**Introgression and Characterization of a Goatgrass Gene for a High Level of Resistance to Ug99 Stem Rust in Tetraploid Wheat**

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**ABSTRACT** The transfer of alien genes to crop plants using chromosome engineering has been attempted infrequently in tetraploid durum wheat (Triticum turgidum L. subsp. durum). Here, we report a highly efficient approach for the transfer of two genes conferring resistance to stem rust race Pgt-TTKSK (Ug99) from goatgrass (Aegilops speltoides) to tetraploid wheat. The durum line DAS15, carrying the stem rust resistance gene Sr47 derived from Ae. speltoides, was crossed, and backcrossed, to durum 5D(5B) aneuploids to induce homeologous pairing. After a final cross to ‘Rusty’ durum, allosyndetic recombinants were recovered. The Ae. speltoides chromosomal segment carrying Sr47 was found to have two stem rust resistance genes. One gene conditioning an infection type (IT) 2 was located in the same chromosomal region of 2BS as Sr39 and was assigned the temporary gene symbol SrAes7t. Based on ITs observed on a diverse set of rust races, SrAes7t may be the same as Sr39. The second gene conditioned an IT 0; and was located on chromosome arm 2BL. This gene retained the symbol Sr47 because it had a different IT and map location from other stem rust resistance genes derived from Ae. speltoides. Allosyndetic recombinant lines carrying each gene on minimal alien chromosomal segments were identified as were molecular markers distinguishing each alien segment. This study demonstrated that chromosome engineering of Ae. speltoides segments is feasible in tetraploid wheat. The Sr47 gene confers high-level and broad spectrum resistance to stem rust and should be very useful in efforts to control TTKSK.

**KEYWORDS** wheat Ug99 Sr47 Aegilops speltoides chromosome engineering

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Common wheat (Triticum aestivum L., 2n = 6x = 42, AABBDD) and durum wheat (T. turgidum L., subsp. durum, 2n = 4x = 28, AABB) are major food sources (Singh et al. 2008). Stem rust (caused by Puccinia graminis Pers.:Pers. f. sp. tritici Eriks. and Henn.) has historically been one of the most important diseases of these crops (Singh et al. 2006). Although resistant cultivars have played a major role in the control of stem rust, the emergence of a new highly virulent race, TTKSK (Ug99), originating in Uganda in 1999, jeopardizes world wheat production (Singh et al. 2006, 2011). TTKSK has proven highly virulent, with an estimate of only 5% of Middle East and Southern Asia wheat acreage planted to resistant cultivars in 2005 to 2006 (Singh et al. 2008). In North America, the majority of wheat cultivars were susceptible to TTKSK (Jin and Singh 2006) or variants TTKST (Jin et al. 2008) and TTTSK (Jin et al. 2009). Finding and deploying stem rust resistance genes effective against the Ug99 lineage of races are vital to protecting the world’s wheat supply.

Wild relatives of wheat are important sources of new genes for cultivated wheat. In the past 40 years, numerous desirable genes, including approximately 20 stem rust resistance genes (McIntosh et al. 2010; Liu et al. 2011; Qi et al. 2011), have been transferred into common wheat from its wild relatives by developing wheat-aliens species chromosome translocations through chromosome engineering (Friebe et al. 1996; Gill et al. 2011). Because homologous chromosome
pairing in wheat is strictly controlled by \( Ph1 \) (pairing homeologous) on chromosome 5B, translocations between a wheat chromosome and its homeologue in wild species are usually induced using \( Ph1 \) deletion stocks such as a 5D(5B) substitution line or the \( ph1b \) mutant (Niu et al. 2011). Compared with its frequent uses in hexaploid wheat, chromosome engineering has been used sparingly in durum wheat, but the successful transfer of genes for high molecular weight glutenins (Ceoloni et al. 1996; Joppa et al. 1998), disease resistance (Huguet-Robert et al. 2001), salt tolerance (Luo et al. 1996), and kernel texture (Morris et al. 2011) have been documented. One major problem with chromosome engineering in a tetraploid background is poor plant vigor and low fertility of interspecific crosses. This may result from reduced genomic buffering and increased linkage drag as the result of durum wheat having only two genomes (AB), rather than three (ABD) as in common wheat (Ceoloni et al. 1996; Gennaro et al. 2007).

The durum wheat line DAS15, developed through \( ph1b \)-induced homeologous recombination by L. R. Joppa, carries the stem rust resistance gene \( Sr47 \) derived from an accession (PI 369590) of \( Aegilops speltoides \) Tausch (2\( n = 2 \times = 14 \), SS). This gene is highly effective against TTKSK, but it was located on a T2BL-2SL-2SS translocation chromosome in which the distal 2BL segment comprised less than 10% of the long arm, with the remainder of the chromosome originating from \( Ae. speltoides \) (Faris et al. 2008). To make \( Sr47 \) usable in wheat breeding, efforts are needed to reduce the \( Ae. speltoides \) segment. A set of aneuploids based on Rusty (PI 639869) (Klindworth et al. 2006), a near-universal stem rust susceptible genetic stock of durum wheat, has been recently established (Klindworth and Xu 2008). One of these aneuploids is the Rusty 5D(5B) double-monosomic (DM), in which one 5B chromosome has been replaced by chromosome 5D. The objectives of this research were to use the Rusty 5D(5B) aneuploids to reduce the \( Ae. speltoides \) segment carrying \( Sr47 \) and to test the feasibility of Rusty aneuploids in chromosome engineering of durum wheat.

### MATERIALS AND METHODS

#### Plant materials

DAS15 and Rusty were used for crossing and population development. Rusty is closely related to Line 47-1, differing mainly by plant ideotype and absence of the minor stem rust resistance gene, \( SrM \) (Klindworth et al. 2006). Rusty aneuploids used in this study included the Rusty 2D(2A) disomic substitution (DS), Rusty 2D(2B) DS, and Rusty 5D(5B) DM. Because the Rusty 5D(5B) DS did not yet exist when crossing was initiated, a 47-1 5D(5B) DS (Klindworth et al. 2007) was used when a 5D(5B) DS was needed for crosses. Like the Langdon 5D(5B) DS (Joppa and Williams 1988), the 47-1 5D(5B) DS is maintained with a 5B monosome.

#### Population development

The Rusty 5D(5B) DM was crossed to DAS15 (Figure 1, Step 1), and 20 \( F_1 \) plants were evaluated for chromosome pairing at metaphase I (MI) of meiosis. Seven DM (12\( ^{+} + 2B-2B/2S^{+} + 5D^{+} + 5D^{+} \)) \( F_1 \) plants with heteromorphic pairing (2B-2B/2S\(^{+}\)) between chromosome 2B and the translocation chromosome 2B/2S were selected and backcrossed as females to the 47-1 5D(5B) DS (Figure 1, Step 2). The rationale for using the \( F_1 \) plants as females was to avoid the high male-transmission rate of the 5B monosome, calculated as 90.3\% by Joppa and Williams (1988). Similarly, the male transmission of the 5B monosome is greater in 5D(5B) DM than in 5D(5B) DS, thus making it preferable to use the 47-1 5D(5B) DS rather than Rusty 5D(5B) DM as the male in the backcross. The \( BC_2 F_1 \) plants were tested for resistance to \( Pgt \).-1

#### Rust inoculation procedures

Following the procedures of Williams et al. (1992), we suspended stem rust urediniospores in nonphytotoxic, paraffinic oil and sprayed on 6- to 8-d-old seedlings. The plants remained in a subdued light mist chamber for 24 hr after inoculation. Seedlings were then moved to a greenhouse at 20 to 23\^\circ C with supplemental fluorescent light to maintain a 14/10-hr (day/night) photoperiod. Seedlings were classified for stem rust infection type (IT) 12-14 d after inoculation by scoring the infected primary leaf from each plant (Stakman et al. 1962; Roelfs and Martens 1988). In this system of notation, 0, fleck (.), 1, or 2 are considered resistant, and 3 or 4 are considered susceptible. For leaves exhibiting combinations of ITs, order indicates predominant types.

### Figure 1 Crossing and selection procedure for production of allosynthetic recombinants of tetraploid wheat carrying stem rust resistance gene \( Sr47 \). The underline symbol (_) indicates a missing 5B chromosome. Blue indicates genes derived from Rusty or 47-1 aneuploids. Red indicates either genes derived from DAS15 or the new allosynthetic recombinant. Ti2B represents intercallary translocation 2B, 2B-2B/2S\(^{+}\) represents heteromorphic pairings between chromosome 2B and the translocation chromosome 2B/2S, and 2B-Ti2B\(^{+}\) represents heteromorphic pairings between chromosome 2B and the intercallary translocation 2B.

#### Table 1 Crossing and selection procedure for production of allosynthetic recombinants of tetraploid wheat carrying stem rust resistance gene \( Sr47 \).

| Step | **Crossing and Selection** |
|------|---------------------------|
| 1    | Rusty 5D(5B) DM (13\(^{+} + 5B^{+} + 5D^{+}\)) × DAS15 (12\(^+\) + 2B/2S\(^{+}\)) Ph1\_Ssr<sup>a</sup> | Select Ph1\_F<sub>1</sub> plants with 13\(^+\)2<sub>1</sub>
| 2    | (12\(^+\) + 2B-2B/2S\(^+\) + 5D\(^+\)) Ph1\_Ssr<sup>a</sup> N<sub>B</sub>C<sub>_1</sub> (12\(^+\) + 2B/2S\(^+\) + 5D\(^+\)) Ph1\_Ssr<sup>a</sup> N<sub>S</sub> | 47-1 5D(5B) DS (13\(^+\) + 5D\(^+\)) Ph1\_Ssr<sup>a</sup> Select __Ssr<sub>1</sub> BC<sub>_1</sub> by testing with stem rust and Ph1 molecular markers
| 3    | Rusty Ph1Ph1Ssr<sup>a</sup> × BC<sub>_1</sub> (12\(^+\) + 2B-2B/2S\(^+\) + 5D\(^+\)) Ph1\_Ssr<sup>a</sup> N<sub>S</sub> | Screen for rust resistance and reduced alien translocation size using SSR markers
| 4    | BC<sub>_1</sub>F<sub>1</sub> (12\(^+\) + 2B-Ti2B\(^{+}\) + 5D\(^+\)) Ph1\_Ssr<sup>a</sup> N<sub>S</sub> | Vertically reduced alien translocation size using SSR markers and FGISH
| 5    | BC<sub>_1</sub>F<sub>1</sub> euploid (13\(^+\) + Ti2B\(^{+}\)) Ph1Ph1Ssr<sup>a</sup> N<sub>S</sub> | TMLKC using inoculation procedures as mentioned in the section Rust inoculation procedures. Resistant BC<sub>_1</sub>F<sub>1</sub> plants were tested for the presence of chromosome 5B using 5BL-specific markers Xpsr128 and Xpsr574 (Roberts et al. 1999).

In hexaploid wheat, marker XAWJL3 can be used as a positive amplification check (Roberts et al. 1999), but we found that this marker was unreliable in tetraploid wheat (supporting information, Figure S1). Instead, simple sequence repeat (SSR) marker Xedm80 was used as a positive check (Figure S1 and Figure S2) (Mullan et al. 2003). Plants negative for the Xpsr128 and Xpsr574 bands were 5D(5B) DS carrying a single 2B/2S translocation chromosome (i.e., 12\(^+\) + 2B-2B/2S\(^+\) + 5D\(^+\)) and they were selected and crossed as males to Rusty to produce a large BC<sub>_1</sub>F<sub>1</sub> population (Figure 1, Step 3). This population was first tested for resistance to TMLKC and then tested for allosynthetic recombination of the \( Ae. speltoides \) chromatin carrying \( Sr47 \) using capillary electrophoresis as described below (Figure 1, Step 4). Plants exhibiting dissociation were self-pollinated to recover euploid progeny and to select homozygous dissociation lines (Figure 1, Step 5).
DNA was extracted from the BC2F1 population developed above using molecular marker analysis or large pustules within a class. The entire 2B/2S chromosome were screened for polymorphism type the BC2F1 population as described by Niu et al. (2009). Five codominant markers (Xgpw4043, Xgwm501, Sr39#22r, Xwmc672, and Xgwm526) were used to map allosyndetic recombinants. Markers shown in red were monomorphic and therefore located on the wheat segment of the 2BL-2SL-2S5S chromosome. Markers in blue were used for capillary electrophoresis of the complete population. Markers in green were used in PAGE to analyze subsets of allosyndetic recombinants identified by capillary electrophoresis. Marker order and distances (cm) generally follow Sourdille et al. (2011). Positions of those markers not shown on the Sourdille et al. (2010) map were inferred from either Somers et al. (2004), Mago et al. (2009), Dobrovolskaya et al. (2011), or Niu et al. (2011). Because of limited published data, order of markers clustered around the IT 2 gene on 2BS/2SS chromosome in DAS15/C1 could not be fully determined, and these markers are listed in no particular order.

Minus (−), double minus (−−), and plus (+) indicated small, very small, or large pustules within a class.

Molecular marker analysis
DNA was extracted from the BC2F1 population developed above using 96-well plates as described by Niu et al. (2011). With the goal of finding SSR markers that are useful for selection of allosyndetic recombinants from the BC2F1 population, 36 SSR markers spanning the entire 2B/2S chromosome were screened for polymorphism among Rusty, DAS15, Rusty 2D(2A)D, and Rusty 2D(2B)D using an ABI 313Oxl Genetic Analyzer (Applied Biosystems, Foster City, CA) as described by Tsoio et al. (2009). Five codominant markers (Xgwm55, Xgwm319, Xwmc474, Xbarc55, and Xcfa2278; Figure 2) were found to be suitable for marker-assisted selection in the capillary electrophoresis system (Table S1), and then they were used to genotype the BC2F1 population as described by Niu et al. (2011).

After completing capillary electrophoresis, additional SSR markers located on wheat chromosome 2B or 2S were reported by Somers et al. (2004), Mago et al. (2009), Sourdille et al. (2010), Niu et al. (2011), and Dobrovolskaya et al. (2011) were evaluated for polymorphisms using polycrylamide gel electrophoresis (PAGE). Four polymorphic markers, Xgwm4043, Xgwm501, Sr39#22r, and Xgwm6614, were used to evaluate all BC2F1 allosyndetic recombinants identified by capillary electrophoresis. For all PAGE, polymerase chain reaction products were run on 10 cm mini-gels composed of 8% acrylamide. Electrophoresis was conducted at 150 V for 40 min if expected products were less than 150 bp. For expected products larger than 150 bp, electrophoresis was conducted for 50–55 min with the exception of Xwmc27, Xrws28, and Xrws29, for which electrophoresis was extended to 135 min. Polymerase chain reaction products were stained with 2X GelRed, and gels were visualized with UV light and photographed.

In progeny evaluations, homozygous rust-resistant BC2F2 plants were identified either through marker analysis or stem rust testing. For marker analysis, homozygous IT 0; and IT 2 plants were identified using markers Xgpw4043 or Sr39#50s, respectively. For rust tests, homozygous BC2F2 plants were identified by BC2F2 progeny tests. DNA from BC2F2 plants identified as homozygous by either selection method was included in additional marker tests.

Tests for segregation distortion and validation of markers
Five allosyndetic recombinant lines were selected to test for segregation distortion. Progeny from plants known to be heterozygous for the translocated segment were tested with race TMLKC and classified as resistant or susceptible. For each family, plants were tested with appropriate SSR markers and classified as homozygous resistant, heterozygous, or susceptible. Data were tested for goodness of fit to a 1:2:1 ratio using $\chi^2$ analysis. Marker validation was tested on Rusty, LMPG6, and the set of eight durum and 32 common wheat cultivars described by Niu et al. (2011). LMPG6 is a common wheat line from Canada with spring growth habit that is near-universally susceptible to stem rust (Knott 1990).

Flourescent genomic in situ hybridization (FGISH) and measurement of translocation breakpoints and fraction lengths
FGISH was used to detect Ae. speltoides segments in the allosyndetic recombinant lines by using the genomic DNA of Ae. speltoides PI 369590 and common wheat cultivar ‘Chinese Spring’ as probe and blocking DNA, respectively. FGISH was performed using the protocol described by Yu et al. (2010). The fraction lengths (FL) of translocation breakpoints relative to chromosomal length (Endo and Gill 1996; Friebe et al. 1996) in nine intercalary translocation (Ti) 2BL-2SL-2BS lines were measured. The distal and proximal FL values were measured as the distance from the 2BL telomere to the distal or proximal breakpoints divided by the chromosomal length. The Ae. speltoides segment FL value was the proximal minus the distal FL value. Lengths were measured in 17 to 21 good-quality mitotic metaphase cells per line. Data were analyzed as a completely randomized design using the SAS GLM procedure (SAS Institute 2004), and means were separated by least significant difference.

RESULTS
Development and selection of allosyndetic recombinants
There were 218 BC1 (Figure 1, Step 2) plants having the pedigree Rusty 5D(5B)/DAS15/47-1 5D(5B) tested for resistance to race TMLKC, and these plants segregated 89 susceptible to 129 resistant. This segregation did not fit a 1:1 ratio ($\chi^2 = 7.34$, $P = 0.007$), and because 47-1 5D(5B) was the male parent of the cross, this result suggested there was minor segregation distortion through female gametes. Resistant plants were tested with chromosome 5BL-specific markers Xpsr128 and Xpsr574 (Figure S2). There were 52 BC1 plants that did not carry chromosome 5B as indicated by the failure to amplify the Xpsr128 and Xpsr574 alleles on 5B. These plants were 5D(5B) DS, which had 28 chromosomes with pairing configurations of 12+2B-2S/2B+5D-. Because of their lack of chromosome 5B, homologous pairing would occur in these 52 BC1 plants, and they were crossed as males to Rusty to produce 1086 BC2F1 seeds for use in selection of recombinant lines.

The 1086 BC2F1 plants were tested with TMLKC. There were 893 resistant and 193 susceptible plants, which did not fit a 1:1 segregation
and 37 plants had IT 2. Among the IT 2 plants, 32 retained the S2) that are summarized in Table 1. Forty-two of the plants had IT 0; and IT 2 recombinant plants comprised 9 and 12 haplotypes, respectively (Table 1). For the IT 0; gene, all 42 allosyndetic recombinants retained the Ae. speltoides allele for either Xgwp4043 alone (9 plants), Xgwm501 alone (6 plants), or both markers (27 plants). For the remaining 7 markers, no marker retained the Ae. speltoides allele in more than 4 of 42 plants (Table 1). This result indicated that the IT 0; gene in DAS15 was located near Xgwp4043 and Xgwm501 on 2BL. Among the 37 IT 2 allosyndetic recombinants (Table 1), the two having the shortest Ae. speltoides segment retained the Sr39#22r allele from Ae. speltoides. The only Ae. speltoides allele retained in all 37 plants was the Sr39#22r allele, indicating that the IT 2 gene was located near Sr39#22r in 2BS.

The 81 BC2F1 plants had chromosome pairing configurations of 12B + 2B-2B/2S’ + 5B’+ 5D’ at MI. Both the 2B-2B/2S heteromorphic bivalent and the DM condition reduce plant fertility. These plants produced on average 13.6 seeds per plant, with nine plants being sterile and one plant (0696) producing 209 seeds (Table S2). On the basis of marker analysis or stem rust testing on BC2F2 progenies, 14 and seven BC2F2 plants that were homozygous for ITs 0; and 2, respectively, were selected for additional marker analysis. In selecting markers to test on IT 0; plants, only markers proximal to Xgwp7506 were studied because Xgwp7506 was located in the wheat segment of the original translocation chromosome in DAS15 (Figure 2 and Figure S4). Polymorphisms for Xgwp4112, Xgwm501, and Xgwm47 were attributable to allele-specific amplification failure, which could be expressed as either a difference in staining intensity or as absence of the amplicon (Figure S5). Line 0406 carried wheat alleles for the four most distal markers (Table 2, Figure S5). Seven lines, including Line 0010, carried wheat alleles for the two most proximal markers. The combination of these results indicated that the IT 0; gene was located between markers Xgwm501 and Xwmc332 (Table 2). The homozygous IT 2 Line 0797 carried the shortest interstitial translocation, with wheat alleles at both the Xbarc183 and Xwmc25 loci (Table 3 and Figure S6). The IT 2 gene lies between these two loci, along with eight markers whose map order was not determined (Figure 2).

**FGISH analysis and measurements of Ti2BL-2SL-2BL 2BS fraction lengths**

Fourteen IT 0; and five IT 2 allosyndetic recombinants were analyzed by FGISH, and micro-photographs for five lines having IT 0; and four lines having IT 2, are shown in Figure 4. All IT 2 lines retained an Ae. speltoides segment in chromosome arm 2BS, with Line 0797 retaining the shortest segment. Lines 0151 and 0902 were IT 2 lines having only small reductions of Ae. speltoides chromatin in the subtelomeric region of 2BL (Figure 4). The IT 0; gene must be located in this small deleted segment; therefore, the FGISH confirmed that the IT 0; gene was located near the break-point of the original 2B/2S translocation chromosome in DAS15. All IT 0; lines retained an Ae. speltoides chromosomal segment in the subtelomeric region of 2BL.

An interesting feature revealed by the FGISH analysis was that some lines had recombination events in both chromosome arms (Figure 4). This finding agreed with results from the marker analysis. For example, Lines 1009 and 0797 retained the Ae. speltoides chromatin around Sr39#22r and Sr39#50s in 2BS and also retained the Ae. speltoides allele for Xgwp4043 in 2BL (Figure 4; Table 3).

The physical positions of the translocation breakpoints in nine Ti2BL-2SL-2BL 2BS allosyndetic recombinant lines were determined
by measuring the distance from the 2BL telomere to the distal and proximal breakpoints. The nine lines carried relatively small *Ae. speltoides* segments (Table 4). The distal FL breakpoints indicated that wheat chromatin comprised between 0.0900 and 0.1238 of the 2BL telomere and subtelomeric region (Table 4). Lines 0406, 0439, and 0801 had high distal breakpoint values, and this was in general agreement with the molecular marker analysis, which showed that, in this region, lines 0406 and 0801 had the wheat allele at *Xgpw4043* (Table 2). Furthermore, the low proximal breakpoint values of Lines 0717, 0790, and 0804 (Table 4) was in agreement with these lines carrying *Ae. speltoides* alleles at *Xgwm501* and *Xgpw4112* (Table 2).

**Segregation distortion and stem rust resistance of selected translocation lines and validation of markers**

Lines 0406, 0696, and 0717, carrying the IT 0; gene, and lines 0406 and 0797 carrying the IT 2 gene, were tested for segregation of resistance and to confirm that the markers could be used for marker-assisted selection (Table S3). Lines 0744 and 0797 were selected because they carried the IT 2 gene on the shortest *Ae. speltoides* segments. Lines 0406, 0696, and 0717 were selected because they carried the IT 0; gene on short, but slightly different, *Ae. speltoides* segments (Table 2).

Progeny from a known heterozygous plant for each of the five lines were tested with stem rust and markers. Results indicated significant segregation distortion in all populations except Line 0406 (Table S3). Segregation distortion resulted in selection against the alien segment in the two lines carrying the IT 2 gene, Lines 0744 and 0797, where only 5.0% and 3.4% of plants were homozygous resistant, respectively. This finding was reversed in Lines 0696 and 0717, where an excess of resistant plants was observed, indicating preferential transmission of the *Ae. speltoides* segment.

Two IT 0; lines (0406 and 0696) and one IT 2 line (1009) with the shortest *Ae. speltoides* segments were tested with race TTKSK and 13 North American races (Table 5). Lines 0406 and 0696 had minor differences from each other on the 14 races. ITs observed on 0406 and 0696 ranged from 0; to 0;12 on the North American races, but when tested with TTKSK, an IT of ;22 was observed on both lines. Although this was a greater IT than observed on DAS15, it still provided a good

### Table 1 Summary of haplotypes for nine SSR markers observed in the BC$_2$F$_1$ generation of Rusty/3/Rusty 5D(5B)/DAS15//47-1 5D(5B) allosyndetic recombinants

| IT     | *Xgpw4043* | *Xgwm501* | *Xcfa2278* | *Xgwm55* | *Xgwm319* | *Xwmc474* | *Xbarc55* | *Sr39#22r* | *Xgwm614* | No. of Plants |
|--------|------------|-----------|------------|----------|-----------|-----------|-----------|------------|-----------|--------------|
| 0;     | S          | W         | W          | W        | W         | W         | W         | S          | W         | 7            |
| 0;     | S          | W         | W          | W        | W         | W         | W         | S          | 2         |              |
| 0;     | W          | S         | W          | W        | W         | W         | W         | S          | W         |              |
| 0;     | S          | S         | W          | W        | W         | W         | W         | S          | S         |              |
| 0;     | S          | S         | W          | S        | W         | W         | W         | S          | S         |              |
| 0;     | S          | S         | S          | S        | W         | W         | W         | S          | W         |              |
| 0;     | W          | S         | S          | S        | S         | S         | S         | S          | W         |              |
| Total  |            |           |            |          |           |           |           |            |           | 42           |
| 2      | S          | S         | S          | S        | S         | S         | S         | S          | S         | 1            |
| 2      | W          | S         | S          | S        | S         | S         | S         | S          | W         |              |
| 2      | S          | W         | S          | S        | S         | S         | S         | S          | S         |              |
| 2      | W          | W         | S          | S        | S         | S         | S         | S          | S         | 20           |
| 2      | W          | W         | S          | S        | S         | S         | S         | S          | S         |              |
| 2      | S          | W         | S          | S        | S         | S         | S         | W          | 2         |              |
| 2      | S          | W         | W          | W        | W         | W         | S         | S          | S         |              |
| 2      | S          | W         | W          | W        | W         | W         | W         | S          | W         |              |
| 2      | W          | W         | W          | W        | W         | W         | W         | S          | W         |              |
| Total  |            |           |            |          |           |           |           |            |           | 37           |
| 34     | S          | S         | S          | S        | S         | S         | W         | W          | S         |              |
| 34     | W          | S         | S          | S        | S         | S         | W         | W          | W         |              |
| Total  |            |           |            |          |           |           |           |            |           | 2            |

Markers are listed in order from most distal on 2BL (left) to most distal on 2BS (right) as suggested by maps of Sourdille et al. (2010) and Mago et al. (2009): S, *Ae. speltoides* allele; W, wheat allele.

### Table 2 Haplotypes for seven SSR markers in 14 homozygous Ti2BL-2SL-2BL/C12BS allosyndetic recombinants carrying the IT 0; (fleck) gene from DAS15

| Line   | *Xgpw4043* | *Xcfa267* | *Xwmc627* | *Xwmc332* | *Xgwm4165* | *Xwmc47* | *Xgwm501* | *Xgwm4112* |
|--------|------------|-----------|-----------|-----------|------------|-----------|-----------|------------|
| 0010   | S          | ?         | S         | S         | S          | S         | S         | W          |
| 0143   | S          | S         | S         | S         | S          | S         | S         | W          |
| 0198   | S          | S         | S         | S         | S          | S         | S         | S          |
| 0225   | S          | S         | S         | S         | S          | S         | S         | W          |
| 0406   | W          | W         | W         | W         | S          | S         | S         | S          |
| 0439   | S          | S         | S         | S         | S          | S         | S         | S          |
| 0466   | S          | W         | S         | S         | S          | S         | S         | W          |
| 0623   | S          | ?         | S         | S         | S          | S         | S         | S          |
| 0696   | S          | S         | S         | S         | S          | S         | S         | S          |
| 0717   | S          | W         | S         | S         | S          | S         | S         | W          |
| 0735   | S          | S         | S         | S         | S          | S         | S         | S          |
| 0790   | S          | S         | S         | S         | S          | S         | S         | W          |
| 0801   | W          | S         | W         | S         | S          | S         | S         | S          |
| 0804   | S          | W         | S         | S         | S          | S         | S         | W          |

Markers are listed in order from most distal (left) to most proximal (right) on 2BL as suggested by maps of Sourdille et al. (2010) and Somers et al. (2004): S, *Ae. speltoides* allele; 1, unknown; W, wheat allele.
level of resistance to TTKSK. Line 1009 having IT 2 was compared with line RWG1 (Niu et al. 2011), which carries Sr39. The results indicated highly similar ITs of Line 1009 and RWG1. Some minor difference could be attributed to genetic background or ploidy level. We concluded that over the 14 races in the test, the ITs conditioned by the genes in Line 1009 and RWG1 did not differ.

Seven markers were tested on a set of 40 diverse common and durum wheat cultivars (Figure 5). Markers Sr39#50s and Sr39#22r were linked to the IT 2 gene on 2BS. Sr39#50s is a codominant marker that amplified a 268-bp fragment from Ae. speltoides and a 236-bp fragment in all 40 cultivars (Figure 5 and Figure S6) and is the preferred marker. Dominant marker Sr39#22r amplified a 1026-bp fragment from Ae. speltoides that was absent in all 40 cultivars (Figure 5). The five remaining markers were all linked to the IT 0; gene on 2BL. Among these markers, the dominant marker Xgpw4112 produced a null allele from the Ae. speltoides and Chinese cultivar Jimai22, but it amplified fragment in 39 of the 40 cultivars (Figure 5). For Xgpw4112, there was polymorphism among cultivars. The dominant marker Xgwm501 amplified a 109-bp fragment from the Ae. speltoides and Chinese cultivar Jimai22, but it amplified fragments in 39 of the 40 cultivars (Figure 5). For Xgwm501, there was polymorphism among cultivars. The dominant marker Xgwm501 amplified a 109-bp fragment from the Ae. speltoides and Chinese cultivar Jimai22, but it amplified fragments in 39 of the 40 cultivars (Figure 5). For Xgwm501, there was polymorphism among cultivars. The dominant marker Xgwm501 amplified a 109-bp fragment from the Ae. speltoides and Chinese cultivar Jimai22, but it amplified fragments in 39 of the 40 cultivars (Figure 5). For Xgwm501, there was polymorphism among cultivars. The dominant marker Xgwm501 amplified a 109-bp fragment from the Ae. speltoides and Chinese cultivar Jimai22, but it amplified fragments in 39 of the 40 cultivars (Figure 5). For Xgwm501, there was polymorphism among cultivars.

However, the eight markers detecting only Ae. speltoides chromatin in all seven lines (Table 3), marker order could not be determined from this study. S, Ae. speltoides allele; W, wheat allele.

In summary, the five markers associated with the IT 0; gene produced good amplification of fragments with durum wheat, but breeders will need to carefully match markers with cultivars to transfer the IT 0; gene to common wheat cultivars.
Table 4 FL of the wheat segments and the Ae. speltoides segments in nine Ti2BL-2SL-2BL-2BS translocation chromosomes

| Dissociation Line | Proximal Breakpoint | Wheat Segment (Distal Breakpoint) | Ae. speltoides Segment |
|-------------------|---------------------|----------------------------------|-----------------------|
| 0406              | 0.1916 AB           | 0.1235 A                         | 0.0680 BCD            |
| 0439              | 0.2031 A            | 0.1238 A                         | 0.0793 A              |
| 0623 (plant 1)    | 0.1886 AB           | 0.1084 C                         | 0.0802 A              |
| 0623 (plant 2)    | 0.1733 CD           | 0.1084 C                         | 0.0649 CD             |
| 0696              | 0.1812 BC           | 0.1098 BC                         | 0.0714 ABC            |
| 0717              | 0.1717 CD           | 0.1111 BC                         | 0.0606 D              |
| 0735              | 0.1728 CD           | 0.1038 CD                         | 0.0690 BCD            |
| 0790              | 0.1648 D            | 0.0900 E                         | 0.0748 AB             |
| 0801              | 0.1968 A            | 0.1200 AB                         | 0.0767 AB             |
| 0804              | 0.1653 D            | 0.0942 DE                         | 0.0711 ABC            |
| DAS15             | 0.0675 F            |                                  | 0.9325                |
| Mean              | 0.1814              | 0.1055                            | 0.0719*               |
| LSD (P = 0.05)    | 0.0149              | 0.0109                            | 0.0097*               |

FL, fraction length; LSD, least square difference.

a Means followed by the same letter were not significantly different as determined by LSD.
b The length from the 2BL telomere to the proximal breakpoint divided by the whole chromosome length.
c The length from the 2BL telomere to the distal breakpoint divided by the whole chromosome length.
d Calculated as the proximal breakpoint minus the distal breakpoint.
e Means and LSD for the size of the Ae. speltoides segment were calculated excluding the data from DAS15.

DISCUSSION

Chromosome engineering of tetraploid wheat has been attempted less frequently than in hexaploid wheat. This has partially been attributable to the fact that more research is conducted on hexaploid than on tetraploid wheat, which results in decreased plant fertility and lower recovery rates of allosyndetic recombinants. We monitored seed fertility of DM plants in this experiment to determine whether low seed set would prevent the use of durum 5D(5B) aneuploids in chromosome engineering. Although the seed set was very low, with a mean of only 13.6 seeds per plant, this was sufficient to conclude that chromosome engineering in tetraploid wheat is feasible. However, the efficiency of chromosome engineering in tetraploid wheat would be more dependent on the strategies for development of allosyndetic recombinants than in hexaploid wheat. Both F2 (Qi et al. 2007) and backcross (Marais et al. 2010; Niu et al. 2011) populations have been successfully used to develop ph1b-induced allosyndetic recombinants in hexaploid wheat. The use of a backcross in the present experiment may have been advantageous over an F2 population in improving seed fertility of progenies derived from the hybrids with 5D(5B) aneuploids and in hastening the transfer of allosyndetic recombinants to a euploid background.

Niu et al. (2011) used a single SSR marker, Xgwm319, to identify allosyndetic recombinants for Sr39. In the present experiment, we used Xgwm319 and four other SSR markers in our initial screening. These five markers were clustered in the pericentromeric regions of 2BS and 2BL (Figure 2). The results indicated that using relatively few markers in the proximal regions of the 2B chromosome was highly effective in recovering allosyndetic recombinants. For example, if we had relied only on marker Xgwm319, we would have recovered 40 of the 42 allosyndetic recombinants that carried the IT 0; gene and would have recovered all five IT 2 lines having the shortest Ae. speltoides chromatin (Table 2). This was caused by lower allosyndetic recombination within the pericentromeric regions as compared with subtelo-meric or telomeric regions. This result agreed with the conclusions of Lukaszewski (1995), who found that ph1b-induced homeologous recombination of chromosomes 7A of wheat and 7S of Ae. speltoides was concentrated in the distal regions and absent near the centromere. We found several double recombinant events, and in this regard, our study differs from Lukaszewski (1995) and Lukaszewski et al. (2004). Our recovery of double recombinant events on a single chromosome may reflect the higher homology of chromosomes 2B and 2S as compared to the rye (Secale cereale L.) 2R chromosome.

Segregation distortion is a common feature in wheat-Ae. speltoides crosses (Zhang and Dvořák 1990). We observed segregation distortion in our initial crosses and also within four of five selected recombinants having short Ae. speltoides segments. In an intervarietal wheat map, Xue et al. (2008) observed 15 wheat segments carrying segregation distortion loci, including one on 2BL and 2BS, with the 2BL locus being centered on Xgwm47. In another intervarietal wheat mapping study, Paillard et al. (2003) noted that segregation distortion in chromosome 2B was greater than in any other chromosome. In addition to segregation distortion genes, gametocidal genes are present on chromosome 2S of Ae. speltoides (Tsujimoto and Tsunewaki 1988). Our results support the conclusion that Sd and/or Gc genes play a large role in the difficulty of recovery of S/B-genome allosyndetic recombinants.

We found that the T2BL-2SL-2BS chromosome in DAS15 actually carried two stem resistance genes. The IT 0; gene was located in a 2SL chromosome segment and lying between Xgwm501 and Xwms332. The size of this interval has been estimated as little as 3 cM on the Wheat Composite-2004 map (GrainGenes), to 8 cM in the Wheat Consensus map (Somers et al. 2004). Therefore, the IT 0; gene may be tightly linked to Xgwm47 and Xgwm165. The stem rust resistance gene Sr9a was mapped to only 0.9 cM distal to Xgwm47 (Tsilo et al. 2007). On the basis of their similar map positions, it is possible that the IT 0; gene is homoeoallelic to Sr9. The IT 2 gene was located to a T2BL-2BS-2BS chromosome and found to lie in the interval between Xbarc183 and Xwms25. We found eight molecular markers that map to this region, including Xgwl148, which has been mapped to bin 2BS3-0.75-0.84 (Sourdille et al. 2010). This is the same region shown by Niu et al.
genes necessitates assigning the DAS15 and are dissimilar from ITs produced by the ITs observed on the new allosyndetic recombinant lines carrying derived from Sr32 based on the map of Sourdille et al. 20 cM from Ae. speltoides.

The gene symbol Sr39 was used to only one of the two. Among markers detecting the IT 0; gene, only Xgpw4043 was codominant. However, the validation test indicated Xgpw4043 was compatible with only 30 of the 40 cultivars, and it cannot be used with Line 0406 (Table 2). For Line 0406, selection must be based on marker Xgwm501 combined with Xgwm47, Xgwm4112, or Xgwp4165. It should also be noted that if the IT 0; gene is homoeoallelic to Sr9, then based on the map of Sourdille et al. (2010), it is located approximately 20 cM from Xgpw4043. Although recombination between wheat and Ae. speltoides chromosomal segments is rare in the presence of Ph1, it has been noted to occur (Yu et al. 2010); and there is a chance of recombination between the IT 0; locus and the Xgpw4043 locus.

The gene symbol Sr47 was previously assigned with the assumption that DAS15 carried a single gene for stem rust resistance (Faris et al. 2008; McIntosh et al. 2010). Our finding that DAS15 carried two genes necessitates assigning the Sr47 symbol to only one of the two. The ITs observed on the new allosyndetic recombinant lines carrying the IT 0; gene show the greatest similarity to the ITs observed in DAS15 and are dissimilar from ITs produced by Sr39 (Table 5) or Sr32 derived from Ae. speltoides (Faris et al. 2008). In addition, the IT 2 gene may not differ from Sr39. The IT 0; gene therefore retained the symbol Sr47 and the IT 2 gene is assigned the temporary gene symbol SrAes7t. Genes conditioning IT 0; to TTKSK are rare, especially for those of wheat origin, although such genes have been identified in Ae. tauschi Cosson (Rouse et al. 2011) and other relatives of wheat (Jin et al. 2007). Thus, with its high level of resistance to TTKSK, Sr47 should be a valuable new gene for the improvement of stem rust resistance in wheat.

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Figure 5 Validation of seven molecular markers on 40 common and durum wheat cultivars. Lanes 1–7 are Chinese common wheat cultivars. Lanes 8–32 are North American common wheat cultivars. Lanes 33–40 are North American durum wheat cultivars. Lanes 41–48 are checks. Lane IDs are MW molecular weight marker, (1) Jimai22, (2) Yangmai16, (3) Shanrong1, (4) Shanrong3, (5) Jinan17, (6) Jinan177, (7) Zhengmai9023, (8) Amidon, (9) Howard, (10) Alsen, (11) Grandin, (12) Glenn, (13) Faller, (14) Glupro, (15) Ernest, (16) Steele, (17) Reeder, (18) Mott, (19) Kulm, (20) Parshall, (21) Granger, (22) Brick, (23) Russ, (24) Briggs, (25) Traverse, (26) Sabin, (27) Oklee, (28) Ulen, (29) Ada, (30) Tom, (31) Newton, (32) ILD6-14262, (33) Divide, (34) Ben, (35) Tioga, (36) Grenora, (37) Lebsock, (38) Monroe, (39) Alkabo, (40) Mountrail, (41) LMPG6, (42) Rusty, (43) DAS15, (44) Line 0744, (45) Line 0406, (46) Line 0696, (47) Line 0717, and (48) Rusty.
