Diagnostic Microsatellite Markers for the Detection of Stem Rust Resistance Gene *Sr36* in Diverse Genetic Backgrounds of Wheat

Toi J. Tsilo,* Yue Jin, and James A. Anderson

ABSTRACT

The wheat stem rust resistance gene *Sr36*, derived from *Triticum timopheevi*, confers a high level of resistance against a new race (TTKS, or commonly known as Ug99) and many other races of *Puccinia graminis* f. sp. *tritici*. Because *Sr36*-virulent races exist, breeding for durable resistance would require pyramiding *Sr36* with other genes, a process that can be facilitated by DNA markers. The aim of this study was to identify and validate microsatellite markers for the detection of *Sr36* in wheat breeding programs. Two populations of 122 F_2 (LMPG × Sr36/9*LMPG) and 112 F_2 ('Chinese Spring' × W2691Sr36-1) were evaluated for stem rust reaction. Both populations exhibited distorted segregation with a preferential transmission of the *Sr36*-carrying segment. Three markers, *Xstm773-2, Xgwm319,* and *Xwmc477*, were in complete linkage with *Sr36* in the LMPG × Sr36/9*LMPG population. In the Chinese Spring × W2691Sr36-1 population, *Xgwm319* was 0.9 cM away from *Xstm773-2, Xwmc477*, and *Sr36*. These codominant markers were easy to score and diagnostic for *Sr36* in a set of 76 wheat cultivars and breeding lines developed in 12 countries. Together, these markers can be used in marker-assisted selection of *Sr36*.

T.J. Tsilo and J.A. Anderson, Dep. of Agronomy and Plant Genetics, 411 Borlaug Hall, Univ. of Minnesota, St. Paul, MN 55108; Y. Jin, USDA-ARS, Cereal Disease Lab., 1551 Lindig Ave., Univ. of Minnesota, St. Paul, MN 55108. Received 12 Apr. 2007. *Corresponding author (tsilo001@umn.edu).

Abbreviations: CS, Chinese Spring; DH, double haploid; HR, homozygous resistant; HS, homozygous susceptible; IT, infection type; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; seg, segregating; SSR, simple sequence repeat.

Puccinia graminis Pers.:Pers. f. sp. *tritici* Eriks. & E Henn., the causal agent of stem rust, can potentially devastate both durum wheat (*Triticum durum* Desf.) and common wheat (*T. aestivum* L.) crops throughout the world. Recently, stem rust reemerged as a serious threat because of a new highly virulent race TTKS (commonly known as Ug99) (Pretorius et al., 2000). The first outbreak was in Uganda in 1999, and the race has also been seen in parts of Kenya and Ethiopia (Wanyera et al., 2006). Currently, researchers with the Global Rust Initiative (http://www.cimmyt.org) have confirmed the existence of TTKS in Yemen in the Arabian Peninsula. This new race has the potential to spread from the affected countries and jeopardize wheat production worldwide (Expert Panel on the Stem Rust Outbreak in Eastern Africa, 2005). The threat of TTKS has resulted in the establishment of the Global Rust Initiative and its recommendation to use and incorporate multiple stem rust resistance genes in commercial cultivars as a strategy to provide durable resistance against stem rust.

The two hard red spring cultivars, CItr 12632 (= W1656) and CItr 12633 (= W1657), carry the stem rust resistance gene *Sr36*, derived from *T. timopheevi* (Allard and Shands, 1954). These

Published in Crop Sci. 48:253–261 (2008).
doi: 10.2135/cropsci2007.04.0204

677 S. Segoe Rd., Madison, WI 53711 USA

cultivars served as the original sources of *Sr36* in wheat breeding programs worldwide (Roelfs, 1988a; Knott, 1989; McIntosh et al., 1995). The *Sr36* gene is one of the 18 stem rust resistance genes that provide a major source of resistance to TTKS (Singh et al., 2005; Wanyera et al., 2006). However, none of the 18 genes occurs at a high frequency in breeding materials, except *Sr36*. In the United States, *Sr36* provides resistance against QFCS, which was the most predominant race in recent surveys (Jin, 2005) and was also found in previous surveys (McVey et al., 1996, 2002). To some races of stem rust, *Sr36* conditions unusual (mixed) infection types (Ashagari and Rowell, 1980), which can make it difficult to distinguish cultivars carrying this gene.

Because *Sr36*-virulent races exist (Knott, 1989), this gene is best deployed when pyramided with other *Sr* genes (Knott, 1988), a process that cannot easily be achieved through the conventional phenotypic screening methods. The drawback of classical breeding methods is that the process of pyramiding genes in a single line can be time consuming or impossible, especially when more than one gene confers resistance against known races of *P. graminis* f. sp. *tritici*; hence, it becomes difficult to identify genotypes carrying combinations of more than one gene. Pyramiding of resistance genes could be facilitated by marker-assisted selection.

Nyquist (1957) used monosomic analysis to locate *Sr36* on chromosome 2B; it was later mapped on the short arm of chromosome 2B (Gyarfas, 1978; McIntosh and Luig, 1973). Bariana et al. (2001) identified 10 molecular markers linked to the *Sr36* locus. Eight were restriction fragment length polymorphism (RFLP) or amplified fragment length polymorphism (AFLP) markers, and two were microsatellites (STM773 and GWM271). These authors reported that STM773 gave a better amplification than GWM271. However, even though the STM773 marker could be directly used to identify homozygous genotypes for *Sr36*, this marker requires careful scoring because the primers also amplify other fragments, making it difficult to distinguish heterozygous from homozygous genotypes. Therefore, more robust, codominant, and easy-to-detect microsatellite markers are needed for *Sr36*.

The objectives of this study were (i) to identify codominant microsatellite markers closely linked to *Sr36*; and (ii) to validate their potential use in marker-assisted selection of *Sr36* using a set of diverse wheat germplasm.

MATERIALS AND METHODS

Plant Materials

Genetic analysis of *Sr36* was performed with two F_2 mapping populations. The 122 F_2 individuals were derived from a cross between a susceptible wheat line, LMPG, and its near-isogenic line Sr36/9*LMPG carrying *Sr36*. The genetic stock Sr36/9*LMPG was developed by Dr. D. Knott at the University of Saskatchewan, Saskatoon, Canada (Knott, 1990). An additional 112 F_2 individuals were derived from a cross between a susceptible wheat cultivar Chinese Spring (CS) and the resistant line W2691Sr36-1, carrying *Sr36* in the genetic background of W2691. The F_2 populations and their subsequent F_3 families were grown in the greenhouse at the University of Minnesota, St. Paul, during spring 2005 and fall 2005, respectively.

In addition to the four wheat lines used for genetic analysis of *Sr36*, a diverse set of 76 wheat cultivars and breeding lines were obtained from the USDA-ARS National Small Grains Collection, Aberdeen, ID. These accessions and breeding lines were selected on the basis of previously published reports that indicated whether they possess *Sr36* (accessions with and without *Sr36*) (Table 1). The information on the pedigree and the presence of *Sr36* was obtained from two USDA Websites (http://www.ars-grin.gov/npgs/acc/acc_queries.html, http://wheat.pw.usda.gov) and McIntosh et al. (1995). It was supplemented with information from previous surveys of seedling resistance conducted at the USDA-ARS Cereal Disease Laboratory. The Chinese Spring nullisomic-tetrasomic (N2B-T2D) line (Sears, 1966) was used to verify the location of the amplified bands of microsatellite markers.

Stem Rust Inoculation and Evaluation

Stem rust screenings were performed on seedlings of parental lines (Sr36/9*LMPG, LMPG, W2691Sr36-1, CS), 122 F_2 (LMPG × Sr36/9*LMPG) lines, and 112 F_2 (CS × W2691Sr36-1) lines. To determine the F_2 genotypes and also to distinguish heterozygous from homozygous resistant F_2 lines, 16 to 30 plants of each $F_{2:3}$ family (seeds derived from bagged F_2 spikes) were tested for segregation at the *Sr36* locus using the race QFCS (isolate 03ND76C), which is avirulent on *Sr6*, *Sr7b*, *Sr9b*, *Sr9e*, *Sr11*, *Sr30*, *Sr36*, and *SrTmp*. For inoculation, urediniospores of QFCS stored at −80°C were heat shocked and suspended in a lightweight mineral oil (soltrol 170) and sprayed on two-leaf stage seedlings (~7 d after planting, when the primary leaves were fully expanded) following protocols described by Jin (2005). Inoculated seedlings were kept overnight in a dew chamber for 16 h with no light and then exposed to 2 to 4 h of light to complete infection. After infection, plants were placed either in a growth chamber with 16 h of light at 20 to 22°C and 8 h of dark at 18 to 20°C or in a greenhouse set at 18 to 21°C under 160-W very high output (VHO) fluorescent tubes with a 16-h photoperiod. Infection types (ITs) were scored from primary leaves approximately 14 d after inoculation based on the scale of 0 to 4 as stipulated by Stakman et al. (1962) and modified by Roelfs (1988b).

The presence and absence of *Sr36* in 76 wheat cultivars and breeding lines was verified on the basis of low infection response (0 = immunity) to QFCS and low infection to MCCF (Table 1). All races used for inoculation were verified on the basis of their avirulence/virulence formula using 16 *Sr* differential lines (Roelfs and Martens, 1988; Roelfs et al., 1993) as checks to verify the standard race designations of all the races.

Molecular Analysis

For molecular mapping of *Sr36*, 2 to 3 cm of leaf tissues were collected from seedlings of parental lines, 122 F_2 (LMPG × Sr36/9*LMPG) lines, 112 F_2 (CS × W2691Sr36-1) lines, and

Table 1. Validation of *Sr36*-linked microsatellite markers using conventional screening methods and polymerase chain reaction–based simple sequence repeat (SSR) markers in wheat cultivars and breeding lines derived from diverse genetic origin.

	Cultivar/breeding line†	Accession number	Origin	*Puccinia graminis* races			*Sr36*‡	SSR marker§		
				QFCS	TPMK	MCCF		GWM 319	STM 773-2	WMC 477
1	Sr36/9*LMPG		Canada	0¶	4	0	+	+	+	+
2	LMPG		Canada	3+	4	4	–	–	–	–
3	W2691Sr36-1		Australia	0	3	1;	+	+	+	+
4	'Chinese Spring'	CItr 14108	China	4	3+	3+	–	–	–	–
5	'GA-Dozier'	PI 591000	USA	2	2	2	–	–	–	–
6	'Fleming'	PI 599615	USA	;1	2	2	–	–	–	–
7	'GA-Stuckey'	PI 591001	USA	0	0;	0	+	+	+	+
8	'Goodstreak'	PI 632434	USA	;1	0;	;	–	–	–	–
9	'Harry'	PI 632435	USA	;	0	;	–	–	–	–
10	'Jaypee'	PI 592760	USA	0	3+	0	+	+	+	+
11	'Sisson'	PI 617053	USA	0	;1	0	+	+	+	+
12	'Morey'	PI 591428	USA	2+	2	2	–	–	–	–
13	'NC-Neuse'	PI 633037	USA	0	;2	0;	+	+	+	+
14	'Patterson'	PI 583825	USA	3	2, 3-	2+	–	–	–	–
15	W1656	CItr 12632	USA	0	4	0	+	+	+	+
16	'Mengavi'	PI 290912	Australia	0	4	0	+	+	+	+
17	'Arthur'	CItr 14425	USA	0	0, 4	0	+	+	+	+
18	'Arthur 71'	CItr 15282	USA	0	0	0	+	+	+	+
19	'Gouritz'	PI 479672	South Africa	0	1	0	+	+	+	+
20	W1657	CItr 12633	USA	0	4	;	+	+	+	+
21	'Maris Fundin'	PI 410869	UK	4	3+	4	–	–	–	–
22	'Zaragoza 75'	PI 519305	Mexico	4	3+	4	–	–	–	–
23	W 3496	PI 520133	Australia	0	2+	0	+	–	–	–
24	'Timson'	PI 404115	Australia	0	0;	0	+	+	+	+
25	NE 73843	PI 519136	USA	0	0	0;	+	+	+	+
26	CI 14050	CItr 14050	USA	u	u	u	+	+	+	+
27	TA 1600	PI 603223	Iran	u	u	u	–	–	–	–
28	RL 6044	CItr 17752	Canada	0	2	1+	–	–	–	–
29	RL 5045	PI 520492	Canada	0;	2, 4	1+	–	–	–	–
30	'Tosca'	PI 479680	South Africa	4	4	4	–	–	–	–
31	Sr 6	CItr 15082	Canada	0;	2, 4	1	–	–	–	–
32	'Eureka'	CItr 17738	USA	0;	2	1	–	–	–	–
33	'Red Egyptian'	CItr 12345	Egypt	u	u	u	–	–	–	–
34	'Excel'	PI 555465	USA	0;	2	2	+/?	±	±	±
35	CK 9803		USA	0	0, 1	0	+	+	+	+
36	'Ernie'	PI 599615	USA	0	;	0	+	+	+	+
37	'Halt'		USA	1;	3+	1	–	–	–	–
38	'Intrada'	PI 631402	USA	;	1	1+	–	–	–	–
39	'Roughrider'	CItr 17439	USA	0	2+	0	+	+	+	+
40	'TAM 200'	PI 578255	USA	1;	1+	1+	–	–	–	–
41	'Vista'	PI 562653	USA	0	2	0	+	+	+	+
42	'Brundage 96'		USA	4	4	4	–	–	–	–
43	'Chukar'	PI 628641	USA	4	4	4	–	–	–	–
44	CK 9877		USA	2+, 4	2+	0, 3+	-	±	±	–
45	'Roane'		USA	4	2+	3+	–	–	–	–
46	'Rosen'	CItr 17607	USA	0	0	0;	+	+	+	+
47	'Truman'	PI 634824	USA	4	4	4	–	–	–	–
48	'TAM 105'	CItr 17826	USA	2-	4	4	–	–	–	–
49	Idead 59	CItr 13631	USA	0, 4	4	4	±	±	±	±
50	'Timvera'	PI 351987	Australia	0	4	0	+	+	+	+

Table 1. Continued.

	Cultivar/breeding line†	Accession number	Origin	*Puccinia graminis* races			*Sr36*‡	SSR marker§		
				QFCS	TPMK	MCCF		GWM 319	STM 773-2	WMC 477
51	'Timgalen'		Australia	0, 1	4	0	±	+	+	+
52	'Hand'	CItr 17288	USA	0	0	0	+	+	+	+
53	'Kenosha'	CItr 14025	USA	0	;	0;	+	+	+	+
54	'Purdue'		USA	2, 4	1	2, 4	–	–	–	–
55	'Centurk'	CItr 15075	USA	1	1	;	–	–	–	–
56	II-53-764	CItr 15711	USA	;2	1+	;	–	–	–	–
57	'Gamut'	PI 329230	Australia	0;	0	0;	–	–	–	–
58	'Songlen'	PI 404114	Australia	0	0	0	+	+	+	+
59	'Timvera'	PI 237648	Australia	0	2, 4	0	+	+	+	+
60	'Oxley'	PI 386167	Australia	;	4	0	–	–	–	–
61	'Gatcher'	PI 377884	Australia	;1	1	;1	–	–	–	–
62	'Tarsa'	PI 422408	Australia	;	u	u	–	–	–	–
63	'Shortim'	PI 422407	Australia	0	;	0;	+	+	+	+
64	'Kenya Plume'	CItr 14335	Kenya	0;	1+	0	–	–	–	–
65	Zaragoza 75	PI 428428	Mexico	4	4	4	–	–	–	–
66	Zaragoza 75	PI 433770	Mexico	0	;1	0;	+	+	+	+
67	Zaragoza 75	PI 479665	Mexico	0;	;	0;	?	–	–	–
68	'Lerma Rojo 64'	CItr 13929	Mexico	;	;1	;	–	–	–	–
69	Red Egyptian	PI 45374	South Africa	0;	2, 2+	0	–	–	–	–
70	Red Egyptian	PI 45403	South Africa	2	2+	4	–	–	–	–
71	Red Egyptian	PI 45415	South Africa	;	2+	;	–	–	–	–
72	Red Egyptian	PI 192020	Ethiopia	;	2	;	–	–	–	–
73	Idaho 1877 NR AE	PI 234233	Zambia	0;	0;	1	+	+	+	+
74	'Kenya 58'	CItr 12471	Kenya	0	;	2-	–	–	–	–
75	'Marquis'/9*RE		Canada	1+	u	2+	–	–	–	–
76	'McMurachy'	PI 122985	Canada	;	2	0;	–	–	–	–
77	ISr9a-Ra	CItr 14177	USA	3	2	2-	–	–	–	–
78	W2691Sr9b	CItr 17386	Australia	1	2+	2+	–	–	–	–
79	ISr9d-Ra	CItr 14177	USA	u	4	1;	–	–	–	–
80	CnsSr9g			4	4	1+	–	–	–	–
Marker alleles								5	6	6

†Represents the order of wheat cultivars and breeding lines as it appears in Supplementary Fig. 1 (lane 1–43).

‡The presence of *Sr36* in these cultivars was based on previously published reports. The status of *Sr36* was verified based on results of QFCS. + or – represents presence or absence, ± indicates heterozygosity at the *Sr36* locus, ? indicates uncertainty.

§For SSR markers, + or – indicates presence or absence of the *Sr36*-linked marker allele, ± indicates heterozygosity.

¶Infection types as described by Stakman et al. (1962) and modified by Roelfs (1988b); u = missing data.

76 wheat cultivars and breeding lines. Total genomic DNA was extracted from the ground tissues following protocols described by Riede and Anderson (1996) and modified by Liu et al. (2006). Since *Sr36* was mapped on the short arm of chromosome 2BS (Gyarfas, 1978; Bariana et al., 2001), microsatellite markers used in this study were based on previously published wheat genetic maps of chromosome 2BS (Röder et al., 1998; Somers et al., 2004; Song et al., 2005). A total of 51 microsatellite primer pairs were screened for polymorphisms between near-isogenic lines LMPG and Sr36/9*LMPG, and between two diverse lines, Chinese Spring and W2691Sr36-1. Two microsatellite markers (STM773 and GWM271) were previously reported to be linked to *Sr36* in a double haploid population of 'Sunco' × 'Tasman' (Bariana et al., 2001). The marker STM773 has since been converted into two SSR markers, STM773-1 and STM773-2, and were included in the analysis. The sequences of STM773-1 and STM773-2 were kindly provided by Dr. Matthew Hayden, University of Adelaide, South Australia.

Polymerase Chain Reaction and Electrophoresis

Polymerase chain reaction (PCR) was performed in a 96-well plate with 10 μL of final reaction mixture containing 2.75 μL ddH_2O, 1 μL 10X PCR buffer, 0.6 μL of 25 mM $MgCl_2$, 1.6 μL of 1.25 mM dNTPs, 1 μL of each 1 μM primer, 0.05 μL of 5U μL^{-1} Taq DNA polymerase (Applied Biosystems, Branchburg, NJ), and 3 μL of 15 ng μL^{-1} genomic DNA. For all the SSR markers, except STM773-1 and STM773-2, the PCR reaction mixture was initially denatured at 94°C for 10 min, followed by 35 cycles of 94°C for 1 min, 48 to 61°C (depending on annealing temperature specific to individual primer pairs) for 1 min, and 72°C for 2 min, with a final extension step of 72°C

for 10 min and 4°C indefinitely. The PCR reaction protocol for STM773-1 and STM773-2 primers was provided by Dr. Matthew Hayden as a touchdown PCR program with 94°C for 10 min, followed by touchdown PCR program with 7 cycles of 60 s at 92°C, 60 s at 64°C, 60 s at 72°C, and then five cycles with 60 s at 92°C, 60 s at 57°C, and 60 s at 72°C (conditions identical to the previous cycles, but with an annealing temperature of 57°C). The program also included an additional 10 to 25 cycles each of 30 s at 92°C, 60 s at 55°C, and 60 s at 72°C. The PCR was ended by an extra incubation for 10 min at 72°C and 4°C indefinitely. Polymerase chain reaction thermal cycling was performed in PerkinElmer/Applied Biosystems (Foster City, CA) thermo cyclers. About 5μL of 3X loading dye (0.02 g bromophenol blue, 0.02 g xylene cyanol, 1.6 mL 0.5 M EDTA, 38.4 mL formamide) was added to the PCR products to make a final volume of 15 μL. All samples were denatured for 5 min at 95°C. The PCR products were subjected to electrophoresis in a polyacrylamide gel (6% [w/v] acrylamide/bisacrylamide, 20:1, 8 M urea in TBE, pH 8.3) in 1X TBE buffer (90 mM Tris-borate [pH 8.3], 2 mM EDTA) at a constant power of 110 W for 90 min. Gels were silver stained (Bassam et al., 1991) and photographed.

Genetic Linkage Analysis

For the genetics analysis of *Sr36*, F_2 genotypes inferred from seedling reactions of $F_{2:3}$ and $F_{3:4}$ families were classified as homozygous resistant (HR), segregating (Seg) and homozygous susceptible (HS). Chi-squared (χ^2) distribution analyses were used to test if the observed segregation ratios for *Sr36* and marker loci fit the Mendelian ratio of 1:2:1. Genetic linkage analysis was performed between polymorphic microsatellite markers and the *Sr36* segregation data using Mapmaker computer program version 3.0b (Lander et al., 1987).

RESULTS

Segregation Analysis of *Sr36* in the Two F_2 Populations

The wheat lines Sr36/9*LMPG and W2691Sr36-1 were highly resistant to race QFCS (infection type 0), and lines LMPG and CS were susceptible (infection types 3+ and 4). The F_2 genotypes were inferred from $F_{2:3}$ plants that were tested and grouped based on their rust reaction when inoculated with QFCS. The LMPG × Sr36/9*LMPG and the CS × W2691Sr36-1–derived populations segregated 43HR:54Seg:24HS and 54HR:35Seg:10HS, respectively (Table 2). The segregation patterns in both populations were significantly different than the expected segregation ratio of 1HR:2Seg:1HS ($\chi^2 = 7.36$, $P = 0.025$; and $\chi^2 = 47.6$, $P < 0.001$).

Genetic Mapping of the *Sr36* Gene

Of 53 microsatellite markers which were previously shown to be located on chromosome 2BS, the same 21 markers showed polymorphism between LMPG and Sr36/9*LMPG, and between Chinese Spring and W2691Sr36-1 lines. Because of the reduced informativeness of dominant markers that cannot distinguish heterozygous and homozygous allele states, only codominant markers were used for further analysis. Four markers, *GWM429*, *GWM319*, *WMC477*, and *STM773-2*, were codominant and gave clear, readable fragments of 220, 170, 190, and 155 bp in the resistant F_2 plants and 210, 180, 160, and 190 bp in the susceptible F_2 plants, respectively (Fig. 1). However, the primers for WMC477 also amplified an additional fragment of 158 bp, which is visible in homozygous resistant but not heterozygous plants (Fig. 1C). The marker *GWM429* was codominant only in the LMPG × Sr36/9*LMPG population and was dominant in the CS × W2961Sr36-1 population. The SSR marker data, together with rust screening data, displayed a similar distortion trend that favored the *Sr36*-containing segment over the non-*Sr36* segment (Table 2). This implies that both populations segregated for a single gene conferring resistance to QFCS.

A linkage map was generated for each population (Fig. 2). In the LMPG × Sr36/9*LMPG population, the three markers, *Xstm773-2*, *Xgwm319*, and *Xwmc477*, showed complete linkage to the *Sr36* gene (Fig. 2). Also in the CS population, the two markers, *Xstm4773-2* and *Xwmc477*, showed complete linkage to *Sr36*, while *Xgwm319* was 0.9 cM from *Sr36* (Fig. 2).

Validation of Microsatellite Markers Tightly Linked to *Sr36*

To determine the diagnostic value of the microsatellite markers identified in this study, a set of 76 wheat cultivars and breeding lines with diverse origins were genotyped with three microsatellite markers that were tightly linked

Table 2. Segregation ratios of *Sr36* and linked simple sequence repeat marker alleles in F_2 populations derived from crosses between susceptible and resistant parents.

Population	Gene/ marker	Total†	Observed‡			Expected ratio	X^2	*P* value§
			X_1X_1	X_1X_2	X_2X_2			
LMPG × Sr36/ 9*LMPG	*Sr36*	121	43	54	24	1:2:1	7.36	0.025
	Xgwm319	122	43	55	24	1:2:1	7.10	0.029
	Xwmc477	122	43	54	25	1:2:1	6.92	0.031
	Xstm773-2	122	44	54	24	1:2:1	8.16	0.017
	Xgwm429	122	43	56	23	1:2:1	7.38	0.025
Chinese Spring × W2691Sr36-1	*Sr36*	99	54	35	10	1:2:1	47.6	<0.001
	Xgwm319	112	58	44	10	1:2:1	46.3	<0.001
	Xwmc477	112	58	43	11	1:2:1	45.5	<0.001
	Xstm773-2	112	58	43	11	1:2:1	45.5	<0.001
	Xgwm429	112	58	54		1:3	42.9	<0.001

†Due to seedling lethality, the F_2 plants not evaluated at the F_3 generation were recorded as missing data.

‡X_1X_1 = homozygous for resistant parent's allele; X_1X_2 = heterozygous; X_2X_2 = homozygous for susceptible parent's allele. F_2 genotypes were inferred from infection types of $F_{2:3}$ or $F_{3:4}$ families to distinguish X_1X_1, X_1X_2, and X_2X_2 F_2s.

§P value less than 0.05 was used to accept a distorted segregation from expected ratio of 1:2:1.

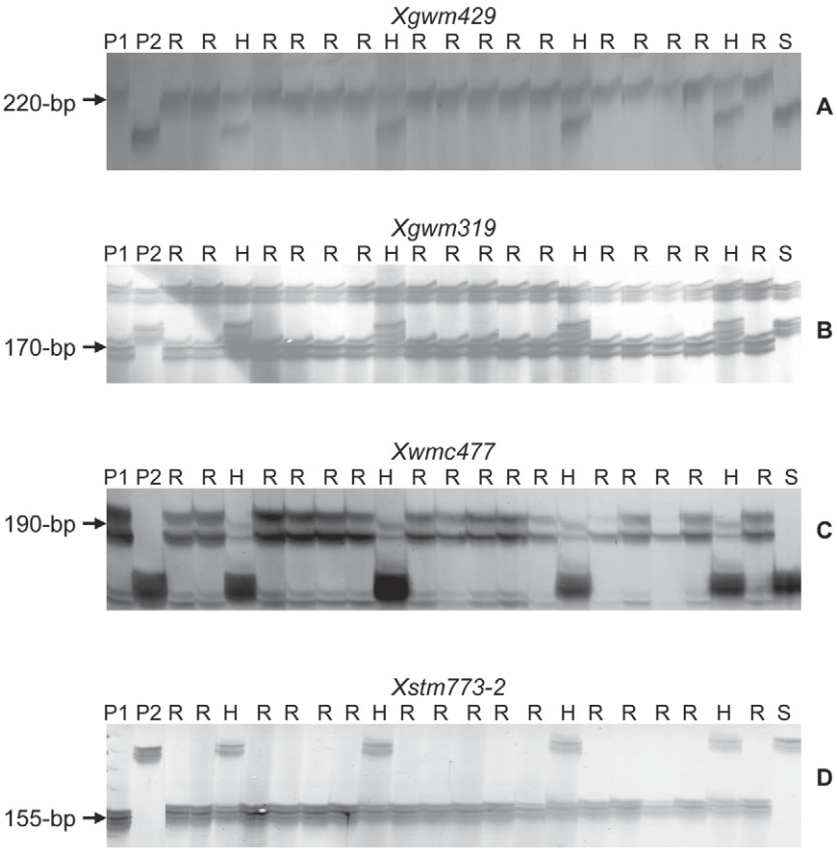

Figure 1. Gel electrophoresis showing segregation pattern of the four SSR markers, (A) *Xgwm429*, (B) *Xgwm319*, (C) *Xwmc477*, and (D) *Xstm773-2*, in a subset of the F_2 progenies from a cross between near-isogenic lines (Sr36/9*LMPG and LMPG); P1, resistant parent; P2, susceptible parent; R, resistant F_2; S, susceptible F_2; H, heterozygous F_2 progenies. The arrow points indicate the size of the band associated with *Sr36*.

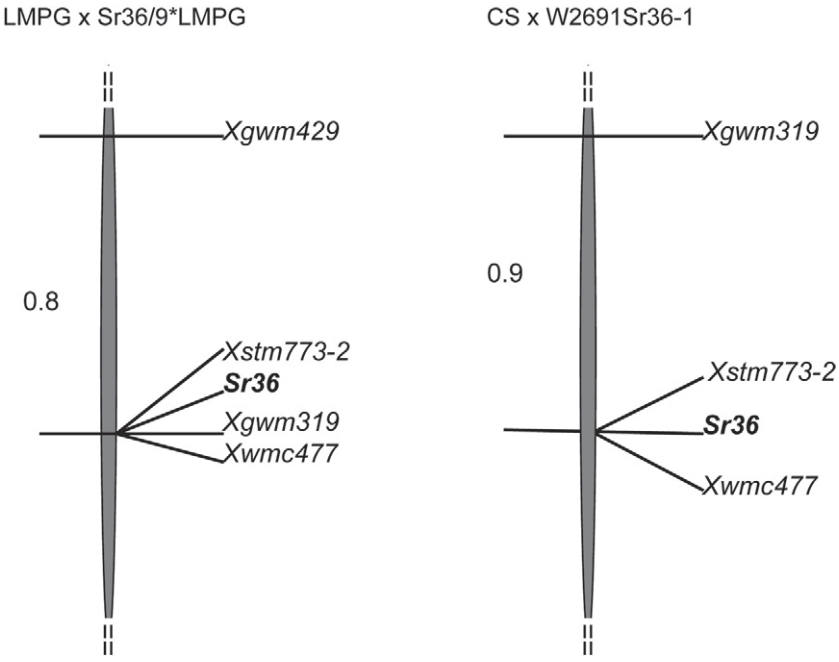

Figure 2. Partial genetic linkage maps of chromosome 2BS depicting the location of *Sr36* with linked codominant simple sequence repeat loci in the LMPG × Sr36/9*LMPG population and the CS × W2691Sr36-1 population. The linkage maps were constructed using map distances (cM) from Kosambi.

to *Sr36*. All accessions that were known to carry *Sr36* were validated based on their reaction as seedlings to races QFCS, TPMK, and MCCF (Table 1). The race QFCS is avirulent on *Sr6*, *Sr7b*, *Sr9b*, *Sr9e*, *Sr11*, *Sr30*, *Sr36*, and *SrTmp*; however, cultivars and lines carrying *Sr36* were clearly distinguishable with zero infection type (immunity) compared with the low infection type (small flecks or IT0;) produced by lines carrying *Sr6* (temperature-sensitive stem rust resistance gene). The DNA fragments of 170, 190, and 155 bp were amplified in cultivars and breeding lines carrying the *Sr36*-containing chromosome segment for the three microsatellite markers, *Xgwm319*, *Xwmc477*, and *Xstm773-2*, respectively (Table 1; Supplementary Fig. 1, online). These fragment sizes were amplified in 30 wheat cultivars and breeding lines known to carry *Sr36* (Table 1). The results indicate that three markers identified *Sr36* correctly in these *Sr36*-carrying cultivars.

Many cultivars and breeding lines that did not carry *Sr36* displayed ITs other than immunity against race QFCS (Table 1). In 'Excel', the marker data reveals *Sr36*-associated alleles in a heterozygous state. In 2 out of 80 wheat cultivars and breeding lines, 'W 3496' and 'CK 9877', we found that the three markers, *Xgwm319*, *Xmwc477*, and *Xstm773-2*, were not in agreement with the stem rust screening results. The W3496 line was developed in Australia and traces back to Verntein/CItr 12632 as ultimate parents in its pedigree. This line showed zero IT to QFCS, meaning that it could be carrying *Sr36* from CItr 12632, a known carrier of this gene. CK 9877 showed 2+/3+ against QFCS, indicating that this line did not have *Sr36*, and only marker *Xwmc477* showed the absence of the *Sr36*-specific marker allele, whereas *Xgwm319* and *Xstm773-2* showed the presence of *Sr36*-specific marker alleles, meaning that there could have been recombination between *Sr36* and the two markers *Xgwm319* and *Xstm773-2*.

DISCUSSION

Mapping *Sr36* in Wheat

McIntosh and Luig (1973) reported a recombination frequency of 20% between *Sr36* and *Sr9*. The two genes were located on different chromosome arms; the *Sr9* locus was mapped on 2BL (Sears and Loegering, 1968; Tsilo et al., 2007). In a recent study, Bariana et al.

(2001) reported two microsatellite markers, *Xstm773* and *Xgwm271A*, together with other RFLP markers that showed complete linkage to the *Sr36* locus in a double haploid (DH) population of 168 lines; the authors reported, however, that the *Xstm773* marker showed better amplification than *Xgwm271*. In their DH population, *STM773* was able to identify HR and HS lines. However, the primers for *Xstm773*, together with primers for several other microsatellite markers identified in our study, also amplified other bands that can make it difficult to distinguish homozygous from heterozygous genotypes. This would complicate its usage in MAS. In the early generations of breeding populations (i.e., F_2, BC_1, BC_2), for example, the majority of individuals are heterozygous and need to be distinguished from homozygous individuals. To date, *STM773* has been converted into two sequence tagged microsatellite markers, *STM773-1* and *STM773-2* (M. Hayden, personal communication, 2005).

In this study, we found that *GWM319*, *STM773-2,* and *WMC477* were diagnostic for *Sr36*. Two marker loci, *Xstm773-2* and *Xwmc477*, were in complete linkage with *Sr36* in both populations. Alleles at the *Xgwm319* locus cosegregated with *Sr36* in one population and were tightly linked (0.9 cM) with *Sr36* in another population. According to the genetic map of Somers et al. (2004), *Xgwm319* and *Xwmc477* were mapped near the centromere and showed no recombination, confirming that these markers are closely linked. Therefore, these three markers would serve as a first step toward the detection of *Sr36* in breeding populations. Preferential transmission of *Sr36*-carrying *T. timopheevi* segment was observed (Table 2). The exact mechanism causing preferential transmission of *T. timopheevi* chromosome segment is unknown. However, Nyquist (1962) hypothesized several possible causes. In our laboratory, studies are in progress to determine the exact cause.

Validation of *Sr36*-Linked Microsatellite Markers

Based on the previous studies, all seven reference stocks that were widely used as sources of *Sr36* in wheat breeding programs (McIntosh et al., 1995) were used in this study: two breeding lines, Sr36/9*LMPG (Knott, 1990) and W2691Sr36-1, and six cultivars, CItr 12632 (= W1656) and CItr 12633 (= W1657) (Allard and Shands, 1954), Idaed 59, Mengavi, Timvera (PI 351987 and PI 237648) (Pridham, 1939), and CItr 14050. In addition to these reference stocks, we examined a range of international germplasm carrying *Sr36* as listed by Roelfs (1988a) and McIntosh et al. (1995), including cultivars developed in Australia, Canada, Mexico, South Africa, and the United States (Table 1). The list included cultivars Songlen, Timgalen, Zaragosa 75 (PI 433770), Gouritz, Hand, Kenosha, Roughrider, Shortim, Timson, Arthur, and Arthur 71. Some of the newly developed *Sr36*-carrying cultivars from the U.S. germplasm were also included—GA-Stuckey, Jaypee, Sisson, NC-Neuse, NE 73843, Vista, Ernie, CK 9803, and Rosen (Table 1). All these cultivars and reference stocks were immune to QFCS and were characterized using *Sr36*-linked marker alleles of *Xgwm319*, *Xwmc477*, and *Xstm773-2* (Table 1; Jin, unpublished data). Other cultivars and breeding lines that were known to carry *Sr36* were developed in Zambia and the United Kingdom, including Idaho 1877 NR AE and Maris Fundin. Both the marker analysis and stem rust screening indicate that the accession of Maris Fundin (PI 410869) obtained from the National Small Grains Collection was incorrect (Table 1). However, another possibility is that Maris Fundin does not carry *Sr36* and that incorrect information about this germplasm exists at the GrainGenes database (http://wheat.pw.usda.gov). Idaed 59 was heterogeneous for both the *Sr36* resistance and *Sr36*-linked marker alleles. The heterogeneity could be the result of seed contamination. The W3496 line, commonly known as Combination III, did not carry any of the *Sr36*-associated marker alleles. This is in agreement with an Australian study based on a recombinant inbred line population derived from Yarralinka/Schomburgk (H.S. Bariana and coworkers, personal communication, 2007). A rare recombinant combining *T. timopheevi* segment with stem rust resistance gene *Sr9e* was present in W3496 and Yarralinka (H.S. Bariana, personal communication, 2007). The CK9877 line does not have *Sr36*, and only marker *Xwmc477* was in agreement; however, loci *Xgwm319* and *Xstm773-2* showed the presence of *Sr36*-associated marker alleles. Therefore, based on these data, *Xwmc477* appears to be the most diagnostic compared with *Xgwm319* and *Xstm773-2*. However, it is important to mention that although WMC477 amplifies 158- and 190-bp fragments in materials containing *Sr36* (Fig. 1C), we consistently observed the 190-bp fragment. We suspect that the quantity of individual PCR products will be lower for one of the fragments; hence, the 158-bp fragment was faint, and only the 190-bp fragment was consistently visible in all accessions that carried *Sr36* (Supplementary Fig. 1). Different PCR-reaction protocols might lead to preferential amplification of one of the two bands (158 and/or 190 bp). A similar PCR discrepancy involving amplifications of multiple fragments was described by Bercovich et al. (1999).

Many accessions with similar names may be confusing, especially when the name is widely used instead of the accession number. In this study, we analyzed four accessions of Zaragoza 75, and only PI43370 carried *Sr36*. The other three accessions could be carrying different stem rust resistance genes. According to Roelfs (1988a) and McIntosh et al. (1995), Purdue carries *Sr36*; however,

there were many accessions of Purdue at the National Small Grains Collection, and the one used in this study did not carry *Sr36* according to the results of reaction to QFCS and *Sr36*-linked markers.

In this study, the information obtained from using races of *P. graminis* f. sp. *tritici* alone was not diagnostic of *Sr36* in the presence of other *Sr* genes. This situation was observed in two cultivars, RL 6044 and Kenya 58, which showed immunity to QFCS, and low IT to TPMK and MCCF (Table 1). However, immunity in these cultivars was due to genes or combination of genes other than *Sr36*. RL 6044 is known to carry *Sr33* from Tetra Canthatch// *Aegilops squarrosa*, whereas Kenya 58 carries *Sr6* and other genes from Red Egyptian and Kenyan cultivars (McIntosh et al., 1995). With conventional screening tests, it would require extensive seedling tests and testcrosses to perform gene postulation in these cultivars by using the appropriate stem rust races—a potentially time-consuming process if two or more genes confer resistance to a particular race. However, both the rust screening results and previously published information were successful in validating the cultivars that carried *Sr36*, and the results were in agreement with the *Sr36*-specific marker alleles. From these results, it is clear that the *Sr36*-linked markers are diagnostic for this gene and can be used to detect its presence during cultivar development.

Even though *Sr36* does not provide a high level of resistance against a wide range of stem rust races, it is still a valuable gene because it is the best available source of resistance to the new race of stem rust, Ug99. Therefore, tightly linked markers identified in this study should be useful in marker-assisted selection of *Sr36* and can be used in selecting for genotypes possessing *Sr36* during cultivar development. These markers will accelerate the use of *Sr36* in commercial cultivars by allowing pyramiding of *Sr36* with other effective genes to confer a more durable resistance. Our results show that these markers are applicable across different genetic backgrounds.

Acknowledgments

We are thankful to Dr. Matthew Hayden for providing the information on the *STM773-1* and *STM773-2* markers, and Dr. Harbans Bariana for his useful comments on the manuscript. This research was supported in part by the Minnesota Annual Conference of the United Methodist Church through the Project AgGrad fellowship awarded to T.J.Tsilo, the Agricultural Research Council of South Africa, and the USDA Cooperative Research, Education and Extension Service, Coordinated Agricultural Project grant number 2006-55606-16629.

References

Allard, R.W., and R.G. Shands. 1954. Inheritance of resistance to stem rust and powdery mildew in cytologically stable spring wheats derived from *Triticum timopheevi*. Phytopathology 44:266–274.

Ashagari, D., and J.B. Rowell. 1980. Postpenetration phenomena in wheat cultivars with low receptivity to infection by *Puccinia graminis* f. sp. *tritici*. Phytopathology 70:624–627.

Bariana, H.S., M.J. Hayden, N.U. Ahmed, J.A. Bell, P.J. Sharp, and R.A. McIntosh. 2001. Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. Aust. J. Agric. Res. 52:1247–1255.

Bassam, B.J., G. Caetano-Anolles, and P.M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. 196:80–83.

Bercovich, D., Z. Regev, T. Ratz, A. Luder, Y. Plotsky, and Y. Gruenbaum. 1999. Quantitative ratio of primer pairs and annealing temperature affecting PCR products in duplex amplification. Biotechniques 27:762–770.

Expert Panel on the Stem Rust Outbreak in Eastern Africa. 2005. Sounding the alarm on global stem rust: An assessment of race Ug99 in Kenya and Ethiopia and the potential for impact in neighboring regions and beyond. Available at http://www.cimmyt.org/english/wps/news/2005/aug/pdf/Expert_Panel_Report.pdf (verified 27 Sept. 2007). CIMMYT, Mexico.

Gyarfas, J. 1978. Transference of disease resistance from *Triticum timopheevi* to *Triticum aestivum*. Master's thesis. University of Sydney, Australia.

Jin, Y. 2005. Races of *Puccinia graminis* in the United States during 2003. Plant Dis. 89:1125–1127.

Knott, D.R. 1988. Using polygenic resistance to breed for stem rust resistance in wheat. p. 39–47. *In* N.W. Simmonds and S. Rajaram (ed.) Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico.

Knott, D.R. 1989. The wheat rusts: Breeding for resistance. Springer-Verlag, Berlin.

Knott, D.R. 1990. Near-isogenic lines of wheat carrying genes for stem rust resistance. Crop Sci. 30:901–905.

Lander, E., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181.

Liu, S., X. Zhang, M.O. Pumphrey, R.W. Stack, B.S. Gill, and J.A. Anderson. 2006. Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Funct. Integr. Genomics 6:83–89.

McIntosh, R.A., and N.H. Luig. 1973. Recombination between genes for reaction to *P. graminis* at or near the *Sr9* locus. p. 425–432. *In* E.R. Sears and L.M.S. Sears (ed.) Proc. of the Fourth Int. Wheat Genetics Symp. Agricultural Experiment Station, University of Missouri, Columbia.

McIntosh, R.A., C.R. Wellings, and R.F. Park. 1995. Wheat rusts: An atlas of resistance genes. CSIRO Press, Victoria, Australia.

McVey, D.V., D.L. Long, and J.J. Roberts. 1996. Races of *Puccinia graminis* in the United States during 1994. Plant Dis. 80:85–89.

McVey, D.V., D.L. Long, and J.J. Roberts. 2002. Races of *Puccinia graminis* in the United States during 1997 and 1998. Plant Dis. 86:568–572.

Nyquist, N.E. 1957. Monosomic analysis of stem rust resistance of a common wheat strain derived from *Triticum timopheevi*. Agron. J. 49:222–223.

Nyquist, N.E. 1962. Differential fertilization in the inheritance of stem rust resistance in hybrids involving a common wheat strain derived from *Triticum timopheevi*. Genetics 47:1109–1124.

Pretorius, Z.A., R.P. Singh, W.W. Wagoire, and T.S. Payne. 2000. Detection of virulence to wheat stem rust gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Phytopathology 84:203.

Pridham, T.J. 1939. A successful cross between *Triticum vulgare* and *Triticum timopheevi*. J. Aust. Inst. Agric. Sci. 5:160–161.

Riede, C.R., and J.A. Anderson. 1996. Linkage of RFLP markers to an aluminum tolerance gene in wheat. Crop Sci. 36:905–909.

Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy, and M.W. Ganal. 1998. A microsatellite map of wheat. Genetics 149:2007–2023.

Roelfs, A.P. 1988a. Resistance to leaf rust and stem rust in wheat. p. 10–22. *In* N.W. Simmonds and S. Rajaram (ed.) Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico.

Roelfs, A.P. 1988b. Genetic control of phenotypes in wheat stem rust. Annu. Rev. Phytopathol. 26:351–367.

Roelfs, A.P., and J.W. Martens. 1988. An international system of nomenclature for *Puccinia graminis* f. sp. *tritici*. Phytopathology 78:526–533.

Roelfs, A.P., D.L. Long, and J.J. Roberts. 1993. Races of *Puccinia graminis* in the United States during 1990. Plant Dis. 77:125–128.

Sears, E.R. 1966. Nullisomic-tetrasomic combinations in hexaploid wheat. p. 29–45. *In* R. Riley and K.R. Lewis (ed.) Chromosome manipulation and plant genetics. Oliver and Boyd, Edinburgh.

Sears, E.R., and W.Q. Loegering. 1968. Mapping of stem rust genes *Sr9* and *Sr16* of wheat. Crop Sci. 8:371–373.

Singh, R.P., M.G. Kinyua, R. Wanyera, P. Njau, Y. Jin, and J. Huerta-Espino. 2005. Spread of a highly virulent race of *Puccinia graminis tritici* in eastern Africa: Challenges and opportunities. p. 51–57. *In* H.T. Buck, J.E. Nisi and N. Salomon (ed.). Wheat Production in Stressed Environments: Proc. of the 7th Int. Wheat Conf., Mar de Plata, Argentina. 27 Nov.–2 Dec. 2005. Springer, Dordrecht, the Netherlands.

Somers, D.J., P. Isaac, and K. Edwards. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum L*). Theor. Appl. Genet. 109:1105–1114.

Song, Q.J., J.R. Shi, S. Singh, E.W. Fickus, J.M. Costa, J. Lewis, B.S. Gill, R. Ward, and P.B. Cregan. 2005. Development and mapping of microsatellite (SSR) markers in wheat. Theor. Appl. Genet. 110:550–560.

Stakman, E.G., D.M. Stewart, and W.Q. Loegering. 1962. Identification of physiological races of *Puccinia graminis* var. *tritici*. Pub. E-617. USDA, Agricultural Research Service.

Tsilo, T.J., Y. Jin, and J.A. Anderson. 2007. Microsatellite markers linked to stem rust resistance allele *Sr9a* in wheat. Crop Sci. 47:2013–2020.

Wanyera, R., M.G. Kinyua, Y. Jin, and R. Singh. 2006. The spread of stem rust caused by *Puccinia gramicis* f. sp. *Tritici*, with virulence on *Sr31* in wheat in eastern Africa. Plant Dis. 90:113.