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Microsatellite Markers Linked to Stem Rust Resistance Allele *Sr9a* in Wheat

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

ABSTRACT

Host resistance to stem rust of wheat (*Triticum aestivum* L.), caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn., is more effective and durable when several stem rust resistance (*Sr*) genes are pyramided into a single line. We studied the *Sr9a* allele, one of six known alleles at the *Sr9* locus on chromosome 2BL, using 116 F_2 plants and their $F_{2:3}$ families derived from the cross of near-isogenic lines (NILs) 'Chinese Spring' and ISr9a-Ra. Four microsatellite markers were identified that mapped within 3.6 cM proximal to the *Sr9a* locus. Fifty-nine wheat accessions were screened with the three codominant and one dominant markers to determine their polymorphism information content (PIC). The marker *Xgwm47* revealed 12 alleles and had the highest PIC value of 0.85. We attempted to postulate the presence of *Sr9a* by phenotypic screening. In accessions that had multiple *Sr* genes, however, it was not possible to postulate *Sr9a* due to masking effects. Despite the ambiguity of phenotypic evaluation, *Xgwm47* was diagnostic for *Sr9a* in additional NILs tested. These results suggest that *Xgwm47* will be a useful tool for marker-assisted selection of *Sr9a* in wheat breeding programs.

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Abbreviations: bp, base pairs; cM, centimorgan; MAS, marker-assisted selection; NIL, near-isogenic lines; PIC, polymorphism information content; SSR, simple sequence repeat.

Stem rust of wheat (*Triticum aestivum* L.), caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn., is a very destructive disease of wheat, reaching devastating epidemic levels in most wheat-growing areas of the world (Knott, 1989). To date, more than 45 stem rust resistance (*Sr*) genes have been identified against different races of this fungus (McIntosh et al., 2003). Even though single-gene resistance may be overcome by the rapidly evolving races, the use of resistant cultivars is still the most effective and economical method of reducing yield losses due to stem rust (McIntosh, 1988). One way to increase the durability of stem rust resistance genes is to pyramid several *Sr* genes to increase broad-spectrum resistance to several races (Pederson and Leath, 1988). With conventional methods in wheat breeding programs, the continual pyramiding of genes in a single genotype will become difficult or even impossible when one or more genes in the background is effective against many races of the pathogen.

Plant breeders have been successful in using many race-specific stem rust resistance genes; however, they have not been

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able to fully exploit resistance conferred by the *Sr9a* allele in breeding programs (McIntosh et al., 1995). This has been due largely to problems of detecting it in the presence of other stem rust resistance genes. Moreover, the wheat cultivar Red Egyptian, the original source of *Sr6*, *Sr8a*, and *Sr9a* (Knott, 1957), has been widely used in many wheat breeding programs (Knott, 1989; McIntosh et al., 1995), and *Sr6* has a masking effect on *Sr9a*. Another difficulty in diagnosing the presence of *Sr9a* is that its infection type is difficult to determine because it is dependent on the background of the cultivar and the particular race of the pathogen (Knott, 1989). Gene *Sr9a* confers moderate resistance against the race TPMK, one of the most predominant and devastating races of *P. graminis* f.sp. *tritici* in North America (McVey et al., 1996, 2002; Jin, 2005), and against other important races such as QCCJ and MCCF. Therefore, this allele is still valuable in North America. The allele was initially characterized as one of the six (identified with suffixes *a*–*g* with the exclusion of *c*) alleles at the *Sr9* locus (Knott, 1989). Sears and Loegering (1968) mapped the *Sr9* locus on the long arm of chromosome 2BL.

The potential benefits of molecular marker-assisted selection (MAS) have been widely discussed (Melchinger, 1990; Paterson et al., 1991; Young, 1996; Mohan et al., 1997; Anderson, 2003), especially to provide solutions to overcome some of the problems faced by classical phenotypic screening approaches in plant breeding programs. For example, to facilitate breeding for durable resistance to stem rust, molecular markers are useful tools in developing resistant cultivars and, especially, pyramiding several disease resistance genes (Anderson, 2003). Marker-assisted selection can be used at an early stage of plant development when multiple DNA markers are used to screen several genes simultaneously.

The objectives of this study were to: (i) determine the precise chromosomal location of the *Sr9a* allele based on genetic markers on 2BL; and (ii) identify codominant microsatellite markers closely linked to *Sr9a* that could be used for MAS.

MATERIALS AND METHODS

Plant Materials

Mapping of the *Sr9a* gene was performed by analysis of 116 F_2 individuals derived from a cross between the near-isogenic lines (NILs) 'Chinese Spring' as a female parent and ISr9a-Ra as a male parent. The ISr9a-Ra (CItr 14169) line is a BC_3F_{11}-derived line resulting from the cross of the original source of *Sr9a*, Red Egyptian, and Chinese Spring (CItr 14108) as the recurrent parent (Loegering and Harmon, 1969). The F_3 seed was obtained from self pollination (bagged spikes) of each F_2 plant. In addition to the mapping of *Sr9a*, a set of 59 wheat cultivars and breeding lines (Table 1) was analyzed to determine polymorphism information of markers and their usefulness as diagnostic markers for *Sr9a*. Seeds of wheat accessions were obtained from the USDA-ARS National Small Grains Collection, Aberdeen, ID. Seeds of breeding lines and germplasm were obtained from the USDA-ARS Cereal Disease Laboratory, St. Paul, MN. This set of accessions included two additional pairs of NILs, Sr9a/9*LMPG (Knott, 1990) and 'Marquis'9*/Red Egyptian (Knott, 1965), carrying the *Sr9a* allele in a different genetic background of LMPG and Marquis, respectively. Red Egyptian (CItr 12345), the original source of the *Sr9a* allele, was also included in the analysis. Because there were five different accessions of Red Egyptian in the NSGC database, all of them were included in the analysis. The Chinese Spring nullisomic–tetrasomic (N2B–T2D) line (Sears, 1966a) was used to determine the chromosomal location of the amplified bands of microsatellite markers.

Stem Rust Inoculation and Evaluation

Seedlings of parents and F_2 progeny were inoculated when the primary leaves were fully expanded (~7 d after planting, depending on whether the seeds were pregerminated in a petri dish or sown directly) with race TPMK (Isolate 74MN1409) (avirulence/virulence formula *Sr6*, *9a*, *9b*, *30/Sr5*, *7b*, *8a*, *9d*, *9e*, *9g*, *10*, *11*, *17*, *21*, *36*, *Tmp*) following protocols described by Jin (2005). Inoculated seedlings were placed in a dew chamber for 16 h with no light and 2 to 3 h light after dark period of incubation. After infection, plants were placed either in growth chambers with 12 h of light at 20 to 22°C and 12 h of dark at 18 to 20°C, or in a greenhouse at 18 to 21°C under 160-W very-high-output fluorescent tubes with a 12-h photoperiod. Infection types were scored approximately 14 d after inoculation using a scale of 0 to 4, as described by Stakman et al. (1962) and modified by Roelfs (1988a). To confirm F_2 genotypes and distinguish between heterozygous and homozygous-resistant F_2 individuals, 16 to 20 plants of each $F_{2:3}$ family were inoculated with race MCCF (Isolate 59KS19) (avirulence/virulence formula *Sr6*, *8a*, *9a*, *9b*, *9d*, *9e*, *11*, *21*, *30*, *36/Sr5*, *7b*, *9g*, *10*, *17*, *Tmp*) and evaluated as described above. To attempt the postulation of *Sr9a* allelic status of wheat accessions and breeding lines, all accessions and breeding lines were inoculated with *Sr9a* avirulent races MCCF and TPMK and virulent race QFCS (Isolate 03ND76C) (avirulence/virulence formula *Sr6*, *7b*, *9b*, *9e*, *11*, *30*, *36*, *Tmp/Sr5*, *8a*, *9a*, *9d*, *9g*, *10*, *17*, *21*), and were then evaluated for their infection types. All isolates used in the study were verified based on avirulence/virulence on the 16 differential lines described by Roelfs and Martens (1988) and Roelfs et al. (1993).

DNA Extraction and Microsatellite Analysis

To investigate the linkage relationship between *Sr9a* and genetic markers, fresh leaf tissues were collected from young seedlings of the 116 F_2 individuals and 59 wheat accessions and breeding lines. Total genomic DNA was extracted following protocols described by Riede and Anderson (1996) and modified by Liu et al. (2006). Gene *Sr9a* was previously located on the long arm of chromosome 2B (Loegering and Harmon, 1969). Forty-four microsatellite primer pairs (GWM, WMC, CFD, and BARC) whose loci mapped on the long arm and centromere region of 2B (Röder et al., 1998; Somers et al., 2004; Song et al., 2005; see also www.scabusa.org/pdfs/BARC_SSRs_011101.html [verified 16 July 2007]) were used to screen for polymorphism between the parental lines that differed in their *Sr9a* allelic

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Table 1. Informativeness of the *Sr9a*-linked simple sequence repeat (SSR) marker alleles in wheat cultivars and breeding lines from diverse genetic origins.

Cultivar or breeding line	Accession no.	Origin	*Puccinia graminis* races			*Sr9a*†	SSR marker‡			
			QFCS	TPMK	MCCF		GWM 47	GWM 120	BARC 101	WMC175
							bp			
1. ISr9a-Ra	CItr 14169	USA	4§	2	2–	+	**190**¶	155	116	–
2. ‘Chinese Spring’	CItr 14108	China	4	3+	3+	–	168	140	125	255
3. Sr9a/9*LMPG		Canada	3	2	2	+	**190**	–	116	255
4. LMPG		Canada	3+	4	4	–	210	–	116/130	–
5. ‘Marquis’/9*RE		Canada	1+	u	2+	+	**190**	–	116	255
6. ‘Red Egyptian’	CItr 12345	Egypt	u	u	u	+	**190**	–	116	–
7. Red Egyptian	PI 45374	S. Africa	0;	2/2+	0	+/?	**190**	155	116	215
8. Red Egyptian	PI 45403	S. Africa	2	2+	4	–	200	170/155	116	255
9. Red Egyptian	PI 45415	S. Africa	;	2+	;	+/?	**190**	155	116	255
10. Red Egyptian	PI 192020	Ethiopia	;	2	;	+/?	**190**	170	116	255
11. ‘Centurk’	CItr 15075	USA	1	1	;	+/?	168	155	116	255
12. ‘Lerma Rojo 64’	CItr 13929	USA	;	;1	;	+/?	168	140	125	255/290
13. ‘NC-Neuse’	PI 633037	USA	0	;2	0;	+/?	150/140	–	110	–
14. ‘Excel’	PI 555465	USA	0;	2	2	+/?	150/140	153	116	220
15. ‘Halt’		USA	1;	3+	1	–	210	–	116	255
16. W2691Sr9b	CItr 17386	Austrailia	1	2+	2+	–	140	130	130	220/255
17. ISr9d-Ra	CItr 14177	USA	u	4	1;	–	–	–	116	290
18. ‘Vernstein’ (*Sr9e*)	PI 442914	Austrailia	;	3+	;	–	140	155	116	220
19. Mengavi	PI 290912	Australia	0	4	0	–	180/150/140	–	110	–
20. W 3496	PI 520133	Australia	0	2+	0	–	–	153	116	220
21. ‘Timson’	PI 404115	Australia	0	0;	0	–	180/150/140	–	110	–
22. ‘Timvera’	PI 351987	Australia	0	4	0	–	165	155	116	220
23. ‘Timgalen’		Australia	0/1	4	0	–	180/150/140	–	110	–
24. ‘Arthur’	CItr 14425	USA	0	0/4	0	–	180/170/150/140	–	110	–
25. ‘Arthur 71’	CItr 15282	USA	0	0	0	–	180/170/150/140	–	110	–
26. W1657	CItr 12633	USA	0	4	;	–	180/150/140	–	110	–
27. CK 9803	PI 591000	USA	0	0/1	0	–	150/140	–	0	–
28. ‘Ernie’	PI 599615	USA	0	;	0	–	150/140	–	–	–
29. ‘Roughrider’	CItr 17439	USA	0	2+	0	?	–	–	116	255
30. ‘Vista’	PI 562653	USA	0	2	0	+/?	**190**/150/140	–	110	–
31. ‘Rosen’	CItr 17607	USA	0	0	0;	–	180/150/140	–	–	–
32. ‘GA-Stuckey’	PI 591001	USA	0	0;	0	–	180/150/140	–	110	–
33. ‘Jaypee’	PI 592760	USA	0	3+	0	–	150/140	–	110	–
34. ‘Sisson’	PI 617053	USA	0	;1	0	–	150/140	–	110	–
35. W1656	CItr 12632	USA	0	4	0	–	180/150/140	–	110	–
36. ‘Hand’	CItr 17288	USA	0	0	0	–	–	–	116	255
37. ‘Kenosha’	CItr 14025	USA	0	;	0;	–	–	–	116	255
38. ‘Gouritz’	PI 479672	S. Africa	0	1	0	–	145	–	116	255
39. TA 1600	PI 603223	Iran	u	u	u	–	–	–	110	–
40. RL 6044	CItr 17752	Canada	0	2	1+	–	–	–	130	220
41. RL 5045	PI 520492	Canada	0;	2/4	1+	–	–	–	130	220
42. Sr 6	CItr 15082	Canada	0;	2/4	1	–	–	153	116	255
43. ‘Eureka’	CItr 17738	USA	0;	2	1	–	–	130	130	220
44. ‘Intrada’	PI 631402	USA	;	1	1+	?	170	–	116/110	255
45. ‘TAM 200’	PI 578255	USA	1;	1+	1+	?	170	–	116	255
46. ‘GA-Dozier’	PI 591000	USA	2	2	2	?	164	155	116	220
47. ‘Fleming’	PI 599615	USA	;1	2	2	?	–	153	116	255
48. ‘Goodstreak’	PI 632434	USA	;1	0;	;	–	–	–	116	255
49. ‘Harry’	PI 632435	USA	;	0	;	–	–	–	116	255
50. ‘Morey’	PI 591428	USA	2+	2	2	?	–	153	116	255
51. CK 9877		USA	2+/4	2+	0/3+	–	180/150/140	–	110	–
52. ‘Patterson’	PI 583825	USA	3	2/3-	2+	–	–	153	116	255
53. ‘Roane’		USA	4	3+	3+	–	168	130	130	220
54. ‘Purdue’		USA	2/4	1	2/4	–	–	130	130	220
55. ‘Chukar’	PI 628641	USA	4	4	4	–	168	–	116	255
56. ‘Truman’	PI 634824	USA	4	4	4	–	0	–	116	255
57. ‘Brundage 96’		USA	4	4	4	–	168	153	116	220
58. ‘Zaragoza 75’	PI 519305	Mexico	4	3+	4	–	168/150	155	116	255
59. ‘Tosca’	PI 479680	S. Africa	4	4	4	–	–	–	116/110	255
No. of alleles							12	6	5	5
PIC no.#							0.85	0.60	0.62	0.67

† For the *Sr9a* gene, + or – represents presence or absence verified based on the results of races TPMK and MCCF. The presence of *Sr9a* in these cultivars was based on previously published reports; ? = no evidence of the presence of *Sr9a* and probably doesn’t contain *Sr9a* based on previous reports; +/? = uncertainty.

‡Sizes of amplified fragments in base pairs (bp). Null alleles are represented by –.

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

¶The 190-bp fragment is diagnostic for *Sr9a* and is in bold.

#Polymorphism information content was calculated as described by Anderson et al. (1993).

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status. We used the primer pairs to screen for polymorphism between two pairs of NILs, ISr9a-Ra and Sr9a/9*LMPG, both carrying *Sr9a* in the genetic background of susceptible lines Chinese Spring and LMPG, respectively. To reveal linkage relationships between *Sr9a* and the microsatellite marker loci, all polymorphic simple sequence repeat (SSR) primer pairs were screened on the 116 F_2 plants.

Polymerase chain reaction (PCR) was performed in a 96-well plate with 10 μL of final reaction mixture containing 1 μL 10× PCR buffer, 25 mmol/L $MgCl_2$, 1.25 mmol/L dNTPs, 1 μmol/L of each primer, 5 units/μL Taq DNA polymerase (Applied Biosystems, Branchburg, NJ), and 15 mg/L genomic DNA. 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 each primer pair) for 1 min, 72°C for 2 min, with a final extension step of 72°C for 10 min and 4°C indefinitely. The PCR thermal cycling was performed using the GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA). About 5 μL of 3× loading buffer (0.02 g bromophenol blue, 0.02 g xylene cyanol, 1.6 mL of 0.5 mol/L ethylenediamine tetraacetic acid [EDTA], and 38.4 mL formamide) was added to the PCR products to make a final volume of 15 μL. Before gel loading, samples were denatured for 5 min at 95°C and chilled on ice. The PCR products were separated by performing electrophoresis on polyacrylamide gels (6% [w/v] 20:1 acrylamide/bisacrylamide, 8 mol/L urea in TBE buffer, pH 8.3) in 1× TBE buffer (90 mmol/L Tris-borate (pH 8.3), 2 mmol/L EDTA) using PowerPac 3000 (Bio-Rad Laboratories, Hercules, CA) at a constant power of 110 W for 90 min. Gels were Ag stained by following the protocol described by Bassam et al. (1991) and photographed using automatic processor compatible film (Promega Corp., Madison, WI).

Genetic Linkage Analysis

For genetic analysis of the *Sr9a* allele, the band scores of all F_2 individuals were classified as being parental types or heterozygous. Analysis of the segregation pattern of *Sr9a* in the F_2 population and F_3 families was based on a χ^2 distribution analysis used to test if the observed segregation ratios of homozygous resistant, heterozygous, and homozygous susceptible phenotypes fit the Mendelian ratios of 3:1 (F_3) or 1:2:1 (F_2) that would be expected if the resistance phenotype was controlled by a single dominant gene. Genetic linkage analysis was performed using Mapmaker Version 3.0b with the linkage groups based on a logarithm of odds score of at least 3.0. A partial genetic linkage map of chromosome 2BL was constructed using genetic distance in centimorgans (cM) as described by the Kosambi mapping function (Lander et al., 1987).

Table 2. Segregation ratios of alleles at *Sr9a* and at the simple sequence repeat marker loci in an F_2 population derived from the cross of 'Chinese Spring' and ISr9a-Ra.

Gene or marker	Observed†			Expected ratio	χ^2	*P* value‡
	X_1X_1	X_1X_2	X_2X_2			
Sr9a	37	46	33	1:2:1	5.24	0.073
Xgwm47	35	48	33	1:2:1	3.52	0.172
Xgwm120	34	50	32	1:2:1	2.28	0.32
Xbarc101	34	50	32	1:2:1	2.28	0.32
Xwmc175	37	79§		1:3	2.94	0.086

†Genotype of F_2 population with X_1X_1 = homozygous resistant or marker allele of resistant parent, X_1X_2 = heterozygous, and X_2X_2 homozygous susceptible or marker allele of susceptible parent. Infection types of $F_{2:3}$ families, against race MCCF, were used to distinguish X_1X_1 from X_1X_2 and also confirm X_2X_2 in the F_2 population.

‡P value > 0.05 significance level is used to accept the observed segregation as a fit to the expected ratio of 1:2:1.

§Combined number of homozygous and heterozygous lines.

Informativeness of Polymorphic Microsatellite Markers

Fifty-nine wheat cultivars and breeding lines of diverse genetic origin (Table 1) were used to determine the informativeness of the SSR markers by calculating their polymorphism information content (PIC) according to the formula described by Anderson et al. (1993).

RESULTS

Phenotype and Segregation Analysis of the F_2 Population

The *Sr9a*-containing NIL parent ISr9a-Ra showed a resistant infection type of 2, whereas the recurrent parent Chinese Spring showed a susceptible infection type of 3+ to 4 against the TPMK and MCCF races of the stem rust pathogen. Among the 116 F_2 plants, 37 were homozygous resistant, 46 were heterozygous, and 33 were homozygous susceptible on the basis of reactions to both TPMK and MCCF races of $F_{2:3}$ families after inoculation with MCCF (Table 2). The ratio of 37:46:33 was consistent with the expected Mendelian segregation ratio of 1:2:1 with $\chi^2 = 5.24$ ($P = 0.073$), indicating that the F_2 population segregated for a single dominant gene conferring resistance.

Genetic Mapping of the *Sr9a* Allele

Based on the parental screening of the NILs (Chinese Spring and ISr9a-Ra) with 44 microsatellite markers whose loci were previously mapped on 2BL and near the centromere on 2BS, only three codominant (*Xgwm47*, *Xgwm120*, and *Xbarc101*) and one dominant (*Xwmc175*) markers were polymorphic between the parents (Fig. 1). For the *Xwmc175* marker, there seemed to be a faint band amplified in resistant F_2 individuals (Fig. 1D); however, this faint band was not repeatable, and it did not amplify in the heterozygous resistant F_2 progeny. The χ^2 test indicated that the segregation pattern of the codominant DNA markers in the F_2 population also fit the expected Mendelian ratio of 1:2:1 segregation (Table 2). The marker *Xwmc175* showed a segregation ratio of 1:3 (Table 2).

A partial genetic linkage map of chromosome 2BL shows the location of the *Sr9a* allele with the four SSR markers (Fig. 2). No recombination was observed between *Xwmc175* and the *Sr9a* allele, indicating that *Xwmc175* cosegregated with the susceptible phenotype

and was linked to *Sr9a* in repulsion (Fig. 1 and 2). The closest codominant microsatellite marker *Xgwm47* was linked to *Sr9a* at a genetic distance of 0.9 cM (Fig. 2). The other two SSR markers, *Xgwm120* and *Xbarc101*, together mapped 2.7 and 3.6 cM proximal to *Xgwm47* and *Sr9a*, respectively (Fig. 2). The results of the analysis of the Chinese Spring nullisomic–tetrasomic (N2B–T2D) line using all the SSR markers (*Xgwm47*, *Xgwm120*, *Xbarc101*, and *Xwmc175*) confirmed that the *Sr9a* allele was mapped on chromosome 2B (data not shown).

Validation and Polymorphism Information of Microsatellite Markers

During phenotypic screening of 59 wheat accessions and breeding lines using three stem rust races, QFCS, TPMK, and MCCF, some of the accessions showed very low infection types against all three races, which revealed that they might be carrying stem rust resistance genes other than *Sr9a* (Table 1). Some of these accessions had no information as to which *Sr* genes were present—the primary purpose of using these accessions was to obtain polymorphism information of the four SSR markers (*Xgwm47*, *Xgwm120*, *Xbarc101*, and *Xwmc175*). The marker *Xgwm47* had the highest polymorphism information content of 0.85 and revealed 12 alleles, while *Xgwm120*, *Xbarc101*, and *Xwmc175* had PIC values of 0.60, 0.62, and 0.67, respectively.

The amplified band (190 base pairs [bp]) of *Xgwm47* was associated with the presence of *Sr9a* in the breeding lines and accessions that were known to carry *Sr9a*, including ISr9a-Ra, Sr9a/9*LMPG, Marquis9*/Red Egyptian, and Red Egyptian as the original source of *Sr9a* (Table 1). Out of five accessions of Red Egyptian, PI 45403 showed a susceptible infection type (4) to race MCCF, indicating that this accession did not carry *Sr9a*, and this concurred with the absence of the *Sr9a*-specific marker allele at the *Xgwm47* locus (Table 1). The *Xgwm47* fragment size varied in most accessions that were known to be non-*Sr9a*-carrying lines. The fragment size of *Xgwm120*, *Xbarc101*, and *Xwmc 175* varied among accessions and breeding lines, and was not associated with the presence of *Sr9a* in breeding lines known to carry *Sr9a* (Table 1).

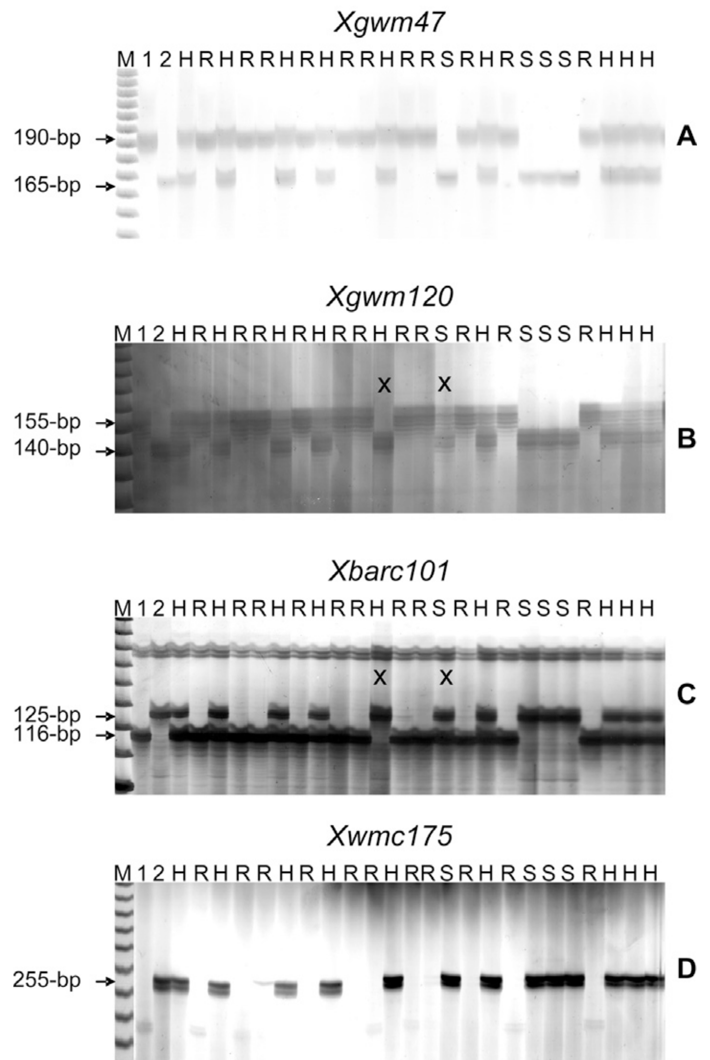

Figure 1. Polyacrylamide gel electrophoresis showing the segregation pattern of the four microsatellite markers (A) *Xgwm47*, (B) *Xgwm120*, (C) *Xbarc101*, and (D) *Xwmc175* in a subset of the F_2 progenies from a cross between *Sr9a* near-isogenic lines ('Chinese Spring' and ISr9a-Ra); M is a 10-base pair (bp) ladder, 1 is resistant parent ISr9a-Ra, 2 is susceptible parent Chinese Spring, R is resistant F_2 progeny, S is susceptible F_2 progeny, and H is heterozygous resistant F_2 progeny. Arrowheads indicate the size of the band associated with the *Sr9a* gene in coupling linkage (A–C) and also in repulsive linkage (D). Recombination or crossing over between the marker allele and the resistance allele is indicated by X.

DISCUSSION

Sears (1962) used a chromosome substitution approach to locate *Sr9a* on chromosome 2B, and *Sr9a* was later mapped on the long arm of chromosome 2B using a telocentric mapping approach (Sears 1966b). The stem rust resistance gene *Sr9a* was identified as part of several alleles that form an allelic series complex at the *Sr9* locus (Knott, 1989). The *Sr9* locus and two other stripe rust resistance genes, *Yr7* and *Yr5*, have been cytologically mapped on chromosome 2B with the same recombination frequency of 15 cM from the centromere (Hart et al., 1993), indicating that these loci were linked. Markers have been identified for *Yr5* (Chen et al., 2003; Yan et al., 2003). In our laboratory, studies are in progress to determine if these sequence tagged site markers can be useful for genetic analysis of the allelic complex at the *Sr9* locus.

We determined the location of *Sr9a* based on the available genetic maps of microsatellite markers. The use of NILs (ISr9a-Ra and Chinese Spring) facilitated the identification of the closest molecular markers, since the two lines have the same genetic background but differ in the presence of the *Sr9a* allele. Given the moderate- to high-

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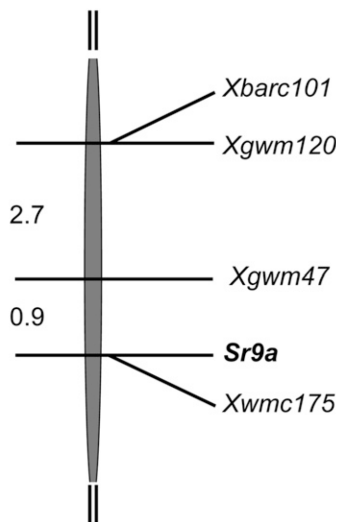

Figure 2. A genetic linkage map of the *Sr9a* locus and the linked simple sequence repeat markers on the long arm of chromosome 2BL. The linkage map was constructed using 116 F_2 individuals derived from a cross between near-isogenic lines ('Chinese Spring' and ISr9a-Ra). Names of markers and the gene are shown on the right.

density coverage of DNA markers on chromosome 2BL (Röder et al., 1998; Somers et al., 2004; Song et al., 2005), a partial linkage map constructed in this study indicated the marker *Xgwm47* as being closely linked to *Sr9a*. These codominant markers were easy to score on polyacrylamide gels, with the exception of *Xgwm120*, which appeared as a faint band and required replication to verify the genotype of the marker allele. There were no codominant markers mapped to the distal side of *Sr9a*, however, owing to a lack of polymorphism rather than a lack of marker coverage. The order of markers in the current study shows some homologies with the map reported by Somers et al. (2004). Out of the four markers, the order of three markers corresponds to that reported by Somers et al. (2004). According to Song et al. (2005), however, the map location of *Xbarc101* was found to be distal to the other markers, which conflicts with the order of markers reported by Somers et al. (2004). In our study, however, we observed no recombination between *Xbarc101* and *Xgwm120*, and the two markers are proximal to *Xgwm47* and *Xwmc175*. Therefore, our results are more consistent with the order of Somers et al. (2004) than Song et al. (2005). According to Somers et al. (2004), *Xwmc175* mapped distal to *Xgwm47*, the same orientation as in Fig. 2. Based on these observations, at this stage we concluded that *Sr9a* is located distal to or in the vicinity of *Xgwm47* and *Xwmc175* (Fig. 2). The *Xwmc175* and *Sr9a* alleles were in complete repulsion linkage. Perhaps this marker also may be useful in selecting individuals that are only homozygous for *Sr9a*. It is often desired to differentiate heterozygous from susceptible individuals, however, indicating a preference for the codominant *Xgwm47*.

Because *Sr9a* shows a moderately resistant reaction to common stem rust races in North America, it may be difficult to detect with phenotypic screening of heterozygous individuals in one generation, which prompts testing further generations. We overcame this complication by inoculating the $F_{2:3}$ families with race MCCF to distinguish and verify the F_2 genotypes. In postulating resistance genes, specific races are required to detect all or specific resistance genes. This requires all individuals to be inoculated with different races—a process that becomes time consuming and laborious. If there are only a few races available to detect all combinations of resistance genes, however, masking effects due to other *Sr* genes cannot be avoided. The problem of detecting the *Sr9a* allele has restricted its use in breeding programs. On the other hand, the *Sr9a* allele does not confer resistance to some races, so there is a need to combine it with other stem rust resistance genes, a process that can be achieved with the use of molecular markers. This will, therefore, avoid the use of a large number of races required to postulate the presence of individual *Sr* genes, in this case *Sr9a*, when it is combined with other *Sr* genes. With MAS, it is possible to develop cultivars with a more broad-based resistance against a wide range of races (Eagles et al., 2001).

The marker alleles of *Xgwm120*, *Xbarc101*, and *Xwmc175* varied among accessions and breeding lines, and were not associated with the presence of *Sr9a* in resistant breeding lines known to carry *Sr9a*. This indicates that the linkage of these marker loci with *Sr9a* had been broken because the breeding lines in this study have undergone several generations of recombination and selfing during inbred line development. The *Xgwm47* marker, however, displayed a marker allele (190 bp) that was specific to Red Egyptian and was validated in all NILs tested. Even though there was recombination between the markers and the *Sr9a* gene, the marker *Xgwm47* should be valuable for MAS because of its diagnostic 190-bp allele and relatively high PIC value. Markers *Xgwm120*, *Xbarc101*, and *Xwmc175* may also be good candidates for MAS if the parents are polymorphic for the marker alleles and one parent is known to contain *Sr9a*.

In the analysis of these accessions, we also tried to postulate the presence of *Sr9a* on the basis of gene-for-gene specificity. There are obvious limitations to this approach of postulating stem rust resistance genes in wheat, and in this study some of the accessions showed very low infection type against all three races (QFCS, TPMK, and MCCF). Hence, we cannot rule out the possibility that some of these accessions may possess the *Sr9a* gene because it could have been masked by other *Sr* genes. Based on these results, further genetic analysis is required to determine if some of the accessions are carriers or noncarriers of *Sr9a*. These include 'Centurk', 'Lerma Rojo 64', 'NC-Neuse', and 'Excel'. The Centurk and Lerma Rojo 64 cultivars were reported as carriers of *Sr9a* together with

other genes, including *Sr2*, *Sr5*, *Sr6*, *Sr7b*, *Sr8a*, and *Sr17* (Roelfs, 1988b; McIntosh et al., 1995); however, these cultivars show a non-*Sr9a*-specific marker allele at the *Xgwm47* locus. Our results showed that 'Vista' has a *Sr9a*-specific marker allele at the *Xgwm47* locus, and this allele might have been inherited from Centurk; however, Centurk did not display the *Sr9a*-linked marker allele (Table 1). One possible explanation is that the Centurk cultivar may be heterogeneous for *Sr9a*.

In summary, although *Sr9a* does not provide a high level of resistance against a wide range of rust races on its own, it can still be a valuable gene when pyramided with other stem rust resistance genes. Moreover, the *Sr9a* gene provides resistance to TPMK, which once was the most common race of stem rust in the USA for many years. The SSR marker *Xgwm47* or other markers identified in this study may be used to pyramid *Sr9a* with other *Sr* genes from different resistance sources into a single line during cultivar development. It may also be possible that the linkage between the *Sr9a* gene and other multiple alleles at the *Sr9* locus can permit an efficient transfer of any one of these alleles based on *Xgwm47*.

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References

Anderson, J.A. 2003. Plant genomics and its impact on wheat breeding. p. 184–215. *In* H.J. Newbury (ed.) Plant molecular breeding. Blackwell Publ., Boca Raton, FL.

Anderson, J.A., G.A. Churchill, J.E. Autrique, S.D. Tanksley, and M.E. Sorrells. 1993. Optimizing parental selection for genetic linkage maps. Genome 36:181–186.

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.

Chen, X., M.A. Soria, G. Yan, J. Sun, and J. Dubcovsky. 2003. Development of sequence tagged site and cleaved amplified polymorphic sequence markers for wheat stripe rust resistance gene *Yr5*. Crop Sci. 43:2058–2064.

Eagles, H., H. Bariana, F. Ogbonnaya, G. Rebetzke, G. Hollamby, R. Henry, P. Henschke, and M. Carter. 2001. Implementation of markers in Australian wheat breeding. Aust. J. Agric. Res. 52:1349–1356.

Hart, G.E., M.D. Gale, and R.A. McIntosh. 1993. Linkage maps of *Triticum aestivum* (hexaploid wheat, $2n = 42$, Genomes A, B, and D) and *T. tauschii* ($2n = 14$, Genome D). p. 204–219. *In* S.J. O'Brien (ed.) Genetic maps. Cold Spring Harbor Lab. Press, Woodbury, NY.

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

Knott, D.R. 1957. The inheritance of rust resistance: II. The inheritance of stem rust resistance in six additional varieties of common wheat. Can. J. Plant Sci. 37:177–192.

Knott, D.R. 1965. A comparison of the reaction to stem rust of wheat lines backcrossed five and nine times to Marquis that carry the same resistance genes. Can. J. Plant Sci. 45:106–107.

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.S., 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.

Loegering, W.Q., and D.L. Harmon. 1969. Wheat lines near-isogenic for reaction to *Puccinia graminis tritici*. Phytopathology 59:456–459.

McIntosh, R.A. 1988. The role of specific genes in breeding for durable stem rust resistance in wheat and triticale. p. 1–9. *In* N.W. Simmonds and S. Rajaram (ed.) Breeding strategies for resistance to the rusts of wheat. CIMMYT, El Batan, Mexico.

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

McIntosh, R.A., Y. Yamazaki, K.M. Devos, J. Dubcovsky, J. Rogers, and R. Appels. 2003. Catalogue of gene symbols. Available at wheat.pw.usda.gov/ggpages/wgc/2003/ (verified 1 Aug. 2007).

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.

Melchinger, A.E. 1990. Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed. 104:1–19.

Mohan, M., S. Nai, A. Bhagwat, T.G. Krishna, M. Yano, C.R. Bhatia, and T. Sasaki. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol. Breed. 3:87–103.

Paterson, A.H., S.D. Tanksley, and M.E. Sorrells. 1991. DNA markers in plant improvement. Adv. Agron. 46:39–90.

Pederson, W.L., and S. Leath. 1988. Pyramiding major genes for resistance to maintain residual effects. Annu. Rev. Phytopathol. 26:369–378.

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. Genetic control of phenotypes in wheat stem rust. Annu. Rev. Phytopathol. 26:351–367.

Roelfs, A.P. 1988b. 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., D.L. Long, and J.J. Roberts. 1993. Races of *Puccinia graminis* in the United States during 1990. Plant Dis. 77:125–128.

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

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Sears, E.R. 1962. The use of telocentrics in linkage mapping. Genetics 47:983.

Sears, E.R. 1966a. 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, UK.

Sears, E.R. 1966b. Chromosome mapping with the aid of telocentrics. p. 370–381. *In* J. Mackey (ed.) Proc. Int. Wheat Genet. Symp., 2nd, Lund, Sweden. 19–24 Aug. 1963. Hereditas Suppl. Vol. 2. Mendelian Soc., Lund.

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

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*. USDA-ARS Bull. E-617. U.S. Gov. Print. Office, Washington, DC.

Yan, G.P., X.M. Chen, R.F. Line, and C.R. Wellings. 2003. Resistance gene-analog polymorphism markers co-segregating with the *Yr5* gene for resistance to wheat stripe rust. Theor. Appl. Genet. 106:636–643.

Young, N.D. 1996. QTL mapping and quantitative disease resistance in plants. Annu. Rev. Phytopathol. 34:479–501.

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