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ORIGINAL PAPER

Identification and validation of SSR markers linked to the stem rust resistance gene *Sr6* on the short arm of chromosome 2D in wheat

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Abstract The wheat stem rust resistance gene *Sr6*, present in several wheat cultivars, confers a high level of resistance against a wide range of races of *Puccinia graminis* f. sp. *tritici*. Resistance conferred by *Sr6* is influenced by temperature, light intensity, and genetic background of the recipient genotype. Here, we report the identification and validation of molecular markers linked to *Sr6* that can be used for the detection of this gene in wheat breeding programs. A mapping population of 136 F_2 plants and their $F_{2:3}$ families derived from a cross between near-isogenic lines, 'Chinese Spring' and ISr6-Ra, were screened for stem rust reaction in the seedling stage. Bulked segregant analysis (BSA) based on seedling tests was used to screen 418 SSR markers that covered the entire genome of wheat. Four markers, *Xwmc453*, *Xcfd43*, *Xcfd77*, and *Xgwm484*, were mapped within a chromosome region that spanned 9.7 cM from *Sr6*. The closest markers, *Xwmc453* and *Xcfd43,* were linked to *Sr6* at a distance of 1.1 and 1.5 cM, respectively. The markers *Xwmc453* and *Xcfd43* amplified *Sr6*-specific marker alleles that were diagnostic for *Sr6* in a diverse set of 46 wheat accessions and breeding lines developed and/or collected in Australia, Canada, China, Egypt, Ethiopia, Kenya, Mexico, South Africa, and USA. These markers can now be used for marker-assisted selection of *Sr6* and for pyramiding it with other stem rust resistance genes.

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Introduction

Stem rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn., historically has been one of the most significant diseases of wheat worldwide (Leonard 2001). The most effective approach to control this disease is to develop varieties carrying more than one resistance gene, thereby providing prolonged resistance against several races (Pederson and Leath 1988). To date, a number of stem rust (*Sr*) resistance genes have been identified and assigned to specific chromosomes (McIntosh et al. 2003). However, pyramiding these genes through traditional phenotypic-screening methods is difficult and imprecise, especially when more than one gene is effective against many races of the pathogen or when different resistance genes produce similar infection types (ITs). This difficulty has been a limiting factor in many wheat breeding programs, and may have contributed to the deployment of only a few stem rust resistance genes that were thought to be durable (Knott 1989).

Stem rust resistance in several American and Australian cultivars is conferred by a combination of *Sr* genes including *Sr6*, a race-specific resistance gene that confers resistance against a wide range of races (Roelfs 1988a; Knott

1989; McIntosh et al. 1995). In North America, *Sr6* has been one of the most important resistance genes because of its high level of resistance against stem rust race TPMK (or race 15B) that caused severe losses in 1953–1954 (Leonard 2001). This gene also confers resistance to other races, including QFCS, MCCF, and QCCJ, which have been the predominant races in the United States based on the recent rust surveys (McVey et al. 2002; Jin 2005). 'Red Egyptian,' the original source of *Sr6*, *Sr8a*, and *Sr9a* (Knott 1957), and its derivatives were used widely as parents in wheat breeding programs (Knott 1989; McIntosh et al. 1995). However, selecting lines carrying *Sr6* in the presence of other genes is difficult. The *Sr6* gene is temperature-sensitive and, to some extent, its resistance is poorly expressed under high light intensity (Forsyth 1956; Luig and Rajaram 1972). To some races of stem rust, the timing and expression of *Sr6* resistance are influenced by genetic background of the recipient genotype (Knott 1981, 2001). Knott (2001) explained anomalous observations suggesting that the inheritance and expression of resistance at this locus are complex. The locus was mapped to the short arm of chromosome 2D (McIntosh and Baker 1968); however, no closely linked molecular markers have been reported so far.

The usage of molecular markers linked to disease resistance genes has made it possible to diagnose, pyramid, and facilitate the development of new resistant varieties through marker-assisted selection (MAS). The benefits of MAS and/or pyramiding of disease resistance genes have been discussed widely (Melchinger 1990; Mohan et al. 1997; Anderson 2003). Currently, diagnostic markers have been reported for several other stem rust resistance genes, including *Sr2* (Hayden et al. 2004), *Sr9a* (Tsilo et al. 2007), *Sr24* and *Sr26* (Mago et al. 2005), *Sr31* (Das et al. 2007), *Sr36* (Tsilo et al. 2008), and *Sr38* (Helguera et al. 2003).

In this paper, we report the identification of *Sr6*-linked microsatellite markers and describe their usefulness in detecting *Sr6* across a diverse range of wheat germplasm developed by different wheat breeding programs.

Materials and methods

Plant material

A population consisting of 136 F_2 plants and their $F_{2:3}$ families was generated from one F_1 plant derived from a cross between a susceptible wheat cultivar 'Chinese Spring' (CI 14164) and its near-isogenic line ISr6-Ra (CItr 14163) carrying *Sr6*. ISr6-Ra was derived from a series of backcrosses (BC_3F_{11}) involving Chinese Spring and the original source of *Sr6*, 'Red Egyptian' (Loegering and Harmon 1969). In addition to ISr6-Ra and Chinese Spring, a set of 25 international wheat accessions and breeding lines was obtained from the USDA-ARS National Small Grains Collection (NSGC), Aberdeen, ID to determine the usefulness of the *Sr6*-linked markers (Table 1). The information on the presence of *Sr6* in these accessions and breeding lines was obtained from the GrainGenes website (http://wheat.pw.usda.gov/GG2/index.shtml), Roelfs (1988a), Knott (1989), and McIntosh et al. (1995). The list included two sets of near-isogenic lines carrying *Sr6*, Sr6/8*LMPG (Knott 1990) and 'Kenya 58'/10*'Marquis' (Green et al. 1960), and their recurrent susceptible parents, LMPG-6 and Marquis (Table 1). A wheat line designated Sr6 (CItr 15082) derived from Kenya 58/10*Marquis//9*LMPG-1 was also included. Five accessions of Red Egyptian, one of which must be the original source of *Sr6*, *Sr8a*, and *Sr9a*, were found in the NSGC database and all of them were also included in the analyses (Table 1). The *Sr6*-carrying wheat cultivars developed in different countries included 'McMurachy,' 'Gamut,' 'Songlen,' 'Gatcher,' 'Shortim,' 'Centurk,' 'Kenya Plume,' 'Lerma Rojo 64,' and Kenya 58, as listed by Roelfs (1988a), Knott (1989), and McIntosh et al. (1995). Non-*Sr6*-carrying wheat accessions included were 'Brundage 96,' 'Chukar,' 'Truman,' 'Zaragoza 75,' 'Timvera,' and 'Halt.' These non-*Sr6*-carrying cultivars were selected based on their susceptibility to at least one of the two *Sr6*-avirulent races QFCS and TPMK in tests by Tsilo et al. (2008).

To determine the usefulness of *Sr6*-linked marker alleles in wheat breeding programs, an additional set of 21 non-*Sr6* wheat cultivars and breeding lines of different classes of wheat obtained from different US wheat breeding programs, as part of the US Wheat Coordinated Agricultural Project (Wheat CAP) (http://maswheat.ucdavis.edu), was included in this study (Table 2). The absence of *Sr6* in these cultivars and lines was based on their susceptibility to at least one of the two *Sr6*-avirulent races, QFCS and TPMK, based on ITs cited from Wheat CAP website.

Stem rust screening

Seedlings of the mapping population (the parents and F_2 progeny) were evaluated for stem rust disease reaction against the stem rust race QFCS (Isolate 03ND76C) (avirulence/virulence formula *Sr6*, *7b*, *9b*, *9e*, *11*, *30*, *36*, *Tmp*/*Sr5*, *8a*, *9a*, *9d*, *9g*, *10, 17*, *21*) at the USDA-ARS Cereal Disease Laboratory, Saint Paul, MN, in 2004. A detailed inoculation protocol was described by Jin (2005). Because *Sr6* is temperature-sensitive and its resistance is rendered ineffective at high temperatures, plants were placed in a growth chamber operated at 19°C for 16 h light and 18°C for 8 h of darkness. About 14 days after inoculation, stem rust ITs were scored based on the scale of 0–4, as described by Stakman et al. (1962) and modified by Roelfs (1988b). The low ITs of 0, 0;, 1, and 2, or combinations thereof,

Table 1 Evaluation of *Sr6*-linked microsatellite markers using conventional screening methods and PCR-based SSR markers in a wide range of international germplasm

Name	Accession Number	Origin	*P. graminis* race		*Sr6*[b]	SSR marker[c]	
			QFCS	TPMK		WMC453 (131 bp)	CFD43 (215 bp)
ISr6-Ra	CI 14163	USA	0;[a]	0	+	+	+
'Chinese Spring'	CI 14108	China	4	4	–	–	–
Sr6/8*LMPG		Canada	;	0	+	+	+
LMPG-6		Canada	4	4	–	–	–
'Kenya 58'/10*'Marquis'		Canada	0	;	+	+	+
Marquis	CI 3641	Canada	2/;[d]	4	±	±	±
Sr6	CI 15082	Canada	;	0	+	+	+
'Red Egyptian'	PI 45374	South Africa	0;	0;	+	+	+
Red Egyptian	CI 12345	Egypt	u	u	+	+	+
Red Egyptian	PI 45403	South Africa	2	2+	–	–	–
Red Egyptian	PI 45415	South Africa	;	0;	+	+	+
Red Egyptian	PI 192020	Ethiopia	;	0;	+	+	+
'McMurachy'	PI 122985	Canada	;	;	+	+	+
'Gamut'	PI 329230	Australia	;	;	+	+	+
'Songlen'	PI 404114	Australia	0	;	+	+	+
'Gatcher'	PI 377884	Australia	;	;	+	+	+
'Shortim'	PI 422407	Australia	0	0	+	+	+
'Centurk'	CI 15075	USA	0;	;	+	+	+
'Kenya Plume'	CI 14335	Kenya	0	0	+	+	+
'Lerma Rojo 64'	CI 13929	Mexico	;	;	+	+	+
Kenya 58	CI 12471	Kenya	0	0/4	±	±	±
'Brundage 96'	PI 631486	USA	4	4	–	–	–
'Chukar'	PI 628641	USA	4	4	–	–	–
'Truman'	PI 634824	USA	4	4	–	–	–
'Zaragoza 75'	PI 519305	Mexico	4	3+	–	–	–
'Timvera'	PI 351987	Australia	0	4	–	–	–
'Halt'	PI 584505	USA	1;	3+	–	–	–

[a] Infection types as described by Stakman et al. (1962) and modified by Roelfs (1988b); *u* no data

[b] The presence of *Sr6* was based on previously published reports and verified using two *Sr6*-avirulent races (QFCS and TPMK). Plus denotes presence and minus denotes absence of *Sr6*; ± denotes heterogeneity

[c] For SSR markers, plus denotes presence and minus denotes absence of the *Sr6*-linked marker allele; ± denotes heterogeneity

[d] Denotes the accession is heterogeneous, the predominant type given first

were considered resistant, whereas high IT 3+, and 4 were considered susceptible. After rust screening, individual F_2 seedlings were transplanted into pots and grown to maturity in a greenhouse. The second screening was conducted on the $F_{2:3}$ families in order to distinguish heterozygous from homozygous F_2 plants. Screening was done with about 16–30 seedlings per family. Segregation ratios of resistant to susceptible plants were analyzed using the Chi-square test (χ^2). The previously reported presence of *Sr6* in selected international wheat accessions and breeding lines was verified using the two *Sr6*-avirulent races QFCS and TPMK (Table 1). The condition for verifying *Sr6* presence was low infection type response (IT 0 for immunity or hypersensitive fleck, IT ;) against QFCS and TPMK (Table 1). The absence of *Sr6* in the US wheat cultivars and breeding lines was based on the absence of typical low ITs (0 to ;) to these races. For all the races used in this study, the avirulence/virulence formula was verified using 16 stem rust differential lines (Roelfs and Martens 1988; Roelfs et al. 1993).

Molecular mapping of *Sr6*

Leaf tissue harvested from young plant material (parents, 136 F_2 plants, and wheat accessions and breeding lines) was frozen in liquid nitrogen. Genomic DNA was extracted following the protocol described by Riede and Anderson (1996) and modified by Liu et al. (2006). In order to rapidly identify markers linked to *Sr6*, the DNA samples of the F_2 plants were bulked into two contrasting pools using 10 homozygous resistant and 10 susceptible lines. Parental DNA and bulked segregant analysis (BSA), as described by Michelmore et al. (1991), were used to screen for polymorphism with 418 SSR markers (GWM, GDM, BARC, CFA, CFD, WMC, and GPW) that covered the entire genome of wheat (Röder et al. 1998; Pestsova et al. 2000; Gupta et al.

Table 2 *Sr6*-specific marker alleles at the *Xwmc453* and *Xcfd43* loci of 21 non-*Sr6* carrying cultivars and breeding lines from different US wheat breeding programs

Name[a]	Market Class	*P. graminis* race		*Sr6*[c]	SSR marker[d]	
		QFCS	TPMK		WMC453 (131 bp)	CFD43 (215 bp)
'Thatcher'	HRS	;12+[b]	4	–	–	–
RSI5	HRS	;	4	–	–	–
'TAM 105'	HRW	2-	2	–	–	–
'Jagger'	HRW	;11+	3	–	–	–
2174	HRW	;	4	–	–	–
'Kanqueen'	HRW	2++	4	–	–	–
CO940610	HWW	4/3 N	4	–	–	–
'Clark's Cream'	HWW	2+	4	–	–	–
IDO556	SWS-club	2+	4	–	–	–
'Penawawa'	SWS	3;	4	–	–	–
'Stephens'	SWW	3	4	–	–	–
'Finch'	SWW	4	4	–	–	–
'Eltan'	SWW	4	4	–	–	–
NY18/Clark's Cream 40-1	SWW	2	3	–	–	–
'Cayuga'	SWW	2+	33+	–	–	–
'Caledonia'	SWW	2++	3	–	–	–
26R46	SRW	4	4	–	–	–
P91193	SRW	0	3;C	–	–	–
P92201	SRW	4	4	–	–	–
25R26	SRW	4	4	–	–	–
'McCormick'	SRW	2	2	–	–	–

HRS hard red spring, *HRW* hard red winter, *HWW* hard white winter, *SWS* soft white spring, *SWW* soft white winter, *SRW* soft red winter

[a] The US wheat cultivars and breeding lines obtained as part of the US Wheat Coordinated Agricultural Project

[b] Infection types as described by Stakman et al. (1962) and modified by Roelfs (1988b)

[c] The absence of *Sr6* (–) on cultivars and lines from the US Wheat Coordinated Agricultural Project was based on susceptibility to either QFCS or TPMK. TAM105 and McCormick showed infection type (IT 2) against QFCS, non-*Sr6* infection type for QFCS

[d] For SSR markers, minus indicates the absence of the *Sr6*-specific marker alleles

2002; Guyomarc'h et al. 2002; Somers et al. 2004; Sourdille et al. 2004; Song et al. 2005; http://wheat.pw.usda.gov/ggpages/SSR/WMC). Of 418 SSR primer pairs, 44 were mapped previously on chromosome 2DS. The reason we used a whole genome screening approach is that in 2004 not all markers on 2DS were available, and we could not resolve the location of *Sr6* on 2DS. PCR was performed with 10 µL per reaction containing 1X PCR buffer (New England Biolabs, Inc. Beverly, MA), 0.125 mM dNTPs, 0.4 pmol forward primer, 0.3 pmol reverse primer, 3.0 pmol of M13 primer labeled with one of the four fluorescent dyes (6-FAM, VIC, NED, and PET), 0.05 units/µL Taq DNA polymerase (NEB), and ~75 ng genomic DNA. The PCR reaction mixture was initially denatured at 94°C for 10 min, followed by 40 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min, with a final extension step of 72°C for 5 min and 4°C indefinitely. The PCR thermal cycling was performed using the GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA). The PCR products amplified with four different dyes (6-FAM, VIC, NED, and PET) were multiplexed to a final volume of 10 µL, including 0.14 µL GeneScan-500 LIZ® size standard (Applied Biosystems) and 6.86 µL Hi-Di™ Formamide (Applied Biosystems). Mixed PCR products were denatured at 94°C for 5 min and chilled on ice. PCR products were separated by capillary electrophoresis using the ABI 3130xl Genetic Analyzer (Applied Biosystems). Separated SSR-amplified fragments were analyzed using GeneMapper software v3.7 (Applied Biosystems) as described in the user manuals.

Linkage analysis

Genetic linkage analysis between the SSR maker loci and *Sr6* was conducted using MAPMAKER/EXP version 3.0b with LOD value of 3.0 and a maximum genetic distance of

Table 3 Segregation of alleles at the *Sr6* and microsatellite marker loci in an F_2 population derived from a cross between Chinese Spring and ISr9a-Ra

Gene/marker	Observed[a]			Total	χ^{2b}	*P* value
	A_1A_1	A_1A_2	A_2A_2			
Sr6	34	64	36	134	0.33	0.848
Xwmc453	33	64	36	133	0.32	0.851
Xcfd43	31	66	36	133	0.38	0.826
Xcfd77	31	68	34	133	0.20	0.904
Xgwm484	30	72	31	133	0.93	0.630

[a] A_1A_1 refers to homozygous for resistant parent's allele, A_1A_2 for heterozygous, A_2A_2 homozygous for susceptible parent's allele. The F_2 genotypes were inferred from the seedling reaction tests of $F_{2:3}$ families against stem rust race QFCS to distinguish A_1A_1 and A_2A_2 from A_1A_2

[b] χ^2 values based on the expected Mendelian segregation of 1:2:1 with 2 degrees of freedom

50 cM (Lander et al. 1987). The Kosambi mapping function was used to convert recombination frequencies into genetic distances (Kosambi 1944).

Results

Segregation and linkage analyses

The stem rust resistant parent ISr6-Ra was highly resistant, displaying a fleck (;) infection type, whereas the susceptible parent 'Chinese Spring' produced a susceptible infection type of 4, against the stem rust race QFCS. In the F_2 generation, 100 plants were resistant and 36 susceptible, consistent with the 3:1 ratio expected for a single gene (*Sr6*) with a dominant resistance ($\chi^2 = 0.09$, $df = 1$, $P = 0.764$) conferring resistance against QFCS. Thirty-four out of 136 F_2 lines were considered homozygous resistant, 64 were segregating and 36 were homozygous susceptible. This segregation was consistent with a 1:2:1 ratio ($\chi^2 = 0.33$, $df = 2$, $P = 0.849$) (Table 3); however, the 64 segregating $F_{2:3}$ families displayed a wide range of segregation ratios with 15 families showing more susceptible than resistant plants.

BSA of 418 SSR markers led to the identification of two SSR markers (*Xwmc453* and *Xcfd43*) that co-segregated with the bulks and were linked to *Sr6* within a genetic distance of 1.5 cM (Fig. 1). The closest marker *Xwmc453* was linked to *Sr6* at a distance of 1.1 cM (Fig. 1). The two markers *Xcfd77* and *Xgwm484* were mapped proximal to *Sr6* at distances of 2.6 and 9.7 cM, respectively (Fig. 1). No segregation distortion was observed for any of the markers (Table 3). The four microsatellite markers *Xwmc453*, *Xcfd43*, *Xcfd77* and *Xgwm484* amplified marker alleles with fragment sizes of 131, 215, 221 and 164 bp, respectively, on the resistant parent (ISr6-Ra) (all fragment sizes included the additional 20 bp due to the M13 tail) (Fig. 2). All markers were co-dominant, allowing homozygote and heterozygote genotypes to be distinguished easily. Marker CFD77 generated an unstable and variable amplification of the *Sr6*-linked allele (221 bp) and was repeated several times with the same PCR conditions until the expected fragment was amplified.

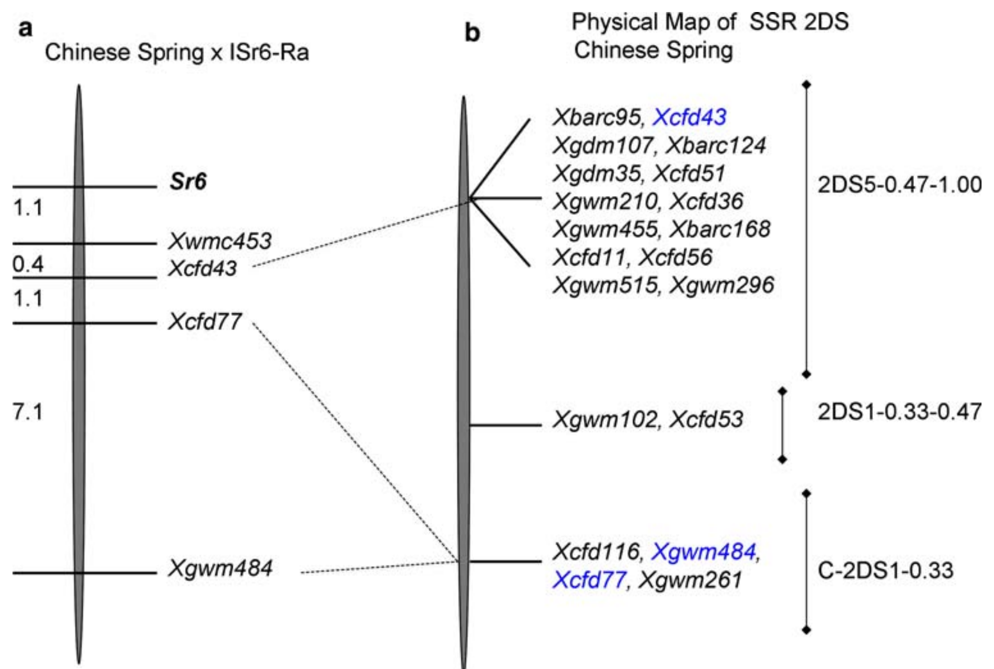

Fig. 1 Alignment of genetic and physical location of *Sr6*-linked SSR markers on chromosome 2DS. **a** Partial genetic linkage map depicting the location of *Sr6* with linked co-dominant SSR loci. The genetic linkage map was constructed using map distances (cM) from Kosambi. **b** The physical map on the right was reproduced from Sourdille et al. (2004) and shows the physical location of all SSR marker loci mapped on deletion breakpoints. Deletion bin breakpoints are indicated on the *right*

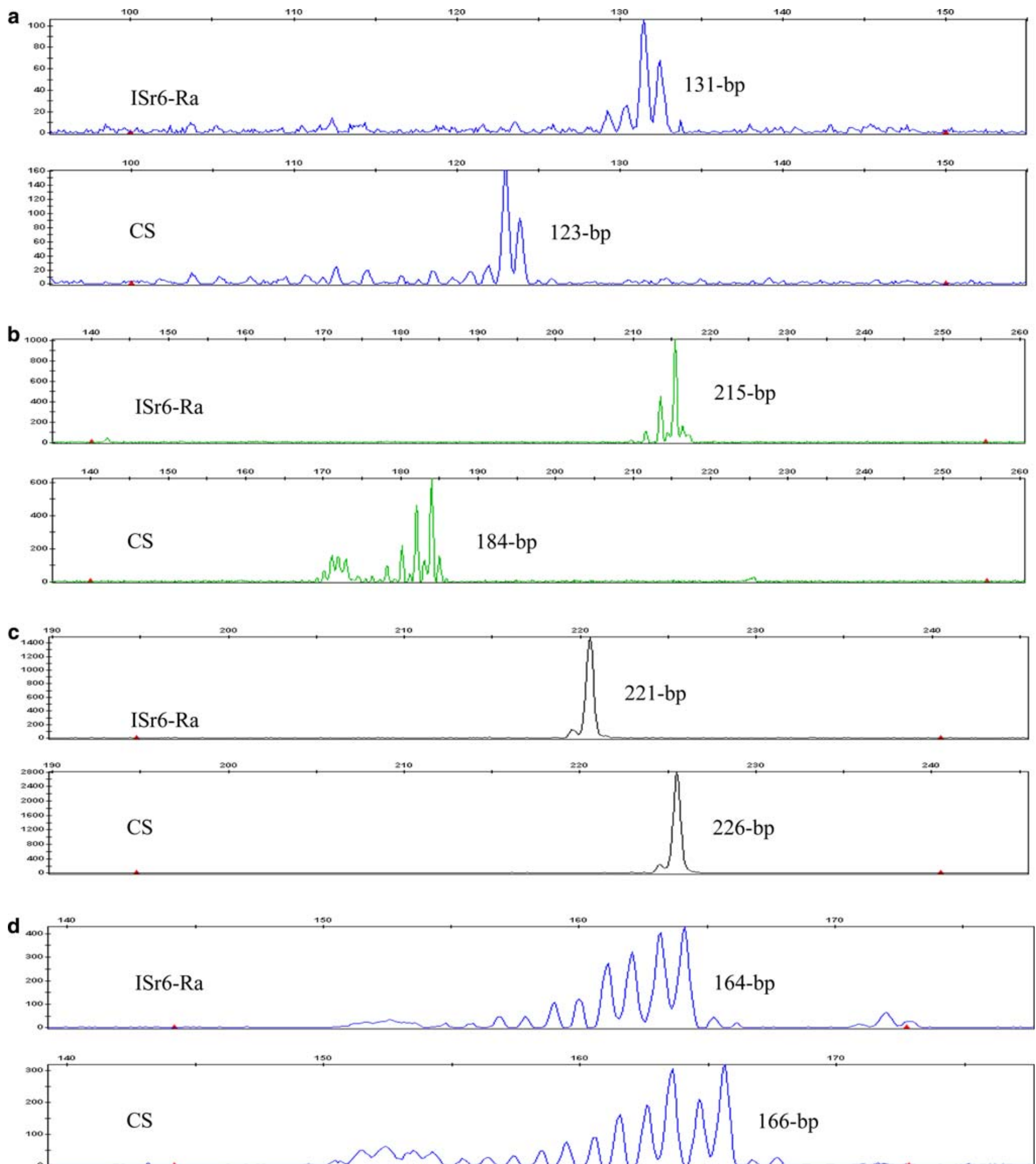

Fig. 2 Electropherograms showing the amplified fragment sizes of the SSR markers on ISr6-Ra (resistant parent) and Chinese Spring (susceptible parent) obtained using GeneMapper software version 3.7. Fragment sizes include a 20 bp M13 tail. The peak represents an amplified fragment, whereas horizontal and vertical scales represent fragment sizes in base pair (bp) and signal intensity, respectively. **a** WMC453, **b** CFD43, **c** CFD77, and **d** GWM484

Validation of SSR markers linked to *Sr6*

In order to determine the diagnostic value of *Sr6*-linked microsatellite markers for the purpose of MAS or pyramiding, a set of 25 international wheat accessions and breeding lines were screened with four SSR markers. Accessions and lines expected to carry *Sr6* showed a high level of resistance to *P. graminis* f. sp. *tritici* from the growth chamber

tests in spring 2007 (Table 1). In wheat accessions and breeding lines, the two *Sr6*-linked markers, *Xcfd77* and *Xgwm484*, amplified marker alleles of different fragment sizes that were not diagnostic of *Sr6* (data not shown). Marker CFD77 was not stable due to a problem of amplification and had to be repeated as described above. However, the *Sr6*-linked marker alleles at the *Xwmc453* and *Xcfd43* loci amplified fragment sizes of 131 and 215 bp, respectively, and were associated with the presence of *Sr6* in all accessions and breeding lines that were known to carry *Sr6*, including Sr6/8*LMPG, 'Kenya 58'/10*'Marquis,' Sr6 (CItr 15082), 'McMurachy,' 'Gamut,' 'Songlen,' 'Gatcher,' 'Shortim,' 'Centurk,' 'Kenya Plume,' 'Lerma Rojo 64,' and Kenya 58 (Table 1). Out of five accessions of Red Egyptian found in the NSGC database, only PI 45403 showed a moderate resistance (IT 2) to QFSC and IT 2+ to race TPMK, indicating that this accession did not carry *Sr6*. This concurred with the absence of the *Sr6*-associated marker alleles at the *Xwmc453* and *Xcfd43* loci (Table 1). All of the wheat accessions and lines known not to carry *Sr6* showed complete susceptibility (IT 3+, 4) against at least one of the two *Sr6*-avirulent races, and none of them displayed the *Xwmc453* and *Xcfd43* marker alleles associated with *Sr6* (Table 1). These results indicate that these two markers identified *Sr6* correctly and were in agreement with the rust screening results. Kenya 58, a known carrier of *Sr6*, showed both types of plants with IT 0 (immunity) and IT 4 (susceptibility) to QFCS, indicating that this cultivar was heterogeneous for *Sr6* and this was in agreement with the *Sr6*-linked marker alleles. Heterogeneity was also observed in Marquis, a non-carrier of *Sr6*, by showing both hypersensitive fleck (;) and IT 2 against QFCS and this was in agreement with the *Sr6*-linked marker alleles. A possible explanation of this would be that the seed source obtained from NSGC was heterogeneous.

Twenty-one non-*Sr6* US wheat cultivars and breeding lines lacked *Sr6*-linked marker alleles at the *Xwmc453* and *Xcfd43* loci (Table 2), further indicating that the *Sr6*-linked marker alleles are specific to *Sr6*.

Discussion

The complexity of *Sr6* resistance

More than 45 stem rust resistance (*Sr*) genes have been reported in wheat (McIntosh et al. 2003). Most of these genes are dominant; however, this is not the case with *Sr6*. When screening 136 $F_{2:3}$ families, 64 heterozygous $F_{2:3}$ families displayed a wide range of segregation ratios with 15 families showing more susceptible than resistant plants. Although the family sizes (16–30 plants) were small in this study, the observation of families showing more susceptible than resistant plants was consistent with the report by Knott (2001). He reported that the dominant inheritance of *Sr6* was reversed to recessive inheritance in some families. Based on the results of Loegering (1966), Browder (1985), and Luig and Rajaram (1972), Roelfs (1988b) suggested that temperature, and perhaps light, might cause the reversal of dominance, thereby causing the heterozygous genotypes to be more susceptible than homozygous resistant genotypes. However, because both temperature and light were controlled in our experiments, the reversal of dominance observed was not caused by temperature and light; hence, the exact cause of the reversed dominance is unknown. Knott (2001) proposed several hypotheses to describe this uncommon phenomenon. He mentioned transposons and paramutations that could explain an excess of susceptible plants in some families.

'Red Egyptian,' the original source of *Sr6*, *Sr8a*, and *Sr9a* (Knott 1957), was used worldwide in wheat breeding programs (Knott 1989; McIntosh et al. 1995). In North America, 'McMurachy,' which inherited *Sr6* from Red Egyptian, was the main contributor for *Sr6* in 'Selkirk,' the main cultivar that overcame the race 15B epidemics in the mid 1950s. The presence of *Sr6* makes it difficult to detect, select and/or transfer the *Sr8a* and *Sr9a* genes into adapted cultivars. This difficult situation was cited by Roelfs (1988b) as an example of how other host genes could impact the detection of some genes.

Molecular mapping of *Sr6*

Conflicting results have been reported about the linkage between *Sr6* and the purple leaf base phenotype conferred by the red auricles (*Ra1*) gene (Knott and Zeven 1987; Gulyaeva 1984; Hart et al. 1993). Zeven (1985) indicated that appearance of red auricles is unstable and can not be relied upon as a morphological marker to diagnose *Sr6*. Consistent with this, we noted that Sr6 (CItr 15082) has red auricles even though neither of its parents ('Kenya 58'/10*'Marquis' and LMPG-1) are expected to have red auricles (Knott and Zeven 1987). We assume that Sr6 (CItr 15082) inherited both *Ra1* and *Sr6* from Kenya 58/10*Marquis.

Genetic linkage analysis in our study positioned *Sr6* on chromosome 2DS. This map location is consistent with the previously reported position of *Sr6* based on monosomic analyses (Sears 1954; Wiggin 1955; Sears et al. 1957). The *Sr6* locus and two other leaf rust resistance genes, *Lr2a* and *Lr15*, were cytologically mapped on chromosome 2DS with the recombination frequency of 27.9% from the centromere (McIntosh and Baker 1968). Recombination values between *Sr6* and the centromere varied from 18.1 to 33.1% (Gfeller and Whiteside 1961; McIntosh and Baker 1968). To our knowledge, none of these genes have been mapped

using DNA-based markers. The map position of *Sr6* also gives the approximate locations of *Lr2* and *Lr15* loci.

Four molecular markers linked to *Sr6* were identified in this study. The use of both BSA and near-isogenic lines facilitated the identification of two diagnostic *Sr6*-linked markers, *Xwmc453* and *Xcfd43*. A partial genetic map comprised of *Sr6*, *Xwmc453*, *Xcfd43*, *Xcfd77*, and *Xgwm484* spanned a distance of 9.7 cM. Based on previous reports of Guyomarc'h et al. (2002) and Somers et al. (2004), all four marker loci were mapped previously on 2DS, and their order in this study was consistent with the physical map of Sourdille et al. (2004) (Fig. 1) and genetic maps of Somers et al. (2004) and Tsilo and Anderson (unpublished map). However, there are differences between these genetic maps in marker intervals. While Somers et al. (2004) reported no recombination between *Xwmc453* and *Xcfd43*, we observed one recombinant in our mapping population. Based on the physical map (Sourdille et al. 2004), the two marker loci *Xcfd77* and *Xgwm484* were located on deletion bin 2DS1-0.33 which is far apart from 2DS5-0.47-1.00, the location of *Xcfd43*.

Validation of *Sr6*-linked SSR markers

For the purpose of MAS of *Sr6* and pyramiding with other *Sr* genes, there is a need to identify breeder-friendly markers that are both linked to resistance genes and useful for breeding. For this purpose, our results showed that all selected wheat accessions and breeding lines carrying *Sr6*, as listed by Roelfs (1988a), Knott (1989), and McIntosh et al. (1995), were resistant to the *Sr6*-avirulent races and amplified the *Sr6*-linked marker alleles at the *Xwmc453* and *Xcfd43* loci (Table 1), indicating that the marker allele sizes of 131 bp for *Xwmc453* and 215 p for *Xcfd43* were diagnostic of *Sr6* in all *Sr6*-carrying wheat accessions and breeding lines tested in this study. This indicates that all of these wheat accessions and breeding lines inherited the *Sr6*-linked marker alleles along with *Sr6* from Red Egyptian, the original source of *Sr6*, *Sr8a*, and *Sr9a*. Of the 5 accessions of Red Egyptian tested, only one (PI 45403) did not have *Sr9a* (Tsilo et al. 2007) or *Sr6* (this study).

Both *Xwmc453* and *Xcfd43* were diagnostic of *Sr6* across a wide range of germplasm developed in different countries. The most distant markers, *Xcfd77* and *Xgwm484*, were not diagnostic. The *Sr6*-linked marker alleles should be useful for MAS and pyramiding *Sr6*. Given the diagnostic nature of the *Sr6*-linked marker alleles, we further determined the usefulness of these markers in different breeding programs. To address this, 21 non-*Sr6* wheat cultivars and breeding lines taken from different breeding programs across the US, as part of the US Wheat Coordinated Agricultural Project, demonstrated that the marker alleles at the *Xwmc453* and *Xcfd43* loci are highly specific to *Sr6* and are not present in germplasm that lacks *Sr6*. Therefore, these markers are useful for detecting *Sr6* in wheat breeding programs. In any given cross that is polymorphic for *Sr6*, these markers should also be polymorphic. Based on the data presented, these markers will be applicable across different genetic backgrounds of wheat.

In the United States, *Sr6*-virulent races exist; however, they have not been predominant. All predominant races of stem rust (TPMK, QFCS, MCCF, and QCCJ), based on recent rust surveys (McVey et al. 2002; Jin 2005), are avirulent on *Sr6*. However, lines carrying *Sr6* alone are susceptible to race TTKS (or Ug99) (Jin et al. 2007), a recently emerged race of stem rust that poses a threat to wheat production worldwide (Singh et al. 2006). Therefore, *Sr6*-specific marker alleles identified in this study will be useful in pyramiding *Sr6* with other *Sr* genes, especially those that are resistant to race TTKS and its derivatives. For the purpose of high-throughput MAS and pyramiding, it is important to mention that any change in PCR annealing temperature might lead to preferential amplification of multiple alleles other than *Sr6*-specific alleles. These markers are co-dominant and easy to score when amplified under appropriate annealing temperatures and PCR conditions. Marker information can be obtained from the GrainGenes website (http://wheat.pw.usda.gov/GG2/index.shtml) and Wheat CAP website (http://maswheat.ucdavis.edu/index.htm).

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