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ORIGINAL PAPER

Identification and validation of molecular markers linked to the leaf rust resistance gene *Lr19* in wheat

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Abstract A leaf rust resistance gene *Lr19* on the chromosome 7DL of wheat derived from *Agropyron elongatum* was tagged with random amplified polymorphic DNA (RAPD) and microsatellite markers. The F_2 population of 340 plants derived from a cross between the leaf rust resistant near-isogenic line (NIL) of Thatcher (Tc + *Lr19*) and leaf rust susceptible line Agra Local that segregated for dominant monogenic leaf rust resistance was utilized for generating the mapping population. The molecular markers were mapped in the F_2 derived F_3 homozygous population of 140 seedlings. Sixteen RAPD markers were identified as linked to the alien gene *Lr19* among which eight were in a coupling phase linkage. Twelve RAPD markers co-segregated with *Lr19* locus. Nine microsatellite markers located on the long arm of chromosome 7D were also mapped as linked to the gene *Lr19*, including 7 markers which co-segregated with *Lr19* locus, thus generating a saturated region carrying 25 molecular markers linked to the gene *Lr19* within 10.2 ± 0.062 cM on either side of the locus. Two RAPD markers $S265_{512}$ and $S253_{737}$ which flanked the locus *Lr19* were converted to sequence characterized amplified region markers $SCS265_{512}$ and $SCS253_{736}$, respectively. The marker $SCS265_{512}$ was linked with *Lr19* in a coupling phase and the marker $SCS253_{736}$ was linked in a repulsion phase, which when used together mimicked one co-dominant marker capable of distinguishing the heterozygous resistant seedlings from the homozygous resistant. The molecular markers were validated on NILs mostly in Thatcher background isogenic for 44 different *Lr* genes belonging to both native and alien origin. The validation for polymorphism in common leaf rust susceptible cultivars also confirmed the utility of these tightly linked markers to the gene *Lr19* in marker-assisted selection.

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Introduction

Leaf rust disease caused by the fungus *Puccinia triticina* Rob. Ex Desm. f. sp. *tritici* Eriks (syn. *Puccinia recondita*) affects the productivity and quality of wheat throughout the world. Depending on the severity and duration of infection, the losses can vary up to 50% depending on the leaf rust resistance genes (*Lr* genes) deployed and the composition of virulence in the region (Nagarajan and Joshi 1975; McIntosh et al. 1995). The utilization of disease resistance genes is the most viable and socially acceptable strategy to minimize the yield loses. Till date, about 55 leaf rust resistance genes (*Lr* genes) including approximately 25 *Lr* genes introgressed from wild relatives have been reported to exist, many of which are being utilized in the wheat breeding program (McIntosh et al. 1995, 2005). The alien *Lr* genes derived from the wild

relatives of wheat contribute as valuable genetic resource for effective resistance against multiple races of the leaf rust pathogen. One such gene *Lr19* has been transferred by Sharma and Knott (1966) to long arm of the wheat chromosome 7D from *Agropyron elongatum* (syn. *Lophopyrum elongatum*). Although, a linkage drag which resulted in an accompanying undesirable yellow flour colour was involved in the initial wheat derivatives, several mutant lines without the yellow pigmentation or other recombinants have been developed (Knott 1980; Marais 1992a, b; Prins et al. 1997; Marais et al. 2001). In addition, unlike many alien gene transfers, the 7DL.7Ag translocation carrying the gene *Lr19* is known to be associated with increased grain yield (Reynolds et al. 2001; Monneveux et al. 2003), making *Lr19* an important gene for wheat breeding against leaf rust.

The alien gene *Lr19* is effective against all the pathotypes of leaf rust in South Africa (Prins et al. 1997), India (Tomar and Menon 1998), Europe (Mesterhazy et al. 2000) and Canada (McCallum and Seto-Goh 2003). Even though virulence for the gene *Lr19* has been reported in some wheat cultivated areas over the past decade (Huerta-Espino and Singh 1994; Sibikeev et al. 1996), the gene is effective in Asia, Australia and Europe and can be utilized in combination with other *Lr* genes world-wide to provide long lasting resistance against leaf rust (Roelfs 1988; Pink 2002). However, breeding efforts to pyramid two or more effective *Lr* genes have been severely restricted by the absence of leaf rust virulences which can help tracking the *Lr* genes in the segregating populations.

Alternatively, molecular markers such as restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) or those which are based on the user friendly polymerase chain reaction (PCR) technology such as random amplified polymorphic DNA (RAPD), sequence characterized amplified region (SCAR), sequence tagged sites (STS) and microsatellite or simple sequence repeat (SSR) provide an important tool to plant breeders to practise indirect selection for the resistance genes in a disease-free environment and pyramid the resistance genes in the absence of distinguish virulences in wheat (Röder et al. 1998; Gupta et al. 1999). Although, a RFLP marker (Autrique et al. 1995) and an AFLP-derived STS marker (Prins et al. 2001) have been identified as linked to the gene *Lr19*, the STS marker could not be validated for consistent polymorphism in the Indian wheat cultivars at this laboratory and the RFLP methodology is restricted by being expensive, time consuming and laborious that requires radio-activity. Therefore, there is a need to identify new PCR based molecular markers linked to the alien gene *Lr19* so that this gene can be rapidly transferred into other leaf rust susceptible wheat cultivars and can be combined with other effective *Lr* genes to increase the durability of the resistance. In this paper, we report the identification of microsatellites and coupling and repulsion phase RAPD markers tightly linked to the gene *Lr19*, conversion of RAPD markers to SCAR markers and their validation for their effective use in marker-assisted selection (MAS).

Materials and methods

Plant materials

A Thatcher near-isogenic line (NIL) of wheat Tc + *Lr19* (Acc. RC 35, sourced from the University of Sydney through Prof. R.G. Saini, Punjab Agricultural University, Ludhiana, India) carrying leaf rust resistance gene *Lr19* from the mutant donor line of Knott (1980) was crossed with universally leaf rust susceptible wheat cultivar Agra Local to generate the mapping population for tagging the gene *Lr19* with RAPD and microsatellite markers. The original donor source for alien gene *Lr19* in the resistant line Tc + *Lr19* was the variety Agatha that originally carried the *A. elongatum* translocation produced by Sharma and Knott (1966). Linkage analysis of the markers with *Lr19* locus was done on a F_2 population segregating for leaf rust and comprising of 340 individuals. Approximately, 50 plants of each F_3 family were tested against leaf rust pathotype to identify the genotype of the corresponding F_2 plants. One hundred and forty F_2 plants homozygous at the *Lr19* locus were used in a proportion of 1:1 for resistance:susceptibility to the leaf rust pathotype for molecular mapping.

The specificity of the markers was checked on an international set of NILs, mostly in the Thatcher background, carrying the different alien source derived leaf rust resistance genes namely *Lr9*, *Lr19*, *Lr21*, *Lr22a*, *Lr23*, *Lr24*, *Lr25*, *Lr26*, *Lr28*, *Lr29*, *Lr32*, *Lr36*, *Lr37*, *Lr39*, *Lr40*, *Lr41*, *Lr42*, *Lr43, Lr44*, *Lr45* and native leaf rust resistance genes namely *Lr1*, *Lr2a*, *Lr3*, *Lr3Ka*, *Lr10*, *Lr11*, *Lr12*, *Lr13*, *Lr14a*, *Lr14b*, *Lr14ab*, *Lr15*, *Lr16*, *Lr17*, *Lr18*, *Lr20*, *Lr22b*, *Lr27+31*, *Lr30*, *Lr33*, *Lr34*, *Lr48*, *Lr49*, received from Sydney, Australia, through Dr R.G. Saini, Punjab Agricultural University, Ludhiana, India. The markers were also tested on a wide range of wheat cultivars known not to possess the gene *Lr19* namely, HD 2285, HD 2329, HUW 234, Kalyansona, Lok-1, PBW 226, WH 147, NI 5439, C-306, WH 542, Sonalika, HUW 468, Vidisha, Vaishali,

Kanchan, Kundan, PBW 343, HS 240, HDR 277, HD 2687, HD 2733, UP 262, Thatcher and Chinese Spring.

Leaf rust evaluation

The parents and the progeny populations of the cross (F_1, F_2 and F_3) were evaluated for leaf rust disease against the leaf rust pathotype 77-5 (syn. 121 R63-1), the most virulent and predominant pathotype of *P. recondita* in southeast Asia. Plants were grown in growth chambers under controlled environmental conditions at National Phytotron Facility, Indian Agricultural Research Institute, New Delhi, India. About 10 days old seedling (single leaf stage) were inoculated with leaf rust pathotype 77-5 by spraying the inoculum suspended in water fortified with Tween-20 (0.75 µl/ml) and were incubated for 36 h in humid glass chambers with a temperature of 23 ± 2°C and more than 85% relative humidity. After incubation, plants were shifted to growth chambers at the same environmental conditions. Disease reaction was scored 12 days after inoculation using the scale of Stakman et al. (1962).

DNA extraction

Leaves collected from all the plant material mentioned above were immediately frozen in liquid nitrogen and lyophilized. Freeze-dried leaf samples were ground in liquid nitrogen and total genomic DNA was extracted from 40 mg of powdered leaf tissue using the procedure described by Prabhu et al. (1998). DNA concentration was quantified spectrophotometrically.

Bulked segregant analysis

Molecular markers putatively linked to the leaf rust resistance gene *Lr19* were identified by using bulked segregant analysis (BSA) (Michelmore et al. 1991). Two contrasting DNA bulks were made by pooling equal amount of DNA from randomly chosen ten F_2 homozygous resistant and ten F_2 homozygous susceptible individuals, respectively. DNA samples of resistant and susceptible parents along with resistant and susceptible bulks were screened for polymorphism with RAPD and microsatellite markers.

RAPD screening

A total of 700 random decamer primers procured from Biobasic Inc., Canada (S1-520 and S1001-1180) were screened on the two bulks as well as parents. The PCR reactions were performed in PTC-200 thermal cycler (MJ Research, Waltham, MA, USA). The 20 µl reaction volume included 10 mM Tris–HCl (pH 8.8), 50 mM KCl, 2.0 mM $MgCl_2$, 0.08% Nonidet P40, 200 µM of each dNTP (MBI Fermentas, St Leon-Rot, Germany), 0.75 U Taq DNA Polymerase (Bangalore Genei Pvt Ltd, Bangalore, India), 0.2 µM of random primer and 20 ng of genomic DNA. The amplification reactions were carried out using the following thermal profile: 95°C for 2 min (1 cycle); 94°C for 1 min, 36°C for 1 min, 72°C for 1 min (45 cycles); 72°C for 7 min (1 cycle). The amplification products were separated on 2% (w/v) agarose gels in 1× TAE buffer by electrophoresis at 80 V for 3 h. Gels were stained in ethidium bromide and photographed on a digital gel documentation system (Alpha Innotech, San Leandro, CA, USA).

SCAR design and analysis

Polymorphic fragments amplified by closely linked RAPD markers were excised from the gel and purified using QIAEX® II Gel Extraction kit (Qiagen, DuÈsseldorf, Germany). The purified DNA was cloned into the pGEM®-T Easy vector (Promega, Madison, WI, USA). The resulting white colonies on X gal-IPTG-LA plates containing 50 µg/ml ampicillin were picked, grown in Luria broth and plasmid DNA was isolated using the QIAprep® Spin Miniprep kit (Qiagen, Hilden, Germany). The cloned fragment was sequenced from both ends using universal primers at the DNA sequencing facility, South Campus, Delhi University, India.

Based on the sequence information, a pair of oligonucleotide primers was designed and synthesized for specific amplification of the loci identified by each RAPD marker (Table 1). PCR amplification was performed in a total volume of 25 µl containing 10 mM Tris–HCl (pH 8.8), 50 mM KCl, 0.08% Nonidet P40, 0.1 mM of each dNTP (MBI Fermentas), 0.75 U Taq DNA Polymerase (Bangalore Genei Pvt Ltd), 20 ng of each SCAR primer and 20 ng of template DNA. PCR amplifications programme consisted of an initial denaturation for 2 min at 95°C, followed by 35 cycles each with 1 min at 94°C, 1 min at 60°C and 1 min at 72°C, with a final extension of 7 min at 72°C. Amplification products were separated on 2% agarose gel, stained with ethidium bromide and photographed on a digital gel documentation system.

Microsatellite screening

Primers of 30 microsatellite markers located on the 7D chromosome of *Triticum aestivum* which included 6 primers (*Xgwm37*, *Xgwm44*, *Xgwm111*, *Xgwm121*, *Xgwm428* and *Xgwm437*) from Röder et al. (1998), 4 primers (*Xgdm46*, *Xgdm67*, *Xgdm84* and *Xgdm150*) from Pestova et al. (2000), 2 primers (*Xwmc94* and

Table 1 Primer sequences for SCAR markers derived from RAPD markers linked to the leaf rust resistance gene *Lr19* in wheat

SCAR marker	RAPD marker	SCAR primer sequence[a] (5′-3′)	SCAR product size (bp)	Phenotype
SCS123	$S123_{688}$	F: CCTGATCACCAATGACGATT R: CCTGATCACCTTGCTACAGA	688	Monomorphic
SCS253	$S253_{737}$	F: GCTGGTTCCACAAAGCAAA R: GGCTGGTTCCTTAGATAGGTG	736	Polymorphic
SCS265	$S265_{512}$	F: GGCGGATAAGCAGAGCAGAG R: GGCGGATAAGTGGGTTATGG	512	Polymorphic
SCS1008	$S1008_{514}$	F: CCAGGTCTTCATGCTATGG R: CCAGGTCTTCTGGGGTACTTC	514	Monomorphic

[a] Underlined sequence represents the part of progenitor RAPD primer sequence

Xwmc157) from Gupta et al. (2002), 1 AFLP-derived SSR primer (*XustSSR2001-7DL*) from Groenewald et al. (2003) and 17 primers (*Xwmc14*, *Xwmc38*, *Xwmc42*, *Xwmc58*, *Xwmc121*, *Xwmc221*, *Xwmc243*, *Xwmc273*, *Xwmc364*, *Xwmc386*, *Xwmc438*, *Xwmc450*, *Xwmc463*, *Xwmc492*, *Xwmc494*, *Xwmc497* and *Xwmc506*) available from USDA/ARS GrainGenes database (http://www.wheat.pw.usda.gov/ggpages/SSR/WMC) were synthesized. The PCR reactions were set up with the recommended protocol for each microsatellite primer pair in a 25-µl reaction volume containing 10 mM Tris–HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP (MBI Fermentas), 0.75 U Taq DNA Polymerase (Bangalore Genei Pvt Ltd), 80 ng template DNA and 40 ng of reverse and forward primer pair for detecting polymorphic microsatellites between the bulks and parents. Amplification products were electrophoresed on 4% Metaphor® agarose (Cambrex, Rockland, ME, USA) gel in 1× TAE buffer at 80 V for 4 h. Gels were stained with ethidium bromide and visualized on a digital gel documentation system.

Linkage analysis

Linkage analysis was performed on a F_2 population segregating for the *Lr19* locus. The putative markers were screened on 70 homozygous resistant F_2 plants and 70 susceptible F_2 plants for molecular mapping. Linkage analysis between the *Lr19* locus and the markers was done using the MAPMAKER computer program, version 3.0 (Lander et al. 1987) with a LOD threshold of 3.0. The standard error for the recombination frequency was calculated using the formula suggested by Adams and Joly (1980).

Results

Genetics of leaf rust resistance

The leaf rust resistant parent Thatcher NIL (Tc + *Lr19*) was highly resistant displaying the fleck reaction (;) and the leaf rust susceptible parent Agra Local produced a susceptible phenotype of the infection type 33^+ against the leaf rust pathotype 77-5. All the F_1 plants of the cross between Tc + *Lr19* and Agra Local were resistant indicating dominance inheritance of the alien gene *Lr19*. Out of 340 F_2 seedlings, 252 were resistant and 88 were susceptible fitting a monogenic segregation ratio of 3:1 (Table 2). Of the 215 resistant F_2-derived F_3 families, 70 belonged to non-segregating resistance group indicating homozygosity at the *Lr19* locus in the respective parental F_2 plants and the rest 145 families belonged to segregating group with heterozygosity at the *Lr19* locus in the parental F_2 plants. All the 88 susceptible F_2-derived F_3 families remained susceptible confirming homozygous status of the susceptibility allele at the *Lr19* locus (Table 2). Since dominant molecular markers like RAPD primers were utilized for tagging, the mapping population was restricted to the homozygous F_2 plants of 140 individuals segregating in 1:1 ratio for resistance:susceptibility, mimicking a backcross progeny.

Identification of RAPD markers

In the BSA, 16 of the 700 RAPD primers screened detected polymorphism between the two parents and bulks suggesting their association with the alien *Lr19* locus (Table 3). Eight of these RAPD markers ($S13_{1600}$, $S193_{800}$, $S265_{512}$, $S325_{750}$, $S348_{750}$, $S1008_{514}$, $S1094_{800}$ and $S1096_{500}$) were associated in a coupling phase linkage with the gene *Lr19*, amplifying the polymorphic fragment only in the resistant parent and resistant F_2 segregants (Fig. 1a). The other eight RAPD fragments ($S36_{1200}$, $S37_{1300}$, $S123_{688}$, $S200_{620}$, $S253_{737}$, $S421_{900}$, $S428_{900}$ and $S470_{700}$) amplified the polymorphic fragment only in the susceptible parent and susceptible F_2 segregants and thus were associated in repulsion phase linkage with the gene *Lr19* (Fig. 1b).

All the 16 RAPD markers co-segregated with the leaf rust resistant locus *Lr19* and amplified the respective polymorphic fragments in all the 140 F_2 individuals of the mapping population. Twelve RAPD markers

Table 2 Reaction of wheat seedlings in F_1, F_2 and F_3 generations of the cross Tc + *Lr19* (resistant) × Agra Local (susceptible) to infection by the leaf rust pathotype 77-5 under controlled conditions

Cross	F_1	No. of F_2 seedlings				
	R	Total tested	*R*	*S*	$\chi^2_{3:1}$	*P*
Tc + *Lr19* × Agra Local	4	340	252	88	0.1410	0.7072

No. of F_2 families for F_3 testing		No. of F_3 families					
		R in F_2				*S* in F_2	
R	*S*	NSeg	Seg	$\chi^2_{1:2}$	*P*	NSeg	Seg
215	88	70	145	0.0355	0.8505	88	0

R leaf rust resistant, *S* leaf rust susceptible, *NSeg* non-segregating family for leaf rust reaction, *Seg* segregating family for leaf rust reaction

Table 3 Details of the RAPD markers linked to the leaf rust resistance gene *Lr19* in wheat

RAPD marker	Primer sequence (5′-3′)	Approximate fragment size (bp)	Linkage phase with *Lr19* locus
S13	TTCCCCCGCT	1,600	Coupling
S36	AGCCAGCGAA	1,200	Repulsion
S37	GACCGCTTGT	1,300	Repulsion
S123	CCTGATCACC	688	Repulsion
S193	GTCGTTCCTG	800	Coupling
S200	TCTGGACGGA	620	Repulsion
S253	GGCTGGTTCC	737	Repulsion
S265	GGCGGATAAG	512	Coupling
S325	TCCCATGCTG	750	Coupling
S348	CATACCGTGG	750	Coupling
S421	CTCACGTTGG	900	Repulsion
S428	ACCTCAGCTC	900	Repulsion
S470	TCCCGCCTAC	700	Repulsion
S1008	CCAGGTCTTC	514	Coupling
S1094	TCGCTCCGTT	800	Coupling
S1096	CTTTCGAGGG	500	Coupling

($S37_{1300}$, $S123_{688}$, $S193_{800}$, $S200_{620}$, $S253_{737}$, $S325_{750}$, $S348_{750}$, $S421_{900}$, $S428_{900}$, $S470_{700}$, $S1008_{514}$ and $S1096_{500}$) did not show any recombination indicating their tight linkage with the gene *Lr19*. However, four RAPD markers ($S13_{1600}$, $S36_{1200}$, $S265_{512}$ and $S1094_{800}$) identified the recombinant genotypes varying from 1/140 in case of marker $S265_{512}$ to a maximum of 3/140 in case of marker $S1094_{800}$.

SCAR markers analysis

The SCAR primer pairs for RAPD markers $S265_{512}$ (coupling) and $S253_{737}$ (repulsion) were found to be polymorphic and give unique band amplification under varying PCR conditions. These SCAR markers were designated as $SCS265_{512}$ and $SCS253_{736}$, respectively. However, SCAR primer pairs for coupling phase RAPD marker $S1008_{514}$ and repulsion phase RAPD marker $S123_{688}$ failed to give polymorphism with different primer pair combinations and PCR conditions. Both SCAR markers $SCS265_{512}$ and $SCS253_{736}$ were screened on all the individuals of the mapping population and gave the similar pattern as progenitor RAPD markers (Fig. 2). The SCAR markers $SCS265_{512}$ and $SCS253_{736}$ were also successfully validated for their specificity on a

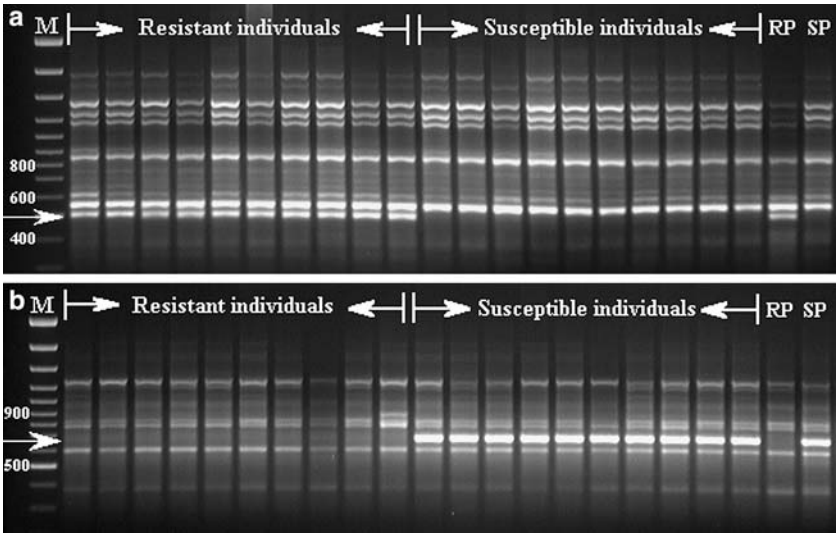

Fig. 1 Screening of the ten resistant and ten susceptible F_2 plant DNA of the cross Tc + *Lr19* × Agra Local with **a** coupling phase RAPD markers $S1008_{514}$ and **b** repulsion phase RAPD marker $S123_{688}$, linked to *Lr19* in wheat. *Lane M* = 100 bp DNA ladder (MBI Fermentas), *RP* leaf rust resistant parent Tc + *Lr19*, *SP* leaf rust susceptible parent Agra Local

set of international wheat NILs for a total of 44 of the known 55 *Lr* genes. All the NILs possessing the 19 alien *Lr* genes and 24 native *Lr* genes did not amplify the *Lr19* specific SCAR markers fragment. The SCAR markers fragment got amplified in the *Lr19* carrying lines Agatha (original source stock of gene *Lr19*) and Cook*6/C80-1 (Australian donor line for gene *Lr19*).

Microsatellite markers analysis

Of the 30 microsatellite primer pairs located on the chromosome 7DL, nine were found to be linked to the alien gene *Lr19*. Two microsatellite markers (*Xgwm221* and *XustSSR2001-7DL*) exhibited a co-dominant pattern amplifying alternate alleles from both the parents. The marker *Xwmc221* amplified a 200-bp fragment from resistant parent Tc + *Lr19* and a 220-bp fragment from susceptible parent Agra Local (Fig. 3a). Similarly, microsatellite marker *XustSSR2001-7DL* amplified a 300-bp fragment from resistant parent and a 310-bp fragment from susceptible parent. Seven microsatellite markers (*Xgwm37*, *Xgwm428*, *Xgwm437*, *Xgdm46*, *Xgdm67*, *Xgdm150* and *Xwmc364*) amplified the *T. aestivum* specific allele only from susceptible parent Agra Local, having no homologous site (null allele) at the resistance allele locus from resistant parent Tc + *Lr19* (Fig. 3b). Of the nine microsatellite markers, seven microsatellite markers (*Xgwm37*, *Xgwm221*, *Xgwm428*, *Xgwm437*, *Xgdm46*, *Xgdm67* and *XustSSR2001-7DL*) co-segregated with the *Lr19* locus when tested on the mapping population, while one recombinant each was identified by the markers *Xgdm150* and *Xwmc364*, respectively.

The specificity of the these microsatellite markers to the gene *Lr19* was confirmed on a set of NILs carrying other leaf rust resistance gene derived from alien and native wheat germplasm. All microsatellite markers amplified the respective marker fragment only in the *Lr19* carrying lines Tc + *Lr19*, Agatha and Cook*6/C80-1, and did not produce the marker fragment in 19 other alien *Lr* genes including the other two *A. elongatum* derived *Lr* genes *Lr24* and *Lr29* (Fig. 4a) and in 24 native *Lr* genes (Fig. 4b). The linked markers were also validated on different wheat cultivars which were found to be polymorphic and did not amplify the marker fragments in any of the wheat cultivars known not to possess the alien gene *Lr19* (Fig. 5).

Linkage analysis

Linkage mapping analysis distributed the 16 RAPD markers and 9 microsatellite markers in one linkage

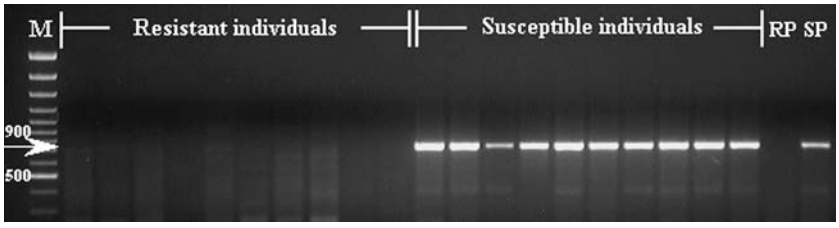

Fig. 2 Amplification of the SCAR marker $SCS253_{736}$ linked to *Lr19* on the ten resistant and ten susceptible F_2 individuals of the cross Tc + *Lr19* × Agra Local. *Lane M* = 100 bp DNA ladder (MBI Fermentas), *RP* leaf rust resistant parent Tc + *Lr19*, *SP* leaf rust susceptible parent Agra Local

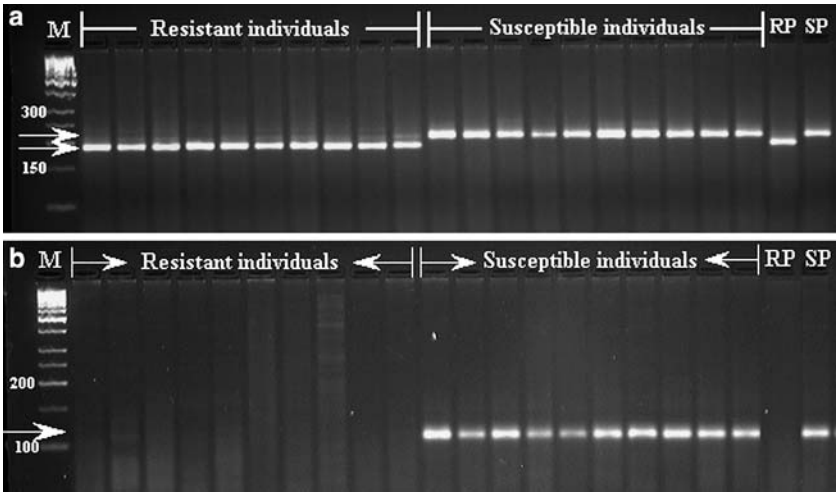

Fig. 3 Screening of the ten resistant and ten susceptible F_2 plant DNA of the cross Tc + *Lr19* × Agra Local with **a** co-dominant microsatellite marker Xwmc221 and **b** null allele microsatellite marker Xgwm437, linked to *Lr19* in wheat. *Lane M* = 50 bp DNA ladder (MBI Fermentas), *RP* leaf rust resistant parent Tc + *Lr19*, *SP* leaf rust susceptible parent Agra Local

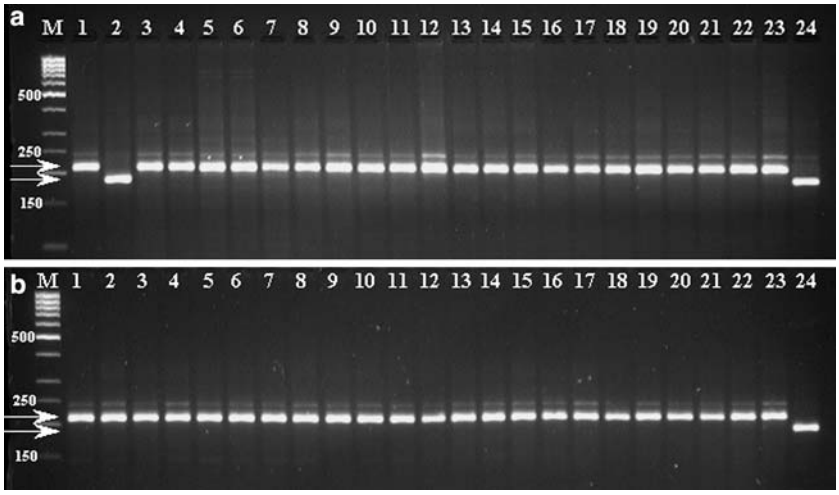

Fig. 4 **a** Validation of the co-dominant microsatellite marker Xwmc221 linked to *Lr19* on wheat genetic stocks possessing different alien *Lr* genes. *Lane M* = 50 bp DNA ladder (MBI Fermentas), *Lanes 1–21—Lr9*, *Lr19*, *Lr21*, *Lr22a*, *Lr23*, *Lr24*, *Lr25*, *Lr26*, *Lr28*, *Lr29*, *Lr32*, *Lr35*, *Lr36*, *Lr37*, *Lr39*, *Lr40*, *Lr41*, *Lr42*, *Lr43*, *Lr44* and *Lr45*, respectively, *22* Thatcher, *23* Chinese Spring, *24* Agatha. **b** Validation of the co-dominant microsatellite marker Xwmc221 linked to *Lr19* on wheat genetic stocks possessing different native *Lr* genes. *Lane M* = 50 bp DNA ladder, *Lanes 1–23—Lr1*, *Lr2a*, *Lr3*, *Lr3ka*, *Lr10*, *Lr11*, *Lr12*, *Lr13*, *Lr14a*, *Lr14b*, *Lr14ab*, *Lr15*, *Lr16*, *Lr17*, *Lr18*, *Lr20*, *Lr22b*, *Lr27 + 31*, *Lr30*, *Lr33*, *Lr34*, *Lr48*, *Lr49*, respectively, *lane 24* = Cook*6/C80-1

group along with the gene *Lr19* with a total map distance of 10.2 ± 0.062 cM (Fig. 6). Twelve RAPD markers ($S37_{1300}$, $S123_{688}$, $S193_{800}$, $S200_{620}$, $S253_{737}$, $S325_{750}$, $S348_{750}$, $S421_{900}$, $S428_{900}$, $S470_{700}$, $S1008_{514}$ and $S1096_{500}$) were tightly linked to the gene *Lr19* and four RAPD markers ($S13_{1600}$, $S36_{1200}$, $S265_{512}$ and $S1094_{800}$) were flanking the gene *Lr19* within a distance of 10.2 ± 0.062 cM. In the case of microsatellite markers, seven microsatellite markers (*Xgwm37*, *Xgwm221*, *Xgwm428*, *Xgwm437*, *Xgdm46*, *Xgdm67* and *XustSSR2001-7DL*) were inseparable from the gene *Lr19* and two microsatellite markers (*Xgdm150* and *Xwmc364*) were flanking the gene *Lr19* at a distance of 0.7 ± 0.007 cM from either side. The recombinants were distinguished by confirming the phenotypic and genotypic status of the F_2 individuals and by repeating their marker amplification profiles.

Discussion

The translocation fragment carrying *Lr19* was saturated with 25 molecular markers (16 RAPD and 9 microsatellite markers), out of which, 12 RAPD markers were co-segregating with the resistance locus *Lr19* and 4 were flanking the gene within a distance of 10 cM. The flanking RAPD markers which were associated in coupling and repulsion phase linkage with the locus, when used together would work genetically equivalent to a co-dominant marker (Kelly 1995) and can overcome the limitation of dominant RAPD markers. In addition, co-dominant flanking marker pair increases the selection efficiency as demonstrated by Cherukuri et al. (2005) and Young and Kelly (1997), and allows the identification of double recombinants not possible with flanking markers alone (Kelly and Miklas 1998).

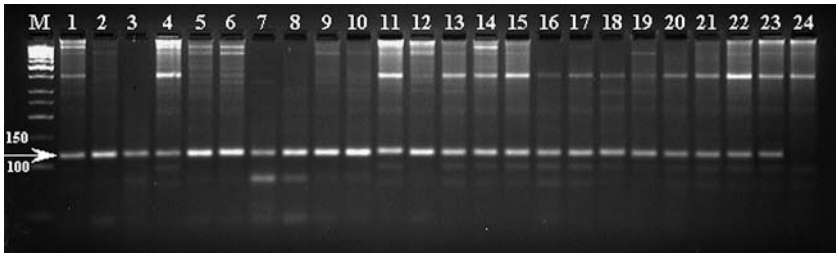

Fig. 5 Validation of null allele microsatellite marker Xgdm150 on different non-*Lr19* wheat genetic backgrounds. *Lanes M* = 50 bp DNA ladder, *1* HD 2285, *2* HD 2329, *3* HUW 234, *4* Kalyansona, *5* Lok-1, *6* PBW 226, *7* WH 147, *8* NI 5439, *9* C-306, *10* WH 542, *11* Sonalika, *12* HUW 468, *13* Vidisha, *14* Vaishali, *15* Kanchan, *16* Kundan, *17* PBW 343, *18* HS 240, *19* HDR 277, *20* HD 2687, *21* HD 2733, *22* UP 262, *23* Chinese Spring, *24* Tc + *Lr19*

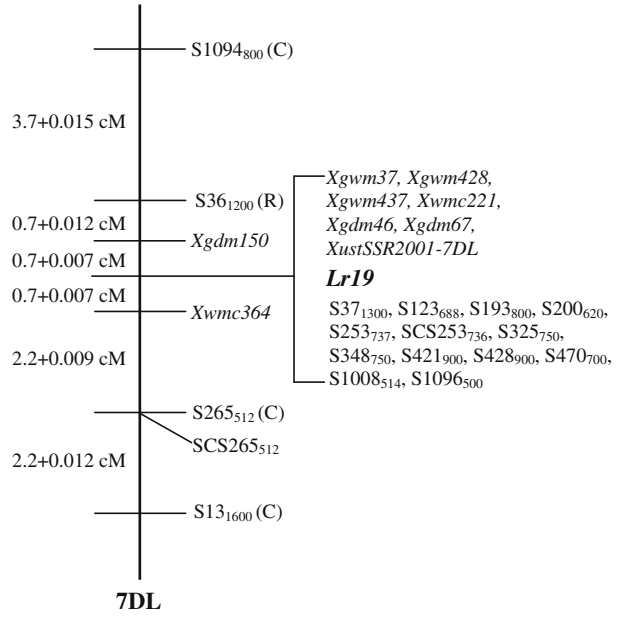

Fig. 6 Genetic linkage map of gene *Lr19* with RAPD, microsatellite and SCAR markers on chromosome 7DL of wheat. *C* coupling phase marker, *R* repulsion phase marker

The two RAPD markers $S265_{512}$ and $S253_{737}$ which were converted to SCAR markers $SCS265_{512}$ and $SCS253_{736}$, respectively, being locus specific markers for single band amplification are expected to provide ease of selection and reproducibility across laboratories and background recipient genotypes (Horejsi et al. 1999). The SCAR markers were polymorphic and produced unique bands linked to the gene *Lr19*. However, in the case of SCAR marker $SCS265_{512}$, where the critical marker fragment amplified a 512-bp long band, a non-specific monomorphic fragment of about 850 bp was also amplified (data not shown). Various PCR protocol changes attempted did not eliminate this non-specific amplification. This however did not interfere with the scoring for the polymorphic SCAR marker fragment of 512 bp linked to *Lr19* and thus, the marker can still be used for MAS. Presence of non-specific amplification with SCAR markers has also been reported in other studies (Kelly and Miklas 1998; Horejsi et al. 1999) which is attributed to the SCAR primers containing ubiquitous sequences in multiple genomic regions that may result in mismatched primer annealing during PCR. The failure of SCAR markers derived from RAPD markers $S1008_{514}$ and $S123_{688}$ to produce polymorphism indicated that original RAPD polymorphisms were caused by mismatches in nucleotides in the priming sites as reported by Paran and Michelmore (1993) and Horejsi et al. (1999).

Even though the microsatellite markers are expected to generally inherit as co-dominant loci, in the present study, seven microsatellite markers inherited as dominant loci amplifying only *T. aestivum* specific allele in susceptible plants and null allele in the resistant plants. A potential cause for the occurrence of microsatellite with null allele is the absence of primer sequences in the targeted genomic region. The 7DL.7Ag translocation being alien to the *T. aestivum* genomic region could have caused the absence of the primer annealing sites of these microsatellite primers resulting in the null alleles as reported in other alien gene transferred wheat lines (Liu et al. 2002; Brown-Guedira et al. 2003; Malik et al. 2003; Adhikari et al. 2004; Vikal et al. 2004). Seven microsatellite markers co-segregated with the *Lr19* locus and two flanked the gene within a distance of 1.4 cM. According to the other published linkage maps (Pestova et al. 2000; Röder et al. 1998; Somers et al. 2004), these microsatellite markers have been binned together in a region that is about 90 cM long on the wheat chromosome 7DL indicating that the 7DL.7Ag segment covered a relatively long portion of the *T. aestivum* chromosome 7DL. Dvorak and Knott (1977) and Kim et al. (1993) also reported that the *Agropyron* translocation represents about half of the length of 7DL chromosome of wheat. A near complete linkage of seven of these microsatellite markers in our study suggested a possible inhibition of recombination in the translocation segment 7DL.7Ag. Inhibition of recombination in the alien translocations has also been observed by Autrique et al. (1995), Dedryver et al. (1996) and Schachermayr et al. (1995). It is therefore likely that the recombination recorded here was possibly due to either smaller translocation region or its partial pairing with the 7DL region unlike the original translocation 7DL.7Ag of Sharma and Knott (1966), where Friebe et al. (1994) reported the presence of nearly entire arm of 7DL comprising the alien translocation that was not expected to recombine. The other mapping efforts by Autrique et al. (1995), Marais and Marais (1990), Prins et al. (1996, 2001) and Groenewald et al. (2005) have observed a lack of recombination and reported that even the smaller translocations on 7DL of *A. elongatum* fragment did not pair with wheat homoeologous chromosome on 7DL region despite the homeology and a possibility of pairing between the translocation and 7DL evidenced in the ph1b/ph1b mutants (Marais et al. 2001). The mutant lines reported by Prins et al. (1997), Groenewald et al. (2005) which carry shorter alien fragment may further resolve among the closely associated markers reported here which did not show any recombination with the 7DL.7Ag translocation carried by the resistance NIL Tc + *Lr19*. In a total mapped distance of 10.2 ± 0.062 cM around the alien

Lr19 locus which included 19 molecular markers in one inseparable region involving the locus *Lr19* that inhibited recombination, appropriate deletion stocks for the translocated *Agropyron* fragment may have to be used to fine map and resolve this genomic region carrying *Lr19*.

The successful validation of the SCAR and microsatellite markers over different *Lr* genes suggested that these markers could be used for MAS. Markers specifically amplified the critical fragment or null alleles in the *Lr19* carrying NILs and did not amplify the marker fragments in the lines carrying other *A. elongatum*-derived genes *Lr24* located on chromosome 3DL and *Lr29* located on chromosome 7DS. This indicated that the markers are highly specific to the gene *Lr19* and can be used for selection of the *Agropyron* translocation carrying the gene *Lr19*. Markers can be effectively utilized for the selection of this gene in different genetic background as indicated by successful validation on different *Lr* genes and wheat cultivars. The co-dominant microsatellite markers *Xwmc221* and *XustSSR2001-7DL* were able to identify the heterozygotes, and would serve as an important tool to rapidly transfer this gene into other wheat cultivars and assist in mapping efforts on the *Lr19* translocation in wheat. These molecular markers can be used with molecular markers tagged to other *Lr* genes like *Lr9* (Schachermayr et al. 1994; Gupta et al. 2005), *Lr24* (Schachermayr et al. 1995; Cherukuri et al. 2003; Prabhu et al. 2004), *Lr28* (Cherukuri et al. 2005) and *Lr35* (Gold et al. 1999) for pyramiding the gene *Lr19* with other effective *Lr* genes.

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