RESEARCH

Molecular and Cytogenetic Characterization of Wheat Introgression Lines Carrying the Stem Rust Resistance Gene *Sr39*

Guo Tai Yu, Qijun Zhang, Daryl L. Klindworth,* Timothy L. Friesen, Ron Knox, Yue Jin, Shaobin Zhong, Xiwen Cai, and Steven S. Xu

ABSTRACT

The stem rust (*Puccinia graminis* Pers.:Pers. f.sp. *tritici* Eriks. and Henn.) resistance gene *Sr39*, which confers resistance to TTKSK (Ug99), has been incorporated into the wheat (*Triticum aestivum* L.) genome from *Aegilops speltoides* in the form of a chromosome translocation but it has not been deployed into adapted cultivars. In this study, we characterized translocation lines carrying *Sr39* in four different wheat backgrounds with fluorescent genomic in situ hybridization (GISH) and simple sequence repeat (SSR) markers. The results indicated that RL5711 and RL6082 had translocation chromosomes of comparable structure. The translocation chromosome in PI 600683 has lost *Ae. speltoides* chromatin in the telomeric end of the 2S long arm. Six translocation lines derived from the cross PI 600683/3*HY438 had translocation chromosomes of comparable structure to the one found in PI 600683. However, one line (P9714-AM03C51), showed a substantial reduction in *Ae. speltoides* chromatin in the short arm of the translocated chromosome. The study demonstrated that it is apparently feasible to shorten *Ae. speltoides* chromatin in some wheat-*Ae. speltoides* translocation lines. These results and the identification of diagnostic SSR markers will be useful in guiding chromosome manipulation efforts to further shorten the *Ae. speltoides* chromosome segments in these materials. Greenhouse inoculation of translocation lines with stem rust indicated that the *Sr39* gene conditions resistance to at least seven stem rust races.

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Abbreviations: GISH, genomic in situ hybridization; SSR, simple sequence repeat.

Stem rust is a destructive disease of common wheat and durum wheat (*T. turgidum* L. ssp. *durum*). Historically, severe epidemics have occurred in 1935, 1953, and 1954 in North America. During those rust epidemics, up to 50% of the wheat crop was destroyed in North Dakota and Minnesota and 20% or more of the crop was lost in South Dakota (Leonard, 2001). Because of this, great effort has been made to find and deploy stem rust resistance genes and to eradicate barberry (*Berberis vulgaris* L.), the alternate host for stem rust. Since the 1950s, only sporadic epidemics have been reported due to the successful deployment of stem rust resistance genes (Leonard, 2001). However, stem rust resistance genes are race specific. Thus, new races of the stem rust pathogen that are virulent to widely deployed stem rust resistance genes can cause serious epidemics if there are no resistance genes specific to the new race in major cultivars. Recently, stem rust re-emerged as a serious threat because a

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new highly virulent race, *Pgt*-TTKSK (commonly known as Ug99), caused a stem rust outbreak in Uganda in 1999 (Pretorius et al., 2000). TTKSK has already spread from Africa into the Middle East (Nazari et al., 2009).

To date, about 45 stem rust resistance genes have been identified (McIntosh et al., 2008). TTKSK is virulent to most of the resistance genes of wheat origin (Singh et al., 2006). Thus, the majority of the genes for resistance to TTKSK have originated from wild wheat relatives. However, only a small portion of these genes, such as *Sr24* and *Sr36*, have been deployed in cultivars due to difficulties in reducing associated alien chromatin causing linkage drag. Furthermore, owing to rapid evolution of the rust fungus, new variants of TTKSK that are virulent on *Sr24* (*Pgt*-TTKST; Jin et al., 2008) and *Sr36* (*Pgt*-TTTSK; Jin et al., 2009) have emerged. Therefore, stem rust resistance genes that are effective against TTKSK, its variants, and the predominant local races are needed in cultivars.

The stem rust resistance gene *Sr39* is effective against TTKSK (Singh et al., 2006), as well as other races, but has not been deployed into cultivars due to linkage drag. *Sr39*, along with the leaf rust (*Puccinia triticina* Eriks.) resistance gene *Lr35*, was derived from *Ae. speltoides* (Kerber and Dyck, 1990; Friebe et al., 1996). Although the S-genome of *Ae. speltoides* is considered to be the putative donor of the B-genome of durum and common wheat (Johnson, 1975), recombination between the homeologous chromosomes of *Ae. speltoides* and wheat rarely occurs. Thus, most of the *Ae. speltoides*-wheat chromosome exchanges have been achieved by *ph1b*-induced homeologous recombination (Friebe et al., 1996) and a small number of the *speltoides*-wheat translocation lines have been created by irradiation (Friebe et al., 1996). RL5711, the original line carrying *Sr39/Lr35*, was developed using conventional crossing and backcrossing (Kerber and Dyck, 1990), and carries a translocation chromosome identified as T2B/2S#2 (Friebe et al., 1996). RL6082 and P8810-B5B3A2A2 (PI 600683) were derived from RL5711 using backcrossing (Gold et al., 1999; Knox et al., 2000). RL6082 is a *T. aestivum* cultivar Thatcher near-isogenic line carrying the genes *Sr39* and *Lr35* (Gold et al., 1999). These lines were developed 10 or more years ago, and all carry *Lr35* and *Sr39* on a translocation chromosome. Mago et al. (2009) determined the size of the T2B/2S#2 chromosome in RL5711, but the amount of the translocated *Ae. speltoides* chromatin in the newer translocation lines is unknown.

The objectives of this study were to characterize the original translocation stock RL5711 harboring *Sr39* and its derived wheat lines using GISH and molecular marker analysis and to determine stem rust races that can be used to detect *Sr39* in the background of the translocation lines.

MATERIALS AND METHODS

Plant Materials

The pedigrees of the lines used in this study are presented in Table 1. The lines RL5711, RL6082, and PI 600683 are wheat-*Ae. speltoides* chromosome translocation lines. We made three single plant selections of RL6082 in the greenhouse, and these plants were designated RL6082–56–1, RL6082–56–6, and RL6082–56–8. PI 600683 also carries *Sr39* (Knox et al., 2000). The *Ae. speltoides* donor of the chromatin harboring the stem rust resistance gene for all three translocation lines was RL5344, which is the *Ae. speltoides* parent of *Ae. speltoides/T. monococcum* amphiploid RL5347 (Kerber and Dyck, 1990).

Another set of breeding lines carrying the *Lr35/Sr39* chromosome translocation were developed from the cross PI 600683/3*HY438, and are therefore referred to in this communication as HY438 lines. The *Lr35/Sr39* rust resistance genes were selected by screening for leaf rust resistance in the BC_1F_1 and BC_2F_5 progeny. In the BC_2F_6 generation, the lines were tested with a dominant sequence characterized amplified region (SCAR) marker (Gold et al., 1999). Lines heterogeneous for leaf rust resistance were selected until the BC_2F_{10} generation, when putative homozygous leaf rust resistant families were selected based on uniformity of leaf rust reaction within progeny rows. We tested 11 lines from these trials to determine whether they still carried *Ae. speltoides* chromatin and to determine if any had a shortened alien segment.

Stem Rust Resistance Evaluation

Stem rust races used in testing were identified following the *Pgt* designation, however the *Pgt* race designations have recently been expanded from four letters to five (Jin et al., 2008). Local races had not yet been converted to the new five letter designation, and so some races reported in this study are identified by a five letter code and others by only a four letter code. Local races used included TMLK, TPMK, TCMJ, THTS, QTHJ, QFCQ, QFMQ, RTQQ, RHTS, HKHJ, LBBL, MCCF, JCMN, and TTTT. Stem rust testing was conducted at Fargo, ND for 13 of these races. For race TTTT, the resistance evaluation was done at the USDA-ARS Cereal Disease Laboratory, University of Minnesota, St. Paul. Seedlings were grown in the greenhouse at 20 to 23°C with a 16/8-h (day/night) photoperiod. Seven-day-old seedlings were inoculated as described in Williams et al. (1992). Inoculated seedlings were transferred to either a greenhouse or growth chamber maintained at 20 to 23°C. Infection types were scored 14 d after inoculation using the scale described by Stakman et al. (1962) where 0 = immune, ; = necrotic flecks, 1 = small necrotic pustules, 2 = small to medium sized chlorotic pustules, 3 = medium sized chlorotic pustules, and 4 = large pustules without chlorosis.

Two stem rust trials were conducted. Eight races were used in the first trial to determine races avirulent to *Sr39* in RL6082 (Thatcher background). Lines tested included Thatcher, RL5711, the three single plant selections of RL6082, and Chinese Spring (CS). Nine races were used in the second trial to determine if any could detect *Sr39* in P9714-AM03C51 (HY438 background). Lines tested included Marquis, RL5711, PI 600683, HY366, HY438, P9714-AM03C51, and LMPG-6. Because seed supplies of HY366 were very limited, we used a mixture of seeds of HY366 and HY366-BL31 (a selection from HY366), to represent HY366.

Genomic in Situ Hybridization

We used the genomic in situ hybridization (GISH) procedures as described by Cai et al. (1998) and Liu et al. (2006). Seeds were

Table 1. Pedigrees of the lines used in the study.

Line	Pedigree/description	Translocation line (±)†	Reference
RL5344	*Ae. speltoides*, *Sr39* source	+	Kerber and Dyck, 1990
Marquis	Hard Red Calcutta/Red Fife	–	
RL5711	Marquis-K*8/RL5347	+	Kerber and Dyck, 1990
Thatcher	Marquis/Iumillo//Marquis/Kanred	–	
RL6082 (*Sr39*)	Thatcher*6/RL5711	+	Gold et al., 1999
HY366	Tobari66/Romany//Pitic62/Gaines/3/RL4137/4/2 *(7722)Tobari66/Romany//Pitic62/Gaines	–	Unpublished data, 2009
HY366-BL31	a selection from HY366	–	Unpublished data, 2009
PI 600683	HY366/RL5711//2*HY366/3/3*HY366-BL31	+	Knox et al., 2000
HY438	HY320*6/7424-BW5B4//KENYA 321/TAKAHE/4/HY320*5/BW553// HY358///HY358/7915-QX76B2		Unpublished data, 2009
P9714-AM03C51	PI 600683/3*HY438	+	Unpublished data, 2009

†+ indicates *Ae. speltoides* or translocation lines, - indicates wheat lines with normal chromosome constitution.

germinated in Petri dishes at 22°C for about 24 h. Roots of 1 to 2 cm in length were collected and pretreated in ice water for 20 to 24 h. After pretreatment, the roots were fixed in a 3:1 mixture of ethanol and acetic acid for 48 h at room temperature. To prepare the slide, root tips were transferred into 1% acetic carmine for 2 to 3 min and then root tips were squashed in 45% acetic acid under a cover glass. Prepared slides were observed under phase contrast optics using an Olympus Research Microscope. The cover glasses on the slides with good chromosome spreads were removed after the slides were frozen at −80°C in an ultralow freezer. The slides were then stored at −80°C for later use.

Genomic DNA of CS and *Ae. speltoides* RL5344 was isolated using the protocol described by Faris et al. (2000). The genomic DNA of *Ae. speltoides* RL5344 was used as probe DNA and labeled with biotin-16-dUTP by nick translation (Roche Diagnostics, Mannheim, Germany). The genomic DNA of CS was used as blocking DNA, which was prepared by shearing total genomic DNA in 0.4 M NaOH in boiling water for 40 to 50 min. The hybridization of the probe DNA to chromosomes and signal detection was conducted using the procedures described by Mukai et al. (1993) and Cai et al. (1998). The *Ae. speltoides* segments were detected with fluorescein isothiocyanate-conjugated avidin (FITC-avidin) (Vector Laboratories, Inc., Burlingame, CA) and wheat chromosomes were counter-stained with 4’-6-Diamidino-2-phenylindole (DAPI) (Sigma, St. Louis, MO) and pseudocolored as red using Axiovision Release 4.5 (Carl Zeiss Light Microscopy, Germany). Slides were mounted in VECTASHIELD (Vector Laboratories) antifading medium containing 2 μg/mL DAPI for counterstaining. The DAPI and FITC were excited at 350 to 490 nm. The GISH images were observed under a Zeiss Axioplan 2 Imaging Research Microscope (Carl Zeiss Light Microscopy, Germany) and captured using an Axiocam HRm CCD camera (Carl Zeiss Light Microscopy, Germany).

Molecular Marker Analysis

The *Ae. speltoides* accession RL5344, Marquis, Thatcher, RL6082, RL5711, PI 600683, HY366, HY366-BL31 and CS were tested for polymorphisms at molecular marker loci using simple sequence repeat (SSR) primers. A total of 72 SSR markers were tested. Markers chosen for study were selected primarily based on the consensus chromosome genetic map (http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=mapdata;query=;name=Wheat,+Consensus+SSR,+2004). DNA isolation was performed as described in Yu et al. (2009). The SSR markers were amplified by polymerase chain reaction (PCR) according to conditions described by Röder et al. (1998). Amplified products were electrophoresed on 6% poly-acrylamide gel in 0.5X TBE, stained with Gel-Red, and scanned with a Typhoon 9410 variable mode imager (GE Healthcare, Inc. Waukesha, WI).

RESULTS

Genomic in situ Hybridization Characterization of Wheat-*Aegilops speltoides* Translocations

We investigated translocation lines RL5711, RL6082–56–1, RL6082–56–6, RL6082–56–8, and PI 600683 with GISH. The sizes of the translocated *Ae. speltoides* chromosome 2S in RL5711 and RL6082–56–6 (as well as RL6082–56–8) were the same (Fig. 1A, B). The translocated chromosomes detected by the probe DNA consisted of the long arm, the centromere, and a large portion of the short arm of chromosome 2S of *Ae. speltoides* with an attached small terminal segment of a wheat chromosome (Fig. 1A). By measuring the physical lengths of the 2S short arm and the wheat segment, the translocation point was located at approximately 85% of the short arm. In the line PI 600683, the short arm of the translocated chromosome was the same as in RL5711 and RL6082–56–6, but the 2SL arm had a small portion of its telomeric end replaced by wheat chromatin (Fig. 1E). In the line RL6082–56–1, unlike its sibling lines, probe DNA detected only two small hybridization sites at the telomere (Fig. 1C), which were not present in the recurrent parent Thatcher (Fig. 1D).

In light of the result of the three translocation lines, we further investigated the 11 HY438 leaf rust resistant lines (*Lr35/Sr39*) by GISH analysis. For four of the HY438 lines, we did not detect *Ae. speltoides* chromatin. Seven lines had translocation chromosomes with substantial amounts of *Ae. speltoides* chromatin. Among these seven lines, six lines had a translocated *Ae. speltoides* chromosome similar to that found in PI 600683, one of which was heterozygous for the

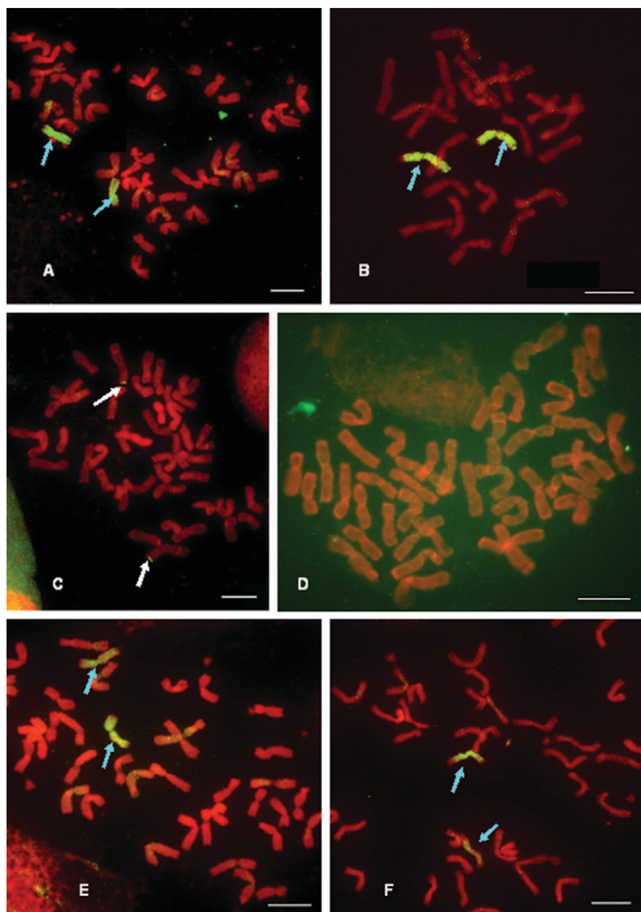

Fig. 1. Fluorescent genomic in situ hybridization of mitotic chromosomes in wheat-*Ae. speltoides* chromosome translocation lines (A) RL5711, (B) RL6082–56–6, (C) RL6082–56–1, (D) Thatcher, (E) PI 600683, and (F) P9714-AM03C51. In (A), *Ae. speltoides* chromatin (yellow-green color) was detected by using biotin-11-dUTP-labeled total genomic DNA of *Ae. speltoides* as probe DNA. The blue arrows indicate the pair of T2BL-2SL·2SS translocation chromosomes that harbor the stem rust resistance gene(s). In (C), the white arrows indicate two small hybridization sites detected with *Ae. speltoides* DNA probe. Scale bar = 10 μm.

chromosome translocation. In one line, P9714-AM03C51, the short arm of the translocation chromosome showed a substantial reduction in *Ae. speltoides* chromatin (Fig. 1F). By measuring the physical lengths of the various translocated segments in P9714-AM03C51, it was found that the translocation chromosome contained *Ae. speltoides* chromatin in 60% of the short arm and 93% of the long arm–the remainder having been replaced with wheat chromatin.

Molecular Characterization of the Translocations

To confirm if the translocated chromosome involved wheat chromosome 2B, SSR markers *Xgwm319* and *Xgwm501* were used to test the lines RL5344, RL6082, and Thatcher, against the CS nullisomic-tetrasomic lines where a pair of missing chromosomes is compensated for by an extra pair of homeologous chromosomes (Sears, 1966). Both *Xgwm319* and *Xgwm501* detected polymorphic loci on chromosomes 2B and 2S. The polymorphism for *Xgwm319* is shown in Fig. 2. Because DNA for all nullisomic-tetrasomic lines except CS N2B-T2A produced a 171bp amplicon, this locus resides on chromosome 2B. Comparisons involving this marker indicated that *Ae. speltoides* chromatin from RL5344 had replaced the corresponding segment of chromosome 2B in RL6082. This result confirmed that the translocation in RL6082 involves wheat chromosome 2B and *Ae. speltoides* chromosome 2S. Using the same approach, it was confirmed that the chromosome translocations in PI 600683 and P9714-AM03C51 also involved chromosomes 2B and 2S.

With respect to these results, we examined the 2B consensus genetic SSR map (http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=mapdata;query=;name=Wheat,+Consensus+SSR,+2004) and the 2B physical SSR map (http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=mapdata&seme=1&name=Wheat,%20Physical,%20SSR). A total of 22 SSR markers were used to characterize the translocation of RL6082 (Fig. 3). Sixteen SSR markers were polymorphic between Thatcher and the *Ae. speltoides* donor line RL5344 or RL6082, but were monomorphic between RL5344 and RL6082. Therefore, these 16 markers detect *Ae. speltoides* chromatin that is present in the T2B/2S#2 translocation chromosome in RL6082. Six SSR markers were monomorphic between Thatcher and RL6082, but were polymorphic between RL5344 and RL6082. These six markers detect the wheat segment that is present in the T2B/2S#2 translocation chromosome in RL6082. The polymorphism and monomorphism revealed by these SSR markers among these three lines further indicated that the majority of chromosome 2B has been replaced by *Ae. speltoides* chromosome 2S.

Based on analysis of the SSR markers on the genetic and physical maps of chromosome 2B, the location of the translocation breakpoint was estimated. In the line RL6082, according to the consensus genetic SSR map (http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=mapdata;query=;name=Wheat,+Consensus+SSR,+2004), marker *Xbarc124*, which is monomorphic between RL6082 and Thatcher, is mapped to the distal 13.6 cM of chromosome arm 2BS, whereas the polymorphic marker *Xwmc25* is mapped to the distal 23.7 cM. Therefore, the translocation breakpoint lies between markers *Xbarc124* and *Xwmc25*. Because two SSR markers *Xbarc200* and *Xgwm257* are located on the physical bin 2BS-0.84~1.00 (Fig. 3), the translocation breakpoint is positioned within the 2BS-4 deletion bin, which is the most distal bin of the short arm and accounts for about 16% of the physical size of the arm. These results together indicate the composition of the T2B/2S#2 translocation chromosome in RL6082 is T2BS-2SS·2SL.

For the line P9714-AM03C51, we used 23 SSR markers to characterize the translocation (Fig. 4). Fourteen SSR

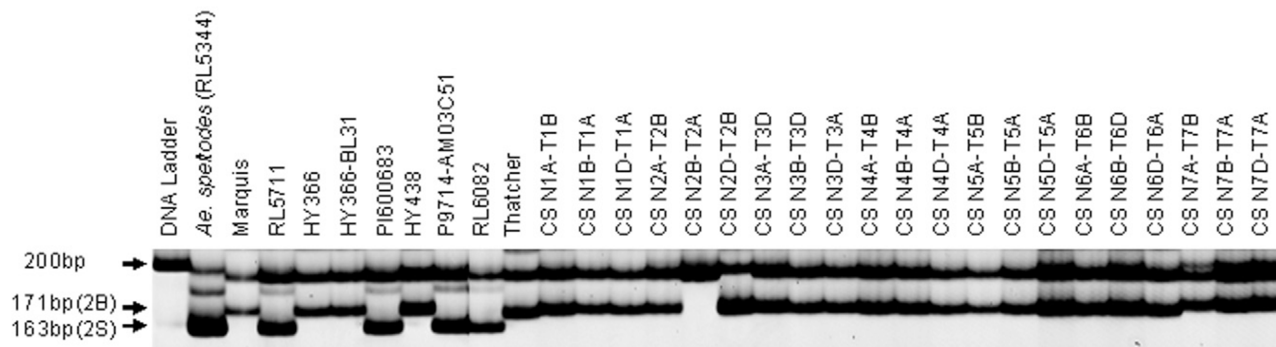

Fig. 2. Scanned image of marker *Xgwm319*. The polymorphism between Thatcher and RL6082 or P9714-AM03C51 indicates that the native Thatcher chromosome 2B amplicon was replaced by the *Ae. speltoides* chromosome 2S amplicon in the translocation line RL6082 and P9714-AM03C51. The Chinese Spring nullisomic-tetrasomic (NT) lines reveal the chromosome assignments of the three amplicons detected by *Xgwm319*.

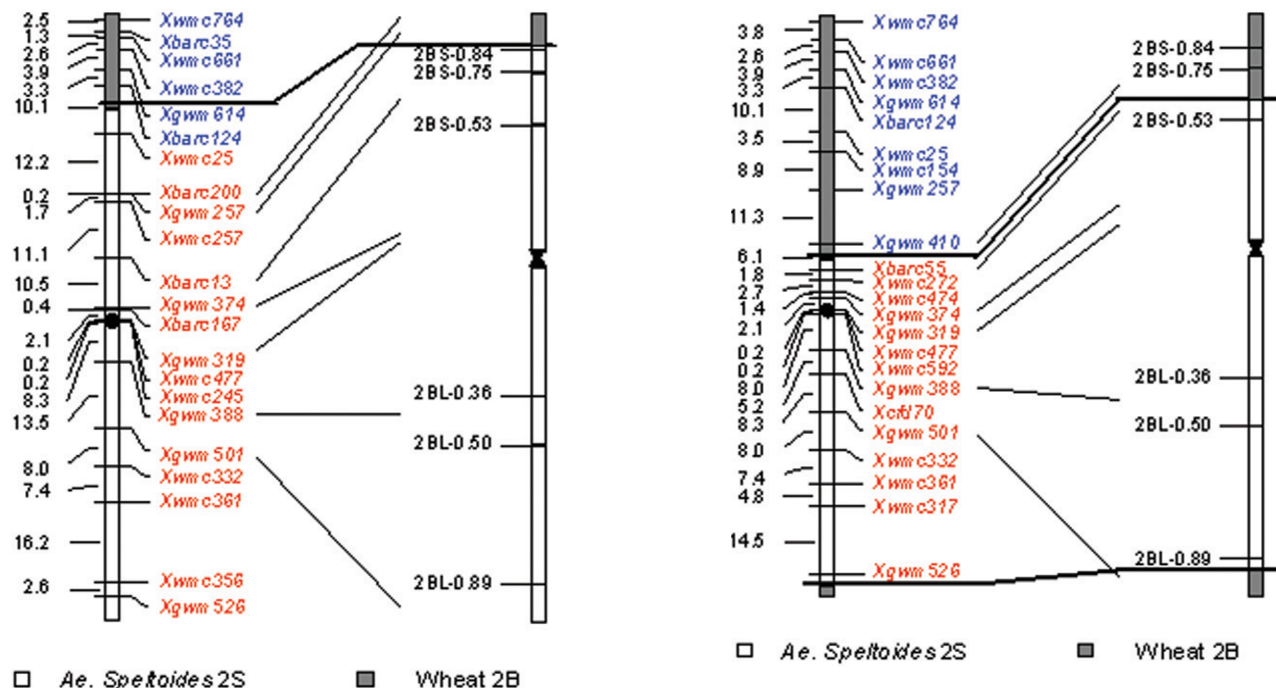

Fig. 3. Wheat chromosome 2B genetic (left) and physical (right) maps of RL 6082. On the physical map, deletion line designations and fraction breakpoints are indicated to the left and bin-mapped markers are indicated by a line between the two maps. CentiMorgan (cM) distances are shown along the left of the genetic map and markers along the right. Markers in red were polymorphic between Thatcher and RL6082 indicating the presence of *Ae. speltoides* chromosome 2S. Markers in blue were monomorphic and indicate the presence of Thatcher chromosome 2B. 2B physical simple sequence repeat (SSR) map was derived from the Graingenes 2.0 (http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=mapdata&seme=1&name=Wheat,%20Physical,%20SSR) and the genetic map was also derived from Graingenes 2.0 (http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=mapdata;query=;name=Wheat,+Consensus+SSR,+2004).

Fig. 4. Wheat chromosome 2B genetic (left) and physical (right) maps of P9714-AM03C51. On the physical map, deletion line designations and fraction breakpoints are indicated to the left and bin-mapped markers are indicated by a line between the two maps. CentiMorgan (cM) distances are shown along the left of the genetic map and markers along the right. Markers in red were polymorphic between HY438 and P9714-AM03C51 indicating the presence of *Ae. speltoides* chromosome 2S. Markers in blue were monomorphic and indicate the presence of HY438 chromosome.

markers that detected polymorphic loci between HY366 and PI 600683 or P9714-AM03C51 were monomorphic between PI 600683 and P9714-AM03C51. These 14 markers detect the *Ae. speltoides* chromatin that is present in the translocation chromosome in P9714-AM03C51. Nine SSR markers were monomorphic between HY366 and P9714-AM03C51, and polymorphic between PI 600683 and P9714-AM03C51. These nine SSR markers detect the wheat chromatin that is present in the translocation chromosome in P9714-AM03C51. The polymorphism and monomorphism among these three lines by these SSR markers further confirmed the replacement of chromosome 2B by a chromosome transloca-

tion from PI 600683 where a segment of the 2S short arm was shortened in the line P9714-AM03C51.

In the line P9714-AM03C51, the SSR marker *Xgwm410* was monomorphic between HY366 and P9714-AM03C51, while the marker *Xbarc55* was monomorphic between PI 600683 and P9714-AM03C51, Therefore, the translocation breakpoint lies between markers *Xgwm410* and *Xbarc55* on the genetic map. Since both markers were mapped to bin 2BS-0.53–0.75 on the deletion map (Fig. 4), the physical location of the recombination breakpoint is positioned within the 2BS-2 deletion bin, which accounts for about 22% of the physical size of the arm. While *Xgwm526* was monomorphic between PI 600683 and P9714-AM03C51, the GISH results indicated that the telomere tip of the translocated *speltoides* chromosome segment was replaced by a 2B segment. Thus, the breakpoint on the long arm is beyond the marker *Xgwm526* on the genetic map. Since *Xgwm501* is located in physical map bin 2BL-0.89–1.00, the breakpoint is positioned within bin 2BL-0.89–1.00, which accounts for about 11% of the physical size of the long arm. Therefore, the configuration of the wheat-*Ae. speltoides* translocation line P9714-AM03C51 is T2BS-2SS·2SL-2BL (Fig. 4).

Among the remaining 10 HY438 lines, five lines still contained the same large *Ae. speltoides* chromosome segment between *Xwmc25* and *Xgwm526*, and were identical to PI 600683. One HY438 line, the same line studied by GISH, was heterozygous for the translocated chromosome. The amount of *Ae. speltoides* chromatin in this line was also identical to that in PI 600683. Four lines did not have a translocated chromosome based on marker analysis.

Stem Rust Resistance Evaluations

In our first stem rust trial, we used eight races of the stem rust pathogen to determine which races could detect *Sr39* in RL6082 (Thatcher background) (Table 2). The susceptible check, CS, was susceptible to all eight races. RL5711 was highly resistant to all eight races of the first test. Thatcher was susceptible to four races including TMLK, TPMK, TCMJ, and THTS, but highly resistant to HKHJ, LBBL, MCCF, and JCMN. The lines RL6082–56–6 and RL6085–56–8 were resistant or highly resistant against all eight races, and were similar to RL5711 which is the source of the wheat-*Ae. speltoides* chromosome translocation in RL6082. This is consistent with the GISH results which indicated that the lines RL6082–56–6 and RL6082–56–8 contained translocated chromosomes identical to that found in RL5711. The selection RL6082–56–1 was susceptible to five races and resistant to three races but this is not the expected result for a wheat line carrying *Sr39*. Based on GISH, RL6082–56–1 did not carry the large wheat-*Ae. speltoides* chromosome translocation with *Sr39* (Fig. 1). Because TMLK, TPMK, TCMJ, and THTS were avirulent on RL6082–56–6 and RL6082–56–8, and were virulent on Thatcher, we concluded that any of these four races could be used to detect *Sr39* in a Thatcher background.

The second stem rust test was intended to determine whether P9714-AM03C51 carries *Sr39* (Table 3). Lines tested included Marquis, RL5711, HY366, PI600683, P9714-AM03C51, HY438, and LMPG-6. Because the recurrent parent HY438 was resistant to all nine races tested, and because differences between HY438 and P9714-AM03C51 were minor, we were not able to determine if *Sr39* was present in P9714-AM03C51.

DISCUSSION

Although stem rust has been under effective control through wide use of resistant cultivars and eradication of barberry since the 1950s, the recently identified new race TTKSK and its variants pose a great threat to global wheat production. Relatively few genes condition resistance to TTKSK and its variants and few commercial wheat cultivars are resistant to TTKSK. For example, TTKSK is virulent to *Sr31*, a predominant gene that protects wheat cultivars against stem rust on a global scale. Moreover, some genes that confer resistance to TTKSK are ineffective against TTKST and TTTSK (Jin et al., 2008). Hence, it is essential that more resistance genes be characterized and deployed into cultivars.

In our study, RL6082, which carries *Sr39,* was resistant to eight North American stem rust races (Table 1). Other genes conditioning resistance to TTKSK include *Sr25, Sr26, Sr32, Sr37, Sr40, Sr43,* and *Sr44* (Singh et al., 2006). However, all of these genes are derived from wild wheat relatives and are located on translocated alien chromosome segments which inevitably introduce linkage drag. To eliminate the linkage drag problem, efforts must be made to shorten the wild translocated segments in wheat.

Sr39 was transferred along with *Lr35,* another valuable gene, from *Ae. speltoides* to wheat in the form of a chromosome translocation involving chromosomes 2B and 2S (Kerber and Dyck, 1990). The translocation breakpoint could not be determined by C-banding (Friebe et al., 1996). In our study, we used GISH and molecular marker analysis and clearly showed the location of the translocation breakpoints, the physical length of the translocated chromosome segments, and their relationship to deletion and genetic maps. Except for 15% of the short arm which is from wheat 2BS, all the rest of the chromosome comes from 2SS and 2SL of *Ae. speltoides*. On the consensus genetic SSR map, the breakpoint lies between markers *Xbarc124* and *Xwmc25.*

Since the original translocation line RL5711 with *Sr39* and *Lr35* was created in a Marquis genetic background, it has been used to develop modern stem/leaf rust resistance germplasm. Lines harboring *Sr39*, including the ones used in our study, RL6082, and PI 600683 have been developed from RL5711. Recently, PI 600683 was backcrossed to HY438, a Canadian spring wheat line. Out of the 11 leaf rust

Table 2. Seedling infection types of RL5711, RL6082–56–1, RL6082–56–6, RL6082–56–8, Thatcher, and Chinese Spring (CS) to eight races of the stem rust pathogen.

Line	Race							
	TMLK	TPMK	TCMJ	THTS	HKHJ	LBBL	MCCF	JCMN
RL5711	1	1	1	1	12	;1	1	1
RL6082–56–6	1	1	1	21	0;	;	;	0;
RL6082–56–8	1	1	1	12	0;	;	;	0;1
Thatcher	34	34	34	34	0;	;	;1	0;
RL6082–56–1	34	34	4	4	;	;	34	0;
CS	34	34	34	34	4	34	34	34

Table 3. Seedling infection types of Marquis, RL5711, HY366, PI600683, HY438, P9714-AM03A51, P9714-AM03C51, P9714-AM03C52, and LMPG-6 to nine races of the stem rust pathogen.

Entry	Race								
	TPMK	THTS	TCMJ	QTHJ	QFCQ	QFMQ	RTQQ	RHTS	TTTT†
Marquis	34	34	32	32	2+	2	3+	34	4
RL5711	2	21	21	2	21	21	2+	2	2
HY366	2+1	34	32	34	2	12	34	34	3
PI600683	;1	23	21	2c	1	21	2	12-	2/2+
P9714-AM03C51	21	;1-	;	12	21	12	12	;1	0;
HY438	2+	;1-	;1-	21	12	21	2	;1-	;1
LMPG-6	4	34	34	4	34	34	4	43	4

† Inoculation TTTT was conducted at St. Paul, MN.

resistant lines developed from this backcross, we identified one line, P9714-AM03C51, which had a shortened chromosome segment from *Ae. speltoides*. Six of 11 lines have the same chromosome translocation as PI 600683. These studies demonstrate that the level of homeologous recombination between 2B and 2S is low, but does occasionally occur even without the use of *Ph1* gene mutants or radiation.

Mago et al. (2009) reported seven lines having shortened *Ae. speltoides* chromatin produced by induction of homeologous recombination between chromosome 2B of wheat and the T2B/2S#2 translocation chromosome. Based on the reactions of these lines to the stem rust pathogen, Mago et al. (2009) localized *Sr39* to the short arm of 2S and near the breakpoint of the T2B/2S#2 translocation chromosome. In the line P9714-AM03C51, that segment is missing, and P9714-AM03C51 may not carry *Sr39*. Because the recurrent parent HY438 was resistant to all nine stem rust races tested, we were not able to determine if P9714-AM03C51 still retains *Sr39* in the current study. The lines will be tested for reactions to more virulent races, including the TTKSK lineage. If P9714-AM03C51 is verified to retain *Sr39*, it might be useful for breeding or a second cycle of chromosome manipulation to further reduce the amount of *Ae. speltoides* chromatin.

Although Mago et al. (2009) developed seven translocation lines with shortened *Ae. speltoides* chromatin, only four of those lines carried *Sr39*. Among those four lines, the line carrying the least amount of *Ae. speltoides* chromatin retained about two-thirds of the physical length of chromosome 2S in both the long and short arms (Mago et al., 2009). At present, it is not yet clear whether the *Ae. speltoides* chromatin in those lines has been sufficiently reduced so that deleterious linkage drag has been eliminated or whether the *Ae. speltoides* chromatin requires additional reduction. The 16 polymorphic SSR markers identified in the present study, which cover the remaining portion of chromosome 2S in the line, can be used in efforts to further shorten chromosome 2S. Gold et al. (1999) developed six SCAR markers associated with *Sr39/Lr35*. However, the positions of these markers were undetermined, which limits their use in marker-assisted selection.

Jin et al. (2007) noted that RL6082 had some off-type plants that apparently did not carry *Sr39*. Rust reactions seen in the experiment by Faris et al. (2008) were not as expected for lines that carry *Sr39*, which suggests that the line used in that study was also an off-type. Our line RL6082–56–1 is also an off-type. Similar to Thatcher, RL6082–56–1 does not carry *Sr39* because it is susceptible to the four races with the common virulence on subset 1 (T) of the stem rust differential set. In addition, the susceptibility of RL6082–56–1 to MCCF indicates that RL6082–56–1 does not carry all of the resistance genes found in Thatcher. The possible explanations for the missing Thatcher gene in RL6082–56–1 is that RL6082 is still segregating, or that RL6082–56–1 was derived from an outcross in the field or greenhouse, and subsequently lost one or more Sr genes from Thatcher.

In this study, we characterized the translocation lines in four different backgrounds using GISH, molecular marker techniques, and stem rust testing. The results gave us information about the size of the *Ae. speltoides* segments on the T2B/2S#2 translocation chromosome, the approximate

breakpoints, and the polymorphic markers between the *Ae. speltoides* and wheat chromosome segments. The information about the molecular markers identified as polymorphic between the wheat and *Ae. speltoides* chromosome segments is particularly important for chromosome manipulation to make *Sr39* useful. We also evaluated the translocation lines against a number of stem rust races. The information about the resistance of *Sr39* to a number of stem rust races is essential to differentiate *Sr39* and other stem rust resistance genes in the various backgrounds.

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