriction site at position 434 (Fig. 2) that divides the 819-bp fragment into two fragments of similar size (397 and 422 bp, Fig. 3). The B genome amplification product has a unique *Bam*HI restriction site at position 111 that cuts the 783-bp fragment into two 672- and 111-bp fragments (Fig. 4).

To validate this CAPS marker in a wide range of wheat cultivars, we evaluated the presence of the diagnostic *Bam*HI and *Pst*I restriction sites in a diverse set of 32 cultivars and breeding lines (Table 1). All of the cultivated wheats from Table 3 showed the 783-bp fragment, which was digested by *Bam*HI into the expected 672- and 111-bp fragments (Fig. 5). None of these amplification products was digested by *Pst*I. These data suggested that the *Lr51* CAPS marker presented here will be useful in a wide range of cultivars.

Development of Leaf Rust Resistant Germplasm using Molecular Markers

Leaf rust resistance has been particularly short-lived in wheat cultivars with single seedling resistance genes. Wheat cultivars with combinations of effective resistance genes should provide resistance for a longer period of time to an increased number of races than single leaf rust resistance genes.

The development of molecular markers for *Lr51* will facilitate combining this gene with additional leaf rust resistance genes. We are currently combining *Lr51* with *Lr47* and *Lr37* before releasing it in a commercial cultivar. This is now possible because molecular markers are available for all three genes (Dubcovsky et al., 1998; Helguera et al., 2000, 2003). We have developed isogenic lines for *Lr51*, *Lr47*, and *Lr37* in Express, Kern, and UC1037 backgrounds and are currently intercrossing the UC1037 lines to develop a single line with all three genes. An alternative strategy that can be used to extend the useful life of *Lr51* is combining this gene with the slow rusting genes *Lr34* and *Lr46*, for which molecular markers also are now available (Schnurbusch et al., 2004; Suenaga et al., 2003; William et al., 2003).

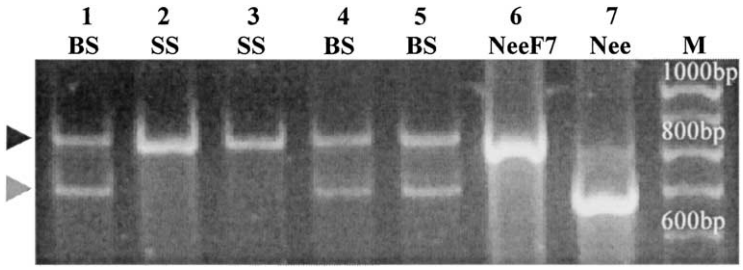

Fig. 4. *Bam*HI digestion of PCR products obtained from genomic DNAs amplified using CAPS primers S30-13L and AGA7-759R. Genomic DNAs were extracted from Neepawa (Nee) and Neepawa*6/Triticum speltoides F-7-3 (NeeF7), and from five BC_6F_2 plants. Letters "BS" and "SS" indicates plants heterozygous and homozygous for the presence of *Lr51*, respectively. Parental line Neepawa (Lane 7) is the only BB plant in this figure. The black arrowhead indicates the 819-bp PCR amplification product from the S genome allele and the gray arrowhead indicates the larger of the two *Bam*HI digested fragments from the B genome allele of *XAga7*. "M" indicates the size molecular marker (100-bp ladder, Biodynamics).

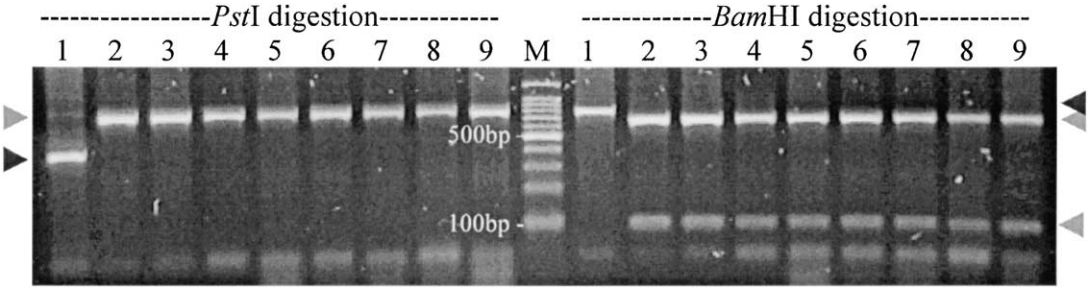

Fig. 5. *Pst*I and *Bam*HI digestion of PCR products obtained with *XAga7* CAPS primers S30-13L and AGA7-759R. Genomic DNAs were extracted from 1) Neepawa*6/Triticum speltoides F-7-3, 2) Neepawa, 3) Madsen, 4) Hyak, 5) Penawawa, 6) Buck Manantial, 7) Prointa Milenium, 8) Prointa Granar, and 9) Klein Pegaso. Gray and black arrowheads indicate amplification products from the B and S genome alleles, respectively. "M" indicates the size molecular marker (100-bp ladder, Biodynamics).

If future comparisons of the *Lr51* isogenic lines reveal the presence of negative effects on yield or end-use quality associated with the introgression of the *T. speltoides* segment, the molecular map presented here can be used to select shorter alien chromosome segments through a second round of homeologous recombination.

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