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Molecular and cytogenetic characterization of a durum wheat–*Aegilops speltoides* chromosome translocation conferring resistance to stem rust

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Abstract

Stem rust is a serious disease of wheat that has caused historical epidemics, but it has not been a threat in recent decades in North America owing to the eradication of the alternative host and deployment of resistant cultivars. However, the recent emergence of Ug99 (or race TTKS) poses a threat to global wheat production because most currently grown wheat varieties are susceptible. In this study, we evaluated a durum wheat–*Aegilops speltoides* chromosome translocation line (DAS15) for reaction to Ug99 and six other races of stem rust, and used molecular and cytogenetic tools to characterize the translocation. DAS15 was resistant to all seven races of stem rust. Two durum–*Ae. speltoides* translocated chromosomes were detected in DAS15. One translocation involved the short arm, centromere, and a major portion of the long arm of *Ae. speltoides* chromosome 2S and a small terminal segment from durum chromosome arm 2BL. Thus, this translocated chromosome is designated T2BL-2SL•2SS. Cytogenetic mapping assigned the resistance gene(s) in DAS15 to the *Ae. speltoides* segment in T2BL-2SL•2SS. The *Ae. speltoides* segment in the other translocated chromosome did not harbour stem rust resistance. A comparison of DAS15 and the wheat stocks carrying the *Ae. speltoides*-derived resistance genes *Sr32* and *Sr39* indicated that stem rust resistance gene present in DAS15 is likely novel and will be useful for developing germplasm with resistance to Ug99. Efforts to reduce *Ae. speltoides* chromatin in T2BL-2SL•2SS are currently in progress.

Abbreviations

CS	Chinese Spring wheat
FGISH	fluorescence genomic in-situ hybridization
FITC	fluorescein isothiocyanate
IT	infection type
LDN	Langdon durum
NT	nullisomic-tetrasomic
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
SSR	simple sequence repeat

Introduction

Stem rust, caused by the pathogen *Puccinia graminis* f. sp. *tritici* Eriks. & Henn., is a potentially devastating disease of durum (*Triticum turgidum* L. ssp.

durum, 2n=4×=28, AABB genomes) and common wheat (*T. aestivum* L., 2n=6×=42, AABBDD genomes). Historical epidemics have occurred. For example, in 1935 more than 50% of the wheat crops in North Dakota and Minnesota were lost owing to stem rust (Leonard 2001). Since the mid 1950s, only localized epidemics have occurred and regional losses have not exceeded 8%. Eradication of the alternate host (*Berberis vulgaris*) and deployment of effective stem rust resistance genes have been the two main factors contributing to the long-term control of stem rust. However, in 1999, a new pathotype of stem rust known as race Ug99 (or TTKS) was detected in Uganda (Pretorius *et al*. 2000). Most currently deployed stem rust resistance genes, including the widely utilized gene *Sr31*, are ineffective against Ug99 (Singh *et al*. 2006). Jin & Singh (2006) reported that only 16% of hard red spring wheat cultivars, 48% of hard red winter wheat cultivars, and 27% of soft winter wheat cultivars in the USA have resistance to Ug99. The genes conferring resistance in most of these cultivars were thought to be *Sr24*, *Sr36*, *SrTmp*, and an unknown gene derived from rye on a chromosome 1AL.1RS translocation in Amigo and its derivatives. However, new variants of Ug99 with virulence on lines containing *Sr24* were identified in 2006 (Jin *et al*. 2008), or on lines with *Sr36* (Y. Jin & T. Fetch, unpublished), rendering a portion of the previously Ug99-resistant wheats ineffective against the new variants.

The wild relatives of wheat are important sources of resistance genes to many diseases and pests including stem rust and, to date, about 20 stem rust resistance genes have been transferred to durum or common wheat from various wild relatives (McIntosh *et al*. 2003). Many of these new alien-derived genes are effective against Ug99 (Jin *et al*. 2007). In particular, *Aegilops speltoides* Tausch (2n=2×=14, SS genomes), a diploid goatgrass, has proved to be an excellent source of genes for stem rust resistance. Anikster *et al*. (2005) reported that all of 181 *Ae. speltoides* accessions collected from Israel were resistant to four common races (QCCJ, QFCS, RCRS, and TPMK) in the USA. A recent evaluation of six amphiploids derived from crosses between Langdon durum and six different *Ae. speltoides* accessions revealed that all of the amphiploids showed near-immunity or high levels of resistance to North American stem rust races and Ug99 (Y. Jin & S. S. Xu, unpublished).

The S genome of *Ae. speltoides* is closely related to the B genome of durum and common wheat (Riley *et al*. 1958, Johnson 1975, Blake *et al*. 1999), but the homoeologous chromosomes of *Ae. speltoides* generally do not recombine with the corresponding homoeologous wheat chromosomes, largely owing to the presence of *Ph1*, a gene on the long arm of wheat chromosome 5B that prevents homoeologous chromosomes from pairing. Therefore, other strategies such as ionizing radiation treatment to induce chromosome breaks, or inducing homoeologous pairing using *ph1* mutants or high pairing lines of *Ae. speltoides* are needed to generate chromosomal translocations. Several useful genes for resistance to leaf rust (*Puccinia recondita* f.sp. *tritici* Rob. Ex Desm.), greenbug (*Schizaphis graminum* Rond.), powdery mildew (*Erysiphe graminis* DC.), and stem rust including genes *Sr32* and *Sr39* have been transferred from *Ae. speltoides* to wheat using radiation treatment or induced homoeologous recombination (Dubcovsky *et al*. 1998; see Friebe *et al*. 1996 for review).

The usefulness of an alien chromosomal translocation line in breeding is largely dependent on whether the introgressed alien segments carry genes for deleterious traits and whether they can compensate for the replaced wheat segments. Generally, translocations with smaller alien segments are genetically more stable and less likely to have deleterious effects. Cytogenetic techniques such as C-banding and fluorescent genomic in-situ hybridization (FGISH) are powerful tools for positioning and measuring the alien chromatin integrated into wheat genomes (see Jiang *et al*. 1994 for review; Cai *et al*. 1998). In addition, molecular marker technologies such as restriction fragment length polymorphisms (RFLPs) and microsatellites (simple sequence repeats; SSRs) have been used to enhance the characterization of alien translocations, leading to a more comprehensive understanding of the chromosomal segments involved in the translocations (Xu *et al*. 2005).

Dr Leonard R. Joppa (USDA-ARS, retired) developed a durum wheat line from crosses involving an *Ae. speltoides* accession with resistance to stem rust. Preliminary results of chromosome pairing analysis suggested that this durum line contained *Ae. speltoides* chromatin and might be a durum–*Ae. speltoides* translocation line (L. R. Joppa, personal communication, 2003). Dr James D. Miller (USDA-ARS, retired) evaluated this line for reaction to five

stem rust races (QTHJ, THTS, TCMJ, JCMN and HKHJ) and found that it was highly resistant to all five races (J. D. Miller, personal communication, 2001). The objectives of this work were (1) to characterize the chromosome constitution of this line using FGISH and molecular markers, (2) to determine the origin of the resistance gene(s) in the line and its relationship with *Ae. speltoides*-derived stem rust resistance genes *Sr32* and *Sr39*, and (3) to determine whether the line harbours resistance to Ug99. The molecular and cytogenetic characterization of this line will facilitate its deployment as a resistance source in wheat breeding.

Materials and methods

Plant materials and stem rust inoculations

The durum–*Ae. speltoides* line DAS15 was developed by Dr. L. R. Joppa (USDA-ARS retired) with a pedigree of LDN 5D(5B)/PI 369590//LDN/3/LDN 5D(5B)/4/3*47–1/5/CS*ph1b*/2*LDN//2*47–1/6/47–1. The durum line 47–1, which is highly susceptible to all known races of stem rust (Klindworth *et al*. 2006), constitutes most of the genetic background of the line. The *Ae. speltoides* accession PI 369590 was evaluated with 15 races of stem rust and found to be highly resistant to all of the races (J. D. Miller, personal communication, 2001). Because the pedigree of 47–1 contains the durum cultivars 'Langdon' (LDN) and 'Marruecos', and the common wheat 'Chinese Spring' (CS), these three genotypes were included in molecular marker analysis along with 47–1, *Ae. speltoides* PI 369590, and DAS15.

Seedlings of DAS15, 47–1, CnsSr32A.s. (donor of *Sr32*), RL6082 (donor of *Sr39*) and DAS15–134 (see below) were evaluated at the Northern Crop Science Laboratory in Fargo, ND for reaction to stem rust isolates A1 of QTHJ, A12 of THTS, and A21 of TCMJ. Seedlings were grown in the greenhouse at 20–23°C with a 16/8-hour (day/night) photoperiod. The preparation of inoculum and the inoculation of seedlings were performed as described in Williams *et al*. (1992). Urediniospore suspensions of individual cultures were used to inoculate seedlings when the primary leaf was fully expanded. After inoculation, seedlings were placed in a dew chamber with 100% relative humidity for 24 h at 21±2°C. After the humidity treatment, seedlings were moved to growth chambers at either 21 or 28°C. Infection types (ITs) were scored 14 days post inoculation using the scale described by Stakman et al (1962), where IT 0, ;, 1, 2 or combinations thereof were considered low infection types, and IT 3 to 4 were considered high infection types. The experiment consisted of three replicates for each temperature regime with three seedlings per replicate.

DAS15 and 47–1 were evaluated for reaction to isolates 74MN1409 of race TPMK, 01SD80A of race QCCJ, 01MN84A of race TTTT, and 04KEN156 and 98UGA1 of race TTKS at the USDA-ARS Cereal Disease Laboratory in St Paul, MN, USA, following the methods described by Jin & Singh (2006).

Fluorescence genomic in-situ hybridization (FGISH)

Fluorescence genomic in-situ hybridization was used to distinguish *Ae. speltoides* chromatin from wheat chromatin in DAS15 as described by Cai *et al*. (1998) and Xu *et al*. (2005). Seeds of DAS15 were germinated in Petri dishes at 25°C for about 24 h. Roots of 1–2 cm in length were collected and pretreated in ice water for 20–24 h. After pretreatment, the roots were fixed in a 3:1 mixture of ethanol and acetic acid for 48 h at room temperature. Somatic chromosomes were prepared from root tips in 45% acetic acid and observed under an Olympus phase-contrast microscope (Olympus Corp., Tokyo, Japan). Slides with good chromosome spreads were then stored at −80°C until needed for FGISH.

Total genomic DNA of LDN and *Ae. speltoides* PI 369590 was isolated as described by Faris *et al*. (2000). Genomic DNA of *Ae. speltoides* PI 369590 was labelled with biotin-16-dUTP as the FGISH probe by nick translation (Vector Laboratories, Inc., Burlingame, CA, USA). Genomic DNA of LDN was sheared as blocking DNA for FGISH in 0.4 M NaOH in boiling water for 40–50 min. Hybridization of the probe DNA to chromosomes and signal detection were conducted following the procedures of Mukai *et al*. (1993) and Cai *et al*. (1998). The *Ae. speltoides* chromatin was detected with fluorescein isothiocyanate-conjugated avidin (FITC-avidin) (Vector Laboratories) and durum wheat chromatin was counter-stained with propidium iodide (Roche Diagnostics Corp, Chicago, IL, USA). Slides were mounted in Vectashield (Vector Laboratories) antifading medium containing 1 μg/ml propidium iodide for counterstaining. FITC and propidium iodide were

excited at 450–490 nm. FGISH images were observed under an Olympus BX51 fluorescence microscope and captured using a CCD camera (Diagnostic Instruments, Inc., Sterling Heights, MI USA).

Molecular marker analysis

DAS15, the *Ae. speltoides* donor accession PI 369590, 47–1, LDN, Marruecos, and CS were tested for polymorphisms at molecular marker loci using RFLP probes and microsatellite PCR primers. DNA isolation, restriction enzyme digestion, gel electrophoresis, Southern blotting, and probe hybridization procedures were performed as described in Faris *et al*. (2000). Probes were tested for polymorphism on blots containing genomic DNA of the six genotypes digested with enzymes *Eco*RI, *Eco*RV, and *Hin*dIII. Microsatellite markers were amplified by PCR according to conditions described by Röder *et al*. (1998b). Amplified products were electrophoresed on 2.3% Metaphor agarose gels in 0.5× TBE, stained with ethidium bromide, and visualized under UV light.

Results

Identification and manipulation of Ae. speltoides chromatin in DAS15

Based on the preliminary results of Drs L. R. Joppa and J. D. Miller, DAS15 was resistant to several races of stem rust and was therefore likely to contain *Ae. speltoides* chromatin. In the present study, we directly visualized *Ae. speltoides* chromatin, which was involved in two pairs of durum–*Ae. speltoides* translocated chromosomes, in DAS15 using FGISH (Figure 1A). One pair of translocated chromosomes had a very small segment of an *Ae. speltoides* chromosome at the distal end of the long arm. The other pair of translocated chromosomes consisted of a large *Ae. speltoides* segment, which included all of one arm of an *Ae. speltoides* chromosome, the *Ae. speltoides* centromere, and a large portion of the other arm of the *Ae. speltoides* chromosome, attached to a small terminal segment of a durum chromosome (Figure 1A).

Because DAS15 was found to possess two *Ae. speltoides* translocations, it was necessary to determine which *Ae. speltoides* segment harbours the stem rust resistance gene(s). To do this, an F_2 population from a cross between DAS15 and 47–1 was developed. Root tips were collected from F_2 plants, and FGISH was conducted on five plants to identify plants homozygous for one translocation or the other. No plants homozygous for only the large translocation were identified, but four F_2 plants homozygous for the small *Ae. speltoides* segment and lacking the large *Ae. speltoides* translocation were identified (Figure 1B). One of these plants, designated DAS15–134 was included in the stem rust inoculation experiments.

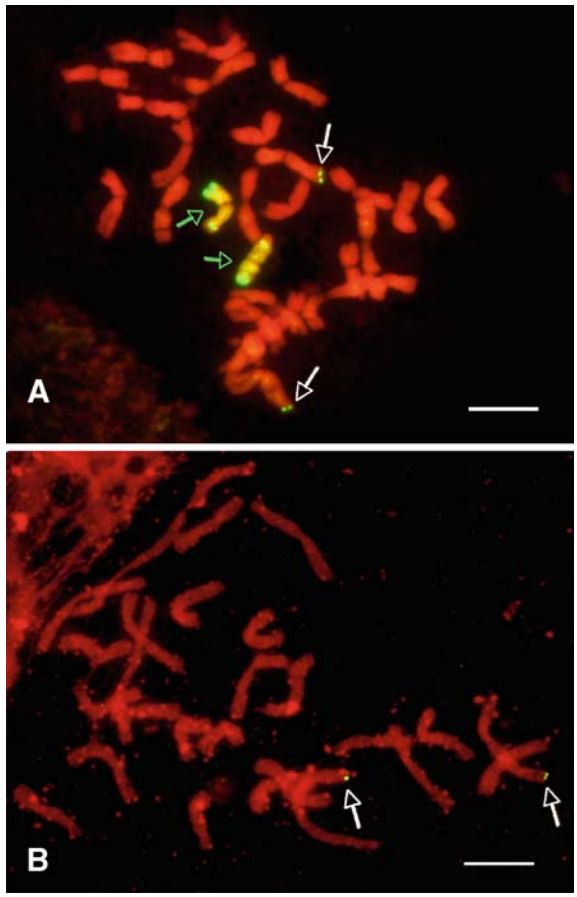

Figure 1. Fluorescence genomic in-situ hybridization patterns of mitotic chromosomes in durum wheat–*Ae. speltoides* chromosome translocation lines DAS15 (A) and DAS15–134 (B). The *Ae. speltoides* chromatin fluoresced yellow-green, and the wheat chromatin fluoresced red. The green arrows indicate the pair of T2BL-2SL•2SS translocation chromosomes, and the white arrows point to the other pair of translocated chromosomes. Scale bar represents 5 μm.

Table 1. Reactions of the durum line 47–1, the durum–*Ae. speltoides* translocation lines DAS15 and DAS15–134, and the *Sr32* and *Sr39* differentials to various races of the stem rust pathogen

Line	Race[a]									
	QTHJ		THTS		TCMJ		TPMK	QCCJ	TTTT	TTKS
	21[b]	28	21	28	21	28	18	18	18	18
47–1	34	34	34	4	34	34	34	4	4	4
DAS15	0;	0;	0	0	0;	0;	0	;	2=	2=
DAS15–134	34	34	34	34	34	34	–[c]	–	–	–
CnsSr32A.s. (*Sr32*)	2	3	23	34	23	34	–	–	–	–[d]
RL6082 (*Sr39*)	34	34	34	34	4	4	–	–	–	–[d]

[a]Inoculations with stem rust races QTHJ, THTS and TCMJ were conducted at Fargo, ND, USA, and inoculations with races TPMK, QCCJ, TTTT and TTKS were conducted at St Paul, MN, USA.
[b]Temperature in °C for the incubation period after inoculation.
[c]– indicates that the races were not tested on these lines.
[d]Data from Kenya indicated that these genes confer resistance to TTKS at the adult stage (Jin *et al*. 2007).

Stem rust reaction of the translocation lines

DAS15, DAS15–134, 47–1, CnsSr32A.s., and RL6082 were evaluated with three stem rust races at Fargo, ND, USA at two temperatures (Table 1). 47–1 and DAS15–134 were susceptible to all three races at both 21 and 28°C, showing infection of types 3 and 4. DAS15 was very resistant to all three races at both temperatures, showing only 0 and 0; infection types. This indicates that the stem rust resistance gene(s) derived from *Ae. speltoides* accession PI 369590 is located within the large *Ae. speltoides* chromosome segment rather than the small segment in DAS15.

CnsSr32A.s. (*Sr32* differential) showed moderately resistant to moderately susceptible infection types to the three races at 21°C, and they were moderately susceptible to susceptible at 28°C (Table 1). RL6082 (*Sr39* differential) was moderately susceptible to susceptible at both temperatures. These results indicate that the resistance gene(s) present in DAS15 differs from the *Ae. speltoides*-derived stem rust resistance genes *Sr32* and *Sr39* by at least one gene.

DAS15 and 47–1 were evaluated for reaction to stem rust races TPMK, QCCJ, TTTT and TTKS (Ug99) at St Paul, MN, USA. DAS15 exhibited low ITs of 0 and ; to TPMK and QCCJ, respectively, and a low IT 2 to races TTTT and TTKS, whereas 47–1 was susceptible to all four races (Table 1). Thus, the stem rust resistance conferred by the large *Ae. speltoides* segment in DAS15 is effective against race TTKS.

Molecular characterization of the large translocation in DAS15

A total of 50 RFLP probes (2–3 probes per wheat chromosome arm) were hybridized to membranes containing DNA of *Ae. speltoides* PI 369590, LDN, CS, Marruecos, 47–1, and DAS15 digested with three enzymes. Two of the 50 probes, BCD855 and KSUD8, detected fragments that were polymorphic between 47–1 and DAS15, and showed that DAS15 harboured a fragment from the *Ae. speltoides* accession PI 369590 (Figure 2A). BCD855 and KSUD8 are known to detect loci on the short and long arms, respectively, of homoeologous group 2 chromosomes in wheat and related species (http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker).

To determine whether the translocated chromosome involved durum chromosome 2A or 2B, we hybridized probe BCD855 to the CS nullisomic-tetrasomic (NT) lines (where a pair of missing chromosomes is compensated for by an extra pair of homoeologous chromosomes) digested with the same enzyme that revealed polymorphism between 47–1 and DAS15. BCD855 detected a single fragment on each of the homoeologous group 2 chromosomes (Figure 2B). The largest fragment was on chromosome 2A, a slightly smaller fragment belonged to 2B, and the smallest fragment was on 2D. Comparison of these fragments with those detected in 47–1 and DAS15 indicated that the chromosome 2A fragments were monomorphic

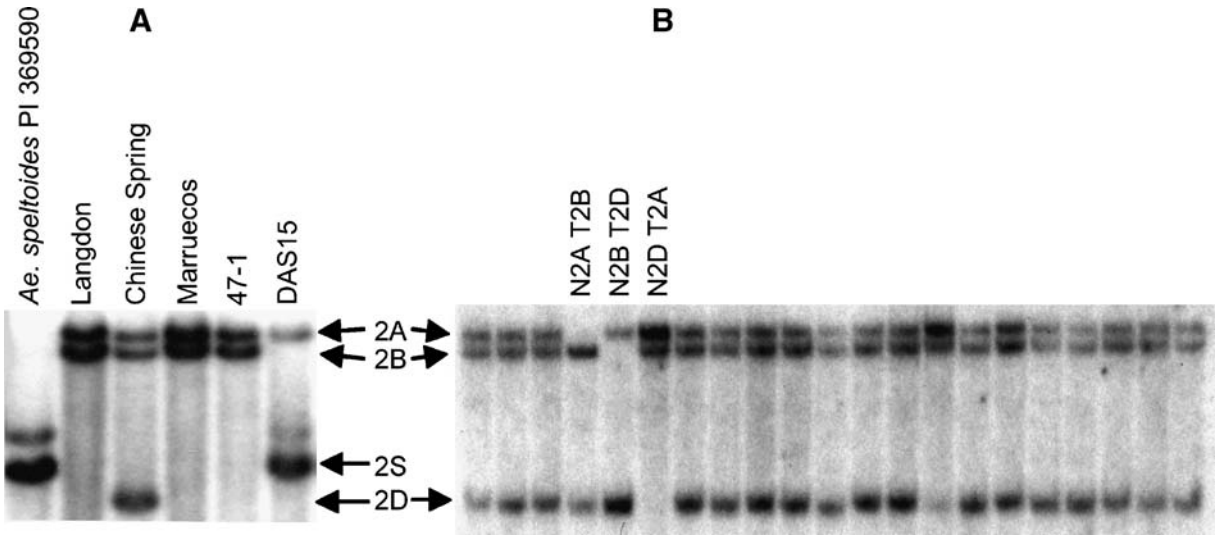

Figure 2. Autoradiograph images of probe BCD855 hybridized to DNA of wheat lines digested with restriction enzyme *Eco*RV. (A) The polymorphism between 47–1 and DAS15 indicates that the native 47–1 chromosome 2B fragment was replaced by the *Ae. speltoides* chromosome 2S fragment in the translocation line DAS15. (B) The Chinese Spring nullisomic-tetrasomic (NT) lines revealing the chromosome assignments of the three fragments detected by BCD855. Lanes for the homoeologous group 2 NT lines are indicated, whereas the NT designations for the other lanes are omitted.

between 47–1 and DAS15, but the 2B fragment of 47–1 was replaced by the *Ae. speltoides* fragment in DAS15. Therefore, the large translocation in DAS15 involves durum chromosome 2B and *Ae. speltoides* chromosome 2S.

In light of this result, we scanned chromosome 2B physical maps (Delaney *et al.* 1995, Röder *et al.* 1998a) and the 2B genetic maps generated by Nelson *et al.* (1995) and Röder *et al.* (1998b) for microsatellite markers and additional RFLP markers to further characterize the translocation. Twenty-one additional RFLP probes and four microsatellite markers (*Xgwm501*, *Xgwm47, Xgwm526*, and *Xgwm382*) were tested on *Ae. speltoides* PI 369590, LDN, CS, Marruecos, 47–1, and DAS15 as described above. Seventeen of the 21 RFLP probes showed polymorphism between 47–1 and DAS15 (Figure 3) and indicated that a fragment derived from 47–1 was replaced by a fragment of the *Ae. speltoides* donor in DAS15. Four of the 21 probes (CDO678, KSUD23, CDO36 and BCD1231), which all detect fragments at the distal end of the long arm of chromosome 2B, failed to reveal polymorphism between 47–1 and DAS15. Of the four microsatellite markers, *Xgwm501* and *Xgwm47*, which map proximal to the 2BL–6 deletion breakpoint, revealed polymporphism between 47–1 and DAS15 (Figure 4). *Xgwm526* and *Xgwm382*, which map distal to the 2BL–6 deletion breakpoint, were monomorphic, indicating that they detected 47–1 chromatin not replaced by the *Ae. speltoides* translocation. The amplification of *Ae. speltoides* DNA by the 2B-specific microsatellites *Xgwm501* and *Xgwm47* further corroborates the identity of the *Ae. speltoides* chromosome involved in the translocation as 2S.

Evaluation of the polymorphic and monomorphic markers along the physical and genetic maps of chromosome 2B allowed us to estimate the location of the translocation breakpoint. All ten markers located on the physical map indicated the presence of the *Ae. speltoides* translocation in DAS15 (Figure 3). This suggests that the physical location of the translocation breakpoint is within the 2BL-6 deletion bin, which is the most distal bin of the long arm and accounts for about 11% of the physical size of the arm. On the genetic map generated in the ITMI mapping population (Nelson *et al.* 1995, Röder *et al.* 1998b), the four monomorphic RFLP markers (*Xcdo678, XksuD23, Xcdo36*, and *Xbcd1231*) and the two monomorphic microsatellite markers (*Xgwm526* and *Xgwm382*) all mapped to the distal 65.3 cM of chromosome arm 2BL, whereas all of the polymorphic markers accounted for the entire short arm and about half of the long arm spanning a

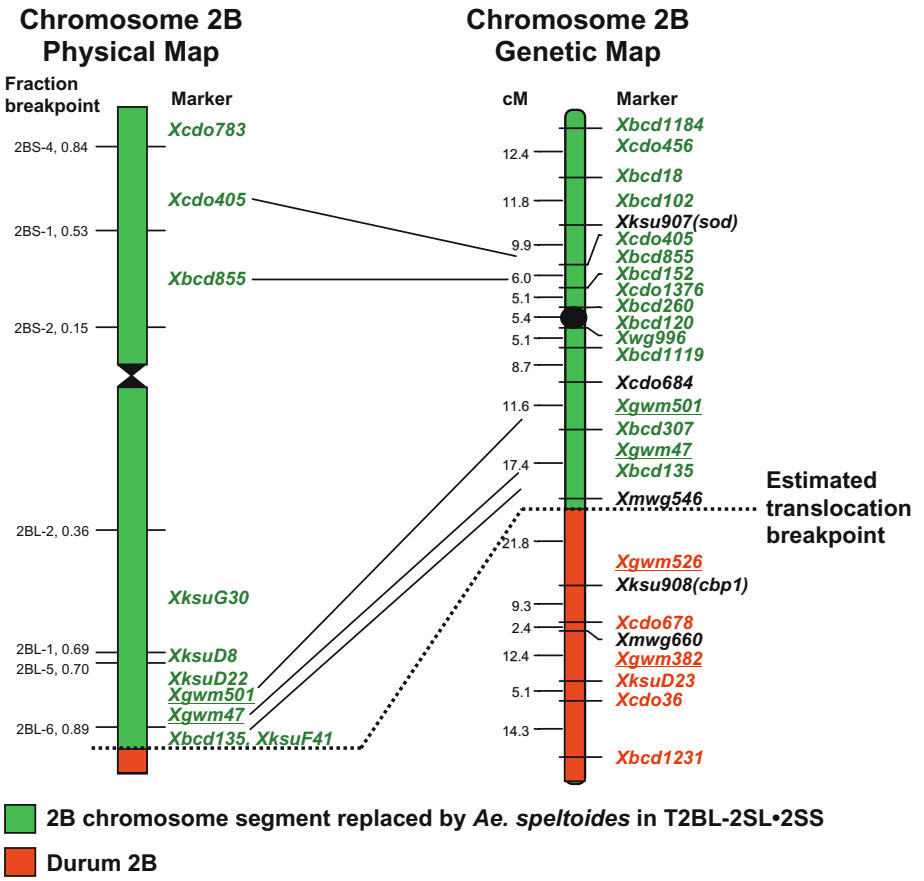

Figure 3. Wheat chromosome 2B physical (left) and genetic linkage (right) maps. On the physical map, deletion line designations and fraction breakpoints are indicated to the left and bin-mapped markers to the right. Centimorgan (cM) distances are shown along the left of the genetic map and markers along the right. Markers in green were polymorphic between 47–1 and DAS15, indicating the presence of *Ae. speltoides* chromosome 2S. Markers in orange were monomorphic and indicate the presence of 47–1 chromosome 2B. Markers in black were not evaluated in this work. Microsatellite markers are underlined and RFLP markers are not. The physical map was derived from Delaney *et al*. (1995) and Röder *et al*. (1998a) and the genetic map was derived from Nelson *et al*. (1995) and Röder *et al*. (1998b).

genetic distance of 93.4 cM. Therefore, based on the genetic map, the translocation breakpoint lies between markers *Xbcd135* and *Xgwm526*. These results together indicate that the composition of this durum-*Ae. speltoides* translocated chromosome in DAS15 is T2BL-2SL•2SS.

Discussion

The occurrence and spread of virulent stem rust races, such as TTKS (Ug99) and its variants, poses a serious threat to global wheat production. Few modern cultivars or adapted germplasms possess adequate resistance. It has been predicted that the route of spread of Ug99 could follow that of a *Yr9*-virulent pathotype of *P. striiformis* which, in the late 1980s, originated in Africa and subsequently spread to the Arabian peninsula, Syria, and eastward to Pakistan and India (Singh *et al*. 2006). It is therefore vital that new sources of resistance and effective resistance genes be identified, characterized, and deployed into adapted germplasm and varieties.

Here, we showed that the stem rust resistance gene(s) present in DAS15 is effective against Ug99, and we characterized the chromosomal segment harbouring the resistance gene in the T2BL-2SL•2SS translocation. Other genes shown to be effective against Ug99 include *Sr25*, *Sr26*, *Sr32*, *Sr37*, *Sr39*, *Sr40*, *Sr43*, and *Sr44* (Singh *et al*. 2006, Jin *et al*. 2007). However, most of these genes, like the one described in this work, are derived from wild relatives

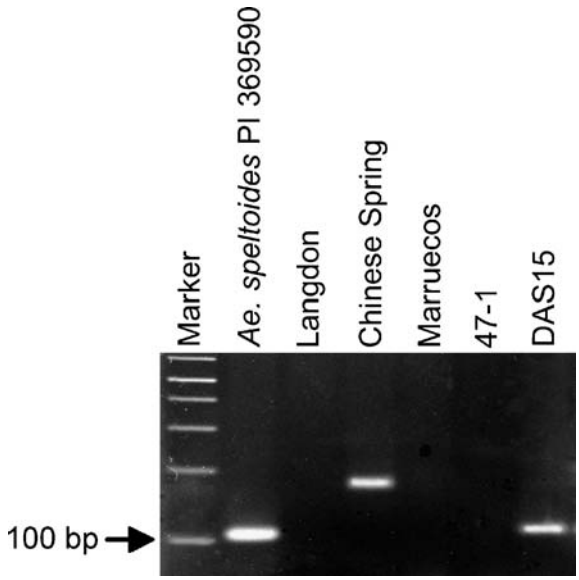

Figure 4. Agarose gel electrophoresis of the microsatellite marker *Xgwm501* on *Ae. speltoides* PI 369590, Langdon, Chinese Spring, Marruecos, 47–1, and DAS15 indicating the presence of the 2S fragment derived from *Ae. speltoides* in DAS15.

of wheat and are located on chromosome translocations that include large donor segments that harbour genes possibly deleterious to agronomic and quality traits. As well as assessing the initial translocations, attempts should be made to further induce homoeologous recombination using *ph1* mutants, irradiation, or other means to produce lines with smaller chromosome segments containing the resistance gene but lacking other genes with deleterious effects.

Of the genes listed above, *Sr32* and *Sr39* were transferred from *Ae. speltoides* to wheat backgrounds, and both genes originated from chromosome 2S of *Ae. speltoides*. The stem rust resistance present in DAS15 also originates from *Ae. speltoides* 2S, but it is likely that at least one gene in DAS15 differs from *Sr32* and *Sr39* because lines containing *Sr32* and *Sr39* gave moderately susceptible to susceptible seedling responses to the three stem rust races tested at Fargo, especially at higher temperature, whereas DAS15 was highly resistant to these races at both temperatures. It is noteworthy that the stem rust resistance of DAS15 resides in a tetraploid background, whereas *Sr32* and *Sr39* both reside in hexaploid backgrounds. Resistance genes are sometimes more effective at lower ploidy levels (R. McIntosh, personal communication, 2008) which lends the possibility that the resistance in DAS15 could be the same as *Sr32* or *Sr39*. However, the differences in the degrees of infection types between DAS15 and the *Sr32* and *Sr39* differentials exceed what might be expected from ploidy level differences. We propose to designate the resistance gene in DAS15 as *Sr47*. Limited seed for DAS15 is available upon request from the corresponding author.

Sears used homoeologous recombination to transfer *Sr32* from *Ae. speltoides* chromosome 2S to wheat chromosomes 2A, 2B, and 2D. The translocation involving wheat chromosome 2B consisted of the complete short arm of *Ae. speltoides* chromosome 2S and most of the long arm of wheat chromosome 2B, whereas the translocations involving wheat chromosomes 2A and 2D consisted of the complete short arm of *Ae. speltoides* chromosome 2S and most of the long arm of 2S with only small terminal segments of 2AL or 2DL, respectively (Friebe *et al*. 1996). These are configurations similar to that observed for the large translocation in DAS15. In work to induce homoeologous recombination and recover plants with shortened *Ae. speltoides* 2S segments, Dundas *et al*. (2007) indicated that the original translocation lines carried two stem rust resistance genes, *Sr32* and *Sr2S#1*. It is also possible that the *Ae. speltoides* segment in DAS15 carries multiple stem rust resistance genes.

Sr39 was transferred along with *Lr35* from *Ae. speltoides* chromosome 2S to wheat chromosome 2B through translocation (Kerber & Dyck 1990). The breakpoint in the translocation could not be determined by C-banding analysis, but the authors concluded that it probably consisted of segments derived from both arms of *Ae. speltoides* chromosome 2S (Friebe *et al*. 1996). Dundas *et al*. (2007) reported the development of several recombinant lines with shortened *Ae. speltoides* chromosome 2S segments and mentioned that *Sr39* is likely to be located on the distal portion of the T2BL-2SL•2SS translocation chromosome. These studies demonstrated that it is feasible to shorten *Ae. speltoides*-derived chromosome translocation segments, and our future research will focus on employing similar strategies to reduce the size of the *Ae. speltoides* segment in DAS15.

We showed that the FGISH technique was effective in identifying the chromosomal translocations and estimating their physical size, but it did not allow us to determine the identity of the chromosomes involved in the translocations. For this, molecular

markers proved very effective and aided in validating the physical size of the translocation determined by FGISH. The molecular markers used in this work, particularly the microsatellites, will be useful for screening new putative recombinants with reduced translocation sizes. In this strategy, the plants may be screened with markers prior to rust screening to ensure that only plants with new recombination events and smaller translocation segments are evaluated for resistance.

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