NOTE

Identification of Flanking Markers for the Stem Rust Resistance Gene *Sr6* in Wheat

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ABSTRACT

The wheat (*Triticum aestivum* L. and *T. turgidum* L.) stem rust resistance gene *Sr6* confers a high level of resistance against a wide range of races of *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. and E. Henn. in North America. In this study, we report the identification of flanking markers for *Sr6*. We used a population of 139 recombinant inbred lines from the cross MN99394 × MN98550. A partial linkage map of chromosome 2D comprised *Xgwm484*, *Xcfd77*, *Xcfd43*, *Xwmc453*, *Sr6*, *XwPt_4381*, *XwPt_0330*, *Xgpw94049*, and *Xgwm102.* Four markers mapped distal to *Sr6*, while the other four were proximal within distances of 4.8 and 6.5 cM, respectively. Marker-assisted selection and pyramiding of *Sr6* with other stem rust resistance genes could benefit from the availability of *Sr6*-flanking markers.

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Abbreviations: DArT, diversity arrays technology; IT, infection type; RIL, recombinant inbred line; SSR, simple sequence repeat.

Early in the 20th century, wheat stem rust (caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. and E. Henn.) was one of the most devastating diseases of wheat (*Triticum aestivum* L.) in North America, sometimes causing yield losses reaching 50 to 70% over large areas (Roelfs, 1982; Knott, 1989). In the United States, the last major epidemics that occurred in the northern Great Plains in 1953 and 1954 were caused by race 15B (Stakman, 1954; Roelfs, 1978) and resulted in a yield loss of 140 million bushels (3.8 million Mg) of wheat. Since the mid-1950s, stem rust has been effectively managed in North America through the deployment of several resistance genes (Knott, 1989), with the estimated percentage yield loss during the period of 2000 to 2006 averaging less than 1% in the United States (http://www.ars.usda.gov/mwa/cdl). The stem rust resistant cultivar Selkirk carrying *Sr6* and grown over millions of acres was one of the main factors for defeating race 15B epidemics in the 1950s (Kolmer, 2001). Since that time, *Sr6* has been one of the most important resistance genes because it confers a high level of resistance against most stem rust races in North America (Leonard, 2001). This gene is widely distributed in northern spring wheat as well as winter wheat in the central and northern Great Plains (Roelfs, 1988a; Knott, 1989).

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The *Sr6* gene was mapped on the short arm of chromosome 2D distal to the *Xwmc453* locus at a distance of 1.1 cM in a population of Chinese Spring near isogenic lines (Tsilo et al., 2009). Two simple sequence repeat (SSR) DNA markers, *Xwmc453* and *Xcfd43*, that were linked to *Sr6* were diagnostic for *Sr6* in a diverse set of 46 wheat accessions and breeding lines collected from nine countries (Tsilo et al., 2009). None of 21 non-*Sr6* wheat cultivars and breeding lines taken from different breeding programs in the United States, as part of the Wheat Coordinated Agricultural Project, amplified *Sr6*-associated marker alleles at the *Xwmc453* and *Xcfd43* loci (Tsilo et al., 2009). The success in marker-assisted selection and/or pyramiding of *Sr6* with other stem rust resistance genes could increase with the availability of additional markers flanking the gene. In this paper, we report several DNA markers that were located distal to the *Sr6* locus in a different population of recombinant inbred lines (RILs). The availability of tightly linked DNA markers that flank *Sr6* will increase the success of marker-assisted selection and pyramiding of this gene.

MATERIALS AND METHODS

Plant Materials

A mapping population of 139 RILs, $F_{6:8}$, was generated from a single F_1 plant derived from a cross between the two University of Minnesota breeding lines MN98550 and MN99394 (Tsilo et al., 2010). The primary purpose of creating this RIL population was to map quantitative trait loci for end-use quality traits. However, there were several reasons for using this population in this study. First, the parents differed in their resistance to stem rust races and resistance was poorly expressed at a higher temperature, which is typical of *Sr6* resistance (Luig and Rajaram, 1972); second, the population amplified *Sr6*-associated marker alleles at the *Xwmc453* and *Xcfd43* loci. With this information, we then decided to pursue further research on *Sr6* and to find markers that could give a precise location of *Sr6* in a different population and also to find markers distal to *Sr6*.

Stem Rust Screening

Stem rust screening was performed on seedlings of the mapping population (the parents and 139 RILs) by inoculating 7-d-old seedlings with the stem rust race QFCS (isolate 03ND76C) (avirulence/virulence formula *Sr6*, *7b*, *9b*, *9e*, *11*, *30*, *36, Tmp/Sr5*, *8a, 9a, 9d, 9 g*, *10*, *17*, *21*) at the USDA-ARS Cereal Disease Laboratory, Saint Paul, MN, in 2008. Thirty seedlings per RIL were used for inoculation following the protocol described by Jin (2005). Inoculated plants were placed in a growth chamber operated at 19°C with 16 h light and 18°C with 8 h of darkness. At 14 d post inoculation, seedlings were scored for stem rust infection types (ITs) using the 0 to 4 scale described by Stakman et al. (1962) and modified by Roelfs (1988b). The low ITs of 0, 0;, 1, and 2, were considered resistant, while high ITs of 3 and 4 were considered susceptible. The chi-square test (χ^2) was used to test the segregation ratios of resistance and susceptible lines. Sixteen stem rust differential lines carrying individual stem rust resistance genes, identified by Roelfs and Martens (1988) and Roelfs et al. (1993), were used as checks to verify the identity of the race used in this study based on the avirulence/virulence formula.

DNA Extraction and Marker Analysis

Genomic DNA was isolated from the youngest leaves of 20 to 30 plants per RIL and parents. Leaf tissue was frozen in liquid nitrogen, ground to a powder, and stored at −80°C. The DNA extraction protocol was previously described by Riede and Anderson (1996) and modified by Liu et al. (2006). The polymerase chain reaction protocol for SSR markers was described by Tsilo et al. (2009). Detection of SSR markers was conducted using a capillary electrophoresis system of ABI 3130x1 Genetic Analyzer (Applied Biosystems, Foster City, CA). Allelic segregation data was analyzed using GeneMapper software v3.7 (Applied Biosystems). For diversity arrays technology (DArT) marker genotyping, genomic DNA of parental lines and 92 random RILs were sent to Diversity Arrays Technology Pty. Ltd. (http://www.diversityarrays.com). DNA was extracted from 20 to 30 plants per RIL following the protocol described by DArT (www.diversityarrays.com/pub/DArT_DNA_isolation.pdf). The marker names (e.g., wPt) were described according to Akbari et al. (2006), where "w" indicates the clone was derived from a wheat library and "Pt" stands for *Pst*I and *Taq*I restriction enzymes used to generate clones. The version 2.0 wheat DArT array of 5137 clones was used for genotyping RILs using the methods previously described by Akbari et al. (2006).

Linkage Analysis

Genetic linkage analysis between the DNA marker loci and *Sr6* was conducted using MAPMAKER/EXP version 3.0b with LOD value of 3.0 and a maximum genetic distance of 37 cM (Lander et al., 1987). Genetic distances between loci were calculated using the Kosambi mapping function (Kosambi, 1944). The order of loci on the linkage group was assigned using COMPARE and RIPPLE commands with the additional command of ERROR DETECTION ON.

RESULTS

Fourteen days after inoculation with race QFCS, the MN98550 parent was highly resistant displaying a zero fleck (0;) IT, whereas the parent MN99394 was susceptible with IT of 3. The RIL population segregated for two main groups of ITs: 74 RILs showed ITs of 0 and 0;, which was typical of *Sr6* ITs against QFCS, and 59 RILs showed a clear IT of 3. This ratio fit a 1:1 ratio (χ^2 = 1.69, P = 0.193) expected for a single gene conferring resistance to QFCS (Table 1).

Since our main goal was to search for DNA markers that map distal to the *Sr6* locus as a way of identifying flanking markers, a mapping population derived from the MN99349 and MN98550 cross generated a partial genetic linkage map of chromosome 2D, which was comprised of nine loci, *Xgwm484*, *Xcfd77*, *Xcfd43*, *Xwmc453*, *Sr6*, *XwPt_4381*, *XwPt_0330*, X*gpw94049*, and *Xgwm102* (Fig. 1b). The total genetic map distance spanned a region

of 11.3 cM. DNA markers on this linkage group covered both sides of the *Sr6* locus. The two DArT marker loci *XwPt_0330* and *XwPt_4381* cosegregated and were mapped distal to *Sr6* at a distance of 2.8 cM, whereas the two SSR marker loci *Xcfd43 and Xwmc453* cosegregated and were mapped proximal to *Sr6* at a distance of 1.2 cM. There was segregation distortion observed for some of the DNA markers on this chromosome region (Table 1). The six SSR marker loci *Xgwm102*, *Xgpw94049*, *Xwmc453*, *Xcfd43*, *Xcfd77*, and *Xgwm484* amplified alleles with fragment sizes of 172, 305, 131, 215, 221, and 153 bp, respectively, on the resistant parent (MN98550) (the sizes included 20 bp of the M13 tail). The two DArT marker loci *XwPt_4381* and *XwPt_0330* amplified the resistant parent (MN98550) and were null alleles on the susceptible parent. Since the mapping population was F_6–derived F_8 with 3.125% expected level of heterozygosity after six generations of selfing, we observed RILs that were heterozygous at all six SSR marker loci, indicating that the SSRs were codominant. These were treated as missing data in constructing the partial linkage map.

DISCUSSION

Several studies have used monosomic stocks to locate *Sr6* on the short arm of chromosome 2D (Sears, 1954; Wiggin, 1955; Sears et al., 1957). The *Sr6* locus and the two leaf rust resistance genes *Lr2a* and *Lr15* were mapped on chromosome 2DS with the recombination frequency of 27.9% from the centromere (McIntosh and Baker, 1968). Recently, two SSR markers, *Xcfd43* and *Xwmc453*, were mapped proximal to *Sr6* at distances of 1.1 and 1.5 cM, respectively (Tsilo et al., 2009). However, no markers mapped distal to *Sr6* in that study. The reason could be that the population originated from near isogenic lines; hence, most marker alleles were monomorphic. A concern when using only the proximal markers was the chance for recombination between the marker(s) and *Sr6*. By using a different mapping population, we were able to identify four DNA markers that mapped distal to *Sr6* within a genetic distance of 6.5 cM. Proximal to *Sr6* were the same four markers *Xwmc453*, *Xcfd43*, *Xcfd77*, and *Xgwm484* as previously reported by Tsilo et al. (2009) (Fig. 1a, 1b). In the RIL population, no recombination was observed between *Xcfd43* and *Xwmc453*, which is in agreement with the consensus map of Somers et al. (2004); however, there was a recombination between these two markers in a population of Chinese Spring near isogenic lines reported by Tsilo et al. (2009) (Fig. 1a).

Of the four markers that mapped distal of *Sr6*, two (wPt_4381 and wPt_0330) are DArT markers, while the other two (gpw94049 and gwm102) are SSRs. The map location of *Xgpw94049* was unknown and in this study this marker was mapped on chromosome 2D. The marker sequence of all the SSRs in this study can be obtained from the GrainGenes website (http://wheat.pw.usda.gov/GG2/index.shtml). We are currently in the process of converting the two DArT markers to sequence-tagged site markers. These four marker loci *XwPt_4381*, *XwPt_0330*, X*gpw94049*, and *Xgwm102* that mapped distal to *Sr6*, within a distance of 6.5 cM, will in combination with either *Xwmc453* or *Xcfd43* provide a choice of flanking

Table 1. Segregation of *Sr6* and DNA marker alleles in a recombinant inbred line (RIL) population derived from a cross between MN99394 and MN98550

Gene/ marker	Observed†		Total	$\chi^{2‡}$	P value
	X_1X_1	X_2X_2			
Xgwm102	81 (172 bp)	55 (166 bp)	136	4.97	0.026
Xgpw94049	76 (305 bp)	55 (307 bp)	131	3.37	0.067
XwPt_0330	47 (present)	33 (null)	80	2.45	0.118
XwPt_4381	48 (present)	37 (null)	85	1.42	0.233
Sr6	74 (resistant)	59 (susceptible)	133	1.69	0.193
Xwmc453	80 (131 bp)	55 (179 bp)	135	4.63	0.031
Xcfd43	80 (215 bp)	56 (185 bp)	136	4.24	0.040
Xcfd77	83 (221 bp)	52 (226 bp)	136	7.12	0.008
Xgwm484	77 (153 bp)	49 (151 bp)	126	6.22	0.013

†X_1X_1 indicates homozygous for resistant parent's allele and X_2X_2 indicates homozygous for susceptible parent's allele. The parental alleles for each marker are provided in parenthesis. The RIL genotypes were based on 20 to 30 plants per RIL that were either tested for seedling reaction against stem rust race QFCS or genotyped with DNA markers.

‡Chi-square values based on the expected Mendelian segregation of 1:1.

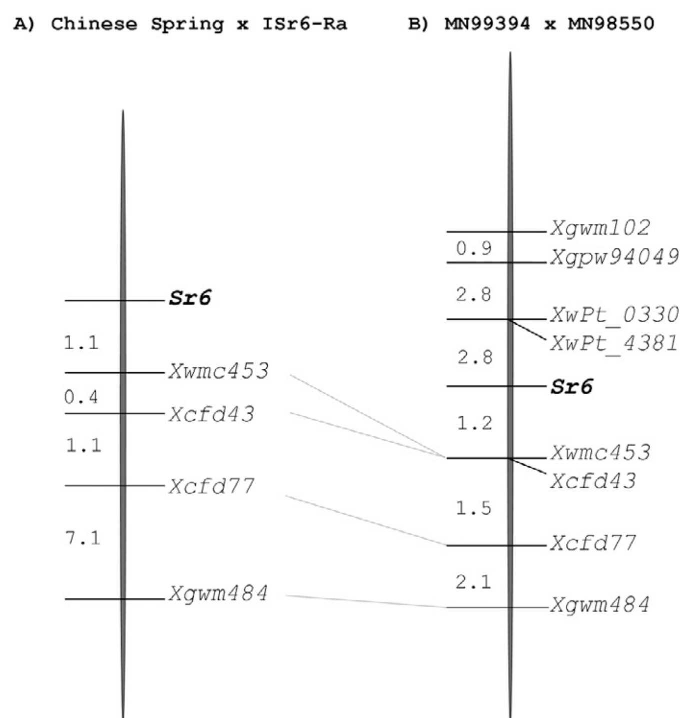

Figure 1. Alignment of Sr6-linked DNA markers on chromosome 2DS. (A) Partial genetic linkage map depicting the location of *Sr6* with linked codominant SSR loci reproduced from Tsilo et al. (2009). (B) The genetic linkage map derived from MN99394 × MN98550 recombinant inbred population with map distances (cM) calculated with the Kosambi mapping function.

markers for the purpose of marker-assisted selection and pyramiding of *Sr6* with other stem rust resistance genes. In North America and other regions where *Sr6* is still effective against predominant races, wheat breeders could consider increasing durability of resistance against the stem rust pathogen by using the DNA markers identified in this study together with DNA markers of other *Sr* genes. We observed segregation distortion in favor of the resistant parent allele for five of the eight markers linked with *Sr6*, but not with *Sr6* per se (Table 1). In our previous study, none of the markers showed distortion (Tsilo et al., 2009).

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