Theor Appl Genet (2013) 126:1167–1177
DOI 10.1007/s00122-013-2044-6

ORIGINAL PAPER

Development and characterization of a compensating wheat-*Thinopyrum intermedium* Robertsonian translocation with *Sr44* resistance to stem rust (Ug99)

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Received: 20 July 2012 / Accepted: 9 January 2013 / Published online: 29 January 2013

Abstract The emergence of the highly virulent Ug99 race complex of the stem rust fungus (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. and Henn.) threatens wheat (*Triticum aestivum* L.) production worldwide. One of the effective genes against the Ug99 race complex is *Sr44,* which was derived from *Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey and mapped to the short arm of 7J (designated 7J#1S) present in the noncompensating T7DS-7J#1L•7J#1S translocation. Noncompensating wheat-alien translocations are known to cause genomic duplications and deficiencies leading to poor agronomic performance, precluding their direct use in wheat improvement. The present study was initiated to produce compensating wheat-*Th. intermedium* Robertsonian translocations with *Sr44* resistance. One compensating RobT was identified consisting of the wheat 7DL arm translocated to the *Th. intermedium* 7J#1S arm resulting in T7DL•7J#1S. The T7DL•7J#1S stock was designated as TA5657. The 7DL•7J#1S stock carries *Sr44* and has resistance to the Ug99 race complex. This compensating RobT with *Sr44* resistance may be useful in wheat improvement. In addition, we identified an unnamed stem rust resistance gene located on the 7J#1L arm that confers resistance not only to Ug99, but also to race TRTTF, which is virulent to *Sr44*. However, the action of the second gene can be modified by the presence of suppressors in the recipient wheat cultivars.

Communicated by P. Heslop-Harrison.

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Introduction

Stem rust of wheat caused by the fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and Henn. (*Pgt*) is one of the most important threats to wheat production worldwide. For the last 30 years, stem rust epidemics have been controlled by the deployment of resistance genes and the removal of the alternate host, *Berberis vulgaris* L. (Singh et al. 2006, 2008a, b; Jin and Singh 2006; Jin et al. 2009).

However, the emergence of a new stem rust race, Ug99, first detected in 1999 from a Uganda *Pgt* collection threatens wheat production worldwide (Pretorius et al. 2000; Wanyera et al. 2006; Jin et al. 2008a, b). Race Ug99 and other members of the Ug99 race complex are virulent to most of the resistance genes deployed in commercial cultivars rendering much of the world wheat crop susceptible (Singh et al. 2006, 2008a). Migration of Ug99 from

East Africa to Sudan and Yemen in 2006 (Yin et al. 2008a) and to Iran in 2007 (Nazari et al. 2009) has increased the urgency of deploying resistant cultivars.

Thus, there is an urgent need to identify new and effective sources of resistance and use them in wheat improvement. Faris et al. (2008) reported a new source of resistance to Ug99 derived from *Aegilops speltoides* Tausch, and chromosome engineering was used to shorten the *Ae. speltoides* segment in the *Sr39* transfer making this gene more useful in cultivar development (Mago et al. 2009; Niu et al. 2011). Another *Ae. speltoides*-derived Ug99 stem rust resistance gene was also transferred to durum wheat (Klindworth et al. 2012). Qi et al. (2011) reported a new source of Ug99 resistance, designated as *Sr52*, derived from *Dasypyrum villosum* (L.) Candargy that was transferred to wheat in the form of the Robertsonian translocation (RobT) T6AS•6V#3L. A second new gene for Ug99 resistance, designated as *Sr51*, was transferred to wheat from *Ae. searsii* Feldman and Kislev ex Hammer, in the form of the Robertsonian translocations (RobTs) T3AL•3S^{s}S, T3BL•3S^{s}S, and T3DL•3S^{s}S by Liu et al. (2011a). A third new gene for Ug99 resistance, *Sr53*, derived from *Ae. geniculata* Roth was transferred to wheat in the form of a T5DL-5M^{g}L•5M^{g}S recombinant chromosome and in the form of an interstitial translocation Ti5DS•5DL-5M^{g}L-5DL (Liu et al. 2011b).

Cauderon et al. (1973) produced a partial wheat-*Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey ($2n = 6x = 42$, JJJ^{s}J^{s}SS) amphiploid and six derived disomic chromosome addition lines in the French wheat cultivar 'Vilmorin 27' background (Friebe et al. 1992). The short arm of the *Th. intermedium* group-7 chromosome in this set, designated as 7Ai#1, conditions purple coleoptiles and harbors a gene conferring resistance to stem rust (*Sr44*) (Friebe et al. 1996), whereas the long arm has a gene conferring resistance to barley yellow dwarf virus (*Bdv2*) (Brettel et al. 1988; Banks et al. 1995; Hohmann et al. 1996). Our previous studies revealed that stem rust resistance gene *SrAgi* (later designated as *Sr44*) on 7Ai#1S was also highly effective against stem rust race Ug99 (Xu et al. 2008). McIntosh (unpublished) used induced homoeologous recombination to transfer *Sr44* from the group-7 *Th. intermedium* chromosome to wheat chromosome 7D. *Sr44* in the wheat germplasm 86.187 is present on a noncompensating wheat-*Th. intermedium* translocation consisting of part of the short arm of wheat chromosome 7D, part of the long arm of 7Ai#1L and the complete short arm of 7Ai#1S (T7DS-7Ai#1L•7Ai#1S) (Friebe et al. 1996). Noncompensating wheat-alien translocations are involving nonhomoeologous chromosome arms with different gene content and gene order and, thus, lead to genomic duplications and deficiencies, which results in poor agronomic performance and, therefore, prohibit their direct use in wheat improvement.

One important step in the transfer of alien genes to wheat is the production of compensating RobTs. These can be produced for the targeted chromosomes by the centric breakage-fusion mechanism of univalents during the meiotic division (Sears 1952). RobTs arise by centric misdivision of univalents during meiotic anaphase I followed by the fusion of the broken ends during interkinesis of the second meiotic division (Friebe et al. 2005). The present study was initiated to produce stem rust-resistant compensating wheat-*Th. intermedium* RobTs as a first step to exploit the *Sr44* gene in wheat improvement.

Materials and methods

Plant material

The stocks used in the present analysis included the wheat-*Th. intermedium* disomic chromosome addition (DA) in Vilmorin 27 (VIL) background VILDA7Ai#1 (TA3647), and the derived ditelosomic addition lines (DtA) VIL-DtA7Ai#1S (TA3656) in Vilmorin 27 background and CORDtA7Ai#1L (TA3659) in 'Courtot' (COR) background (Cauderon et al. 1973). The *Th. intermedium* 7Ai#1S arm is the physically longer arm but is homoeologous to group-7 short arms, has a small distal C-band, conditions purple coleoptiles, and harbors a gene for stem rust resistance (*Sr44*), whereas the physically shorter 7Ai#1L arm is homeologous to group-7 long arms, has a small proximal C-band, and harbors a gene for barley yellow dwarf resistance (*Bdv2*) (Friebe et al. 1996). In addition, the *Sr44* resistant noncompensating CST7DS-7Ai#1L•Ai#1S translocation stock (TA5584) in 'Chinese Spring' (CS) background and the barley yellow dwarf resistant translocation stocks SNRT7DS-7Ai#1S•7Ai#1L (TC6, TA5546) and SNRT7DS•7DL-7Ai#1L in Sunstar (SNR) background (Hohmann et al. 1996) were included together with the recipient wheat cultivars Vilmorin 27, Courtot, Chinese Spring, Sunstar, and the (CS) monosomic stock CSM7D (TA3061) ($2n = 41$, $20'' + 7D'$), the ditelosomic stocks CSDt7DS (TA3130), CSDt7DL (TA3071), the 'Canthach' (CTH) ditelosomic stocks CTHDt7DS (TA3068) and CTHDt7DL (TA3069), and ditelosomic wheat-*Th. intermedium* stock CSDtA7S#3L (TA7700). All materials are maintained by the Wheat Genetic and Genomic Resources Center at Kansas State University, Manhattan, KS, USA (http://www.ksu.edu.wgrc/).

Marker development

For assaying 7Ai#1 and detecting wheat-*Th. intermedium* RobTs, three STS-EST PCR markers were developed by screening CS and VILDA7Ai#1 with primers designed on

the sequences of 109 ESTs mapped to the short arms, and 119 ESTs mapped to the long arms of group-7 chromosomes (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). STS-PCRs were performed in 15 μL of reaction mixture containing 1× PCR buffer (Bioline USA Inc., Taunton, MA, USA), 2 mM $MgCl_2$, 0.25 mM dNTPs, 5 pmol forward and reverse primer, respectively, 0.02 unit/μl of Taq DNA polymerase (Bioline USA Inc., Taunton, MA, USA), and 90 ng of genomic DNA. PCR products were amplified with the program Touch-down 63 (Qi et al. 2007). STS-PCR-amplified products were digested with four-base cutter restriction enzymes (*Msp*I and *Hae*III). A total of 5 μl of enzyme mixture composed of 3.25 μl of ddH_2O, 1.5 μl of 10× NEB buffer 4, 0.15 μl of 100× BSA, 0.1 μl of enzyme stock solution was added to 10 μl PCR products and incubated for 2 h at 37 °C. PCR products were resolved on 1.5 % agarose gels and visualized by Ethidium bromide staining under UV light.

The chromosomal constitution of the wheat-*Th. intermedium* RobTs was confirmed using 7D short-arm markers BARC126, CFD31, CFD66, WMC463 and the 7D long-arm markers GDM46 and GWM428 (Somers et al. 2004).

Production and identification of putative wheat-*Th. intermedium* RobTs

To produce compensating RobTs involving the *Th. intermedium* chromosome 7Ai#1, wheat chromosome 7D monosomics (CSM7D) were crossed as female with VILDA7Ai#1 (Fig. 1). F_1 plants with $2n = 6x = 42$ chromosomes were double monosomic for chromosomes 7D and 7Ai#1 ($20'' + 7D' + 7Ai\#1'$) and were allowed to self pollinate. F_2 progenies were screened for the presence of putative compensating RobTs first using molecular markers and progenies with dissociation of the 7Ai#1S and 7Ai#1L markers were further characterized by genomic in situ hybridization (GISH) and C-banding analysis.

Cytological procedures

C-banding and chromosome identification were according to Gill et al. (1991).

Genomic DNA was extracted using a DNeasy Plant Mini Kit following the manufacturer's instructions (QIAGEN Inc. Valencia, CA, USA). Genomic in situ hybridization (GISH) was performed according to Zhang et al. (2001) using genomic DNA of *Th. intermedium* and *Pseudoroegneria spicata* (Pursh) Love ($2n = 2x = 14$, SS). The ratios of *Th. intermedium* and *Ps. spicata* probes to CS blocking DNA were 1:30-50 and 1:70, respectively with some modifications. Squash preparations were made after staining with acetocarmine. After hybridization at 37 °C overnight, the slides were washed in 2× SSC twice at room temperature for 5 min, twice at 42 °C for 10 min and 5 min each, and once at root temperature for 5 min. A drop (25–30 μl) of Vectashield mounting medium containing 1 μg/ml of PI (Cat. No. H-1400, Vector laboratories Inc, Burlingame, CA, USA) was added to each slide after 15–20 min, then covered with a 24 × 30 mm glass cover slip. Images were captured with a SPOT2.1 charge-coupled device (CCD) camera (Diagnostic Instruments, Sterling Heights, MI, USA) using an epifluorescence Zeiss Axioplan 2 microscope. Images were processed with Adobe Photoshop CS3 (Version 10.0.1) (Adobe Systems Incorporated, San Jose, CA, USA). C-banding and chromosome identification were according to Gill et al. (1991).

For fluorescence in situ hybridization (FISH), somatic chromosome preparations were made using the drop technique, and probe labeling and hybridization conditions were as described in (Kato et al. 2004, 2006). Three probes were used for FISH: for NOR labeling, we used clone pTa71 containing a 9 kb *Eco*RI fragment of 45S rDNA that was isolated from bread wheat (Gerlach and Bedbrook 1979) and for tandem repeat labeling we used the oligonucleotide probes Cy-5$(GAA)_9$, 6-FAM-$(GAA)_9$ and 6-FAM-pAs1 (Danilova et al., in preparation). Clone pAs1 was isolated from *Aegilops tauschii* and inserted into the plasmid pUC8 (Rayburn and Gill 1986) that preferentially hybridized to D-genome chromosome. Images were captured with Zeiss Axioplan 2 microscope using a cooled charge-coupled device camera CoolSNAP HQ2 (Photometrics) and AxioVision 4.8 software (Zeiss). Images were processed using the Photoshop software.

Stem rust resistance screening

Infection types were scored at 12–14 days post-inoculation. The infection type scale was originally developed by Stakman et al. (1962) and modified by Roelfs and Martens (1988) to differentiate between resistance and susceptibility. Infection types of class 3 and 4 were used to denote susceptibility and of classes 0, 0;, 1, and 2 to denote resistance. The primary distinguishing features separating resistance and susceptibility are size of uredinia and effects on plant tissue adjacent to the uredinia. Infection type 2 indicates round shaped (small to medium size) uredinia surrounded by plant tissue exhibiting the green island effect, where plant tissue immediately adjacent to the uredinia is green and surrounded by a border of chlorotic tissue. Infection type 3 indicates elongated uredinia (not round) without the green island effect. Plus and minus signs indicate variability of uredinia size within an infection type class. Disease reactions to Ug99 complex stem rust races TTKSK, TTSKT, and TTTSK, along with TRTTF, were evaluated on homozygous translocation stocks together with the appropriate controls at the USDA-

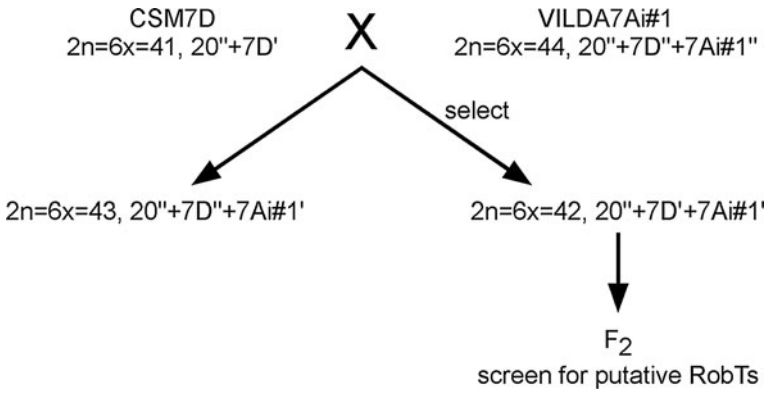

Fig. 1 Crossing scheme for producing compensating RobTs involving wheat chromosome 7D and the *Th. intermedium* chromosome 7Ai#1. The Chinese Spring stock monosomic for chromosome 7D is crossed as a female with the 7Ai#1 disomic addition stock in Vilmorin 27 background. F_1 plants with $2n = 6x = 42$ chromosomes are selected that are double monosomic for 7D and 7Ai#1, allowed to self pollinate and their progenies are screened for putative RobTs

ARS Cereal Disease Laboratory, St. Paul, MN, USA, following procedures reported previously (Jin and Singh 2006). A total of five plants were phenotyped for reaction to each isolate of *Puccinia graminis* Pers. f. sp. *tritici*. Three isolates of the Ug99 lineage were assayed (races TTKSK, TTKST, and TTTSK).

Results

Genomic affinity of the *Th. intermedium* 7Ai#1 chromosome

The *Th. intermedium* chromosome pair in TA3647 was previously shown to be homoeologous to group-7 chromosomes of wheat and was designated as 7Ai#1 (The and Baker 1970; Figueiras et al. 1986; Forster et al. 1987; Friebe et al. 1992). However, its genomic affinity remained to be determined. GISH using the diploid progenitor species *Ps. spicata* as a probe was shown previously to allow discrimination between the J-, J^s-, and S-genome chromosomes of *Th. intermedium*. Whereas the S-genome chromosomes are labeled over their entire lengths, the J-genome chromosomes only have hybridization sites at the telomeres and J^s-genome chromosomes are labeled in their pericentromeric and telomeric regions (Chen et al. 1998a, b, 1999, 2003). GISH using total genomic *Ps. spicata* DNA as a probe labeled the *Th. intermedium* chromosomes in TA3647 at both telomeres, indicating that this chromosome belongs to the J genome of *Th. intermedium* and, thus, was re-designated as 7J#1 (Fig. 2).

Developing PCR-based markers specific to 7J#1

For assaying 7J#1 and detecting wheat-7J#1 RobTs, three STS-PCR markers were developed. Two reliable short-arm polymorphic markers, *Xbe404728* and *Xbe473884*, were selected from the centromeric (C-7BS1-0.27) and distal bins (7AS1-0.89-1.00), and a long-arm polymorphic marker *Xbe498418* (C-7DL5-0.30) detected polymorphic fragments in TA3647 (Fig. 3); all three were used for screening progenies derived from double-monosomic plants (Table 1; Fig. 3).

Identification of wheat- *Th. intermedium* recombinants by molecular markers

A total of 2,402 F_2 plants and $F_{2:3}$ lines were screened for the presence of putative wheat-*Th. intermedium* RobTs. Three selection procedures were used. In the first selection procedure, F_2 progenies derived from double-monosomic plants were screened by the 7J#1S markers *Xbe473884* and *Xbe404728* and 7J#1L marker *Xbe498418* and plants that were lacking the long-arm marker were further characterized by GISH. In the second selection scheme, only plants with purple coleoptiles conditioned by the 7J#1S arm were screened by the 7J#1 long-arm marker *Xbe498418*, and plants that were lacking this marker were further analyzed by GISH. In the third selection scheme, $F_{2:3}$ families were first screened by their coleoptile color and families that were either homozygous or heterozygous for purple coleoptiles were kept and further analyzed. Genomic DNA of these plants was pooled in each family and used to screen for the presence of 7J#1S and 7J#1L markers. Plants that were positive for the 7J#1S and negative for the 7J#1L markers were further characterized by GISH.

A total of 1,152 F_2 plants derived from double-monosomic plants were screened with the three STS-PCR markers as outlined in the first selection scheme. Twenty-six plants were positive for both short-arm markers and were missing the long-arm marker, indicating that they had putative RobTs and were further characterized by GISH.

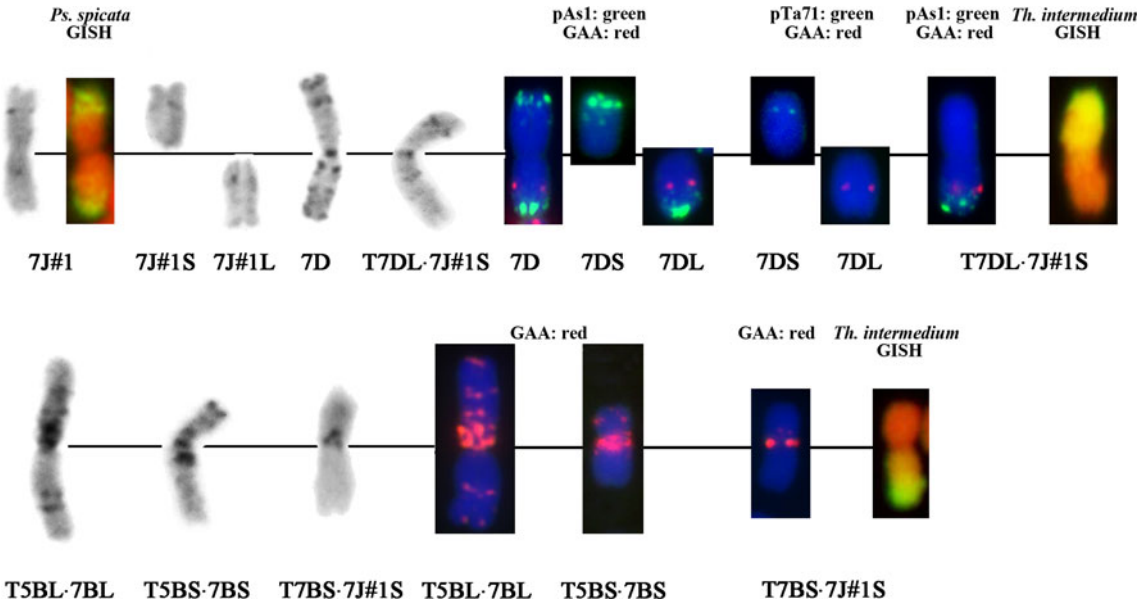

Fig. 2 C-banding, GISH and FISH patterns of the critical chromosomes involved in the *Sr44* transfer. *Upper panel* from *left* to *right*: C-banding and GISH of *Th. intermedium* chromosomes and telosomes from VILDA7J#1 (TA3647), VILDtA7J#1S (TA3656) and CORDtA7J#1L (TA3659); C-banding of chromosome CS7D and the compensating RobT T7DL•7J#1S present in U6032-359; FISH of CS7D (TA3008), CSDt7DS (TA3130), CSDt7DL (TA3071) and FISH and GISH of the compensating RobT present in U6032-359. *Lower panel*: C-banding and FISH of the reciprocal RobTs T5BL•7BL and T5BS•7BS present in U6032-359 and U6032-637 and FISH and GISH of the noncompensating RobT T7BS•7J#1S present in U6032-637

Two plants (U6032-359, U6032-1444) had a wheat-*Th. intermedium* RobT (Fig. 4), whereas the remaining plants had either telosomes, isochromosomes, had no hybridization signals, or were unidentified (Table 2; Fig. 4).

A total of 840 F_2 plants were screened by the second selection scheme and 13 plants had purple coleoptiles and were missing the long-arm marker *Xbe498418*. Three of these plants (U6032-176, U6032-286, U6032-321) had a wheat-*Th. intermedium* dicentric chromosome and two plants (U6032-633, U6032-637) had wheat-*Th. intermedium* RobTs (Table 2; Fig. 4). Of the remaining eight plants, five plants had telosomes, two plants had isochromosomes, and one plant had no GISH signal.

A total of 410 $F_{2:3}$ families were screened using the third selection scheme outlined above and none of these families had either wheat-*Th. intermedium* dicentric chromosomes or RobTs (Table 3).

Selection and identification of homozygous RobT stocks

The molecular marker analyses identified three plants with wheat-*Th. intermedium* dicentric chromosomes. The F_3 progenies derived from these three plants (U6032-176, U6032-286, and U6032-321) were analyzed by GISH. The dicentric chromosome in U6032-176 was stabilized as a wheat-*Th. intermedium* recombinant chromosome that was mostly derived from 7J#1 with only a small distal region of the long arm derived from wheat. The dicentric in U6032-286 was stabilized as a *Th. intermedium* telosome and the progeny U6032-321 as a RobT (Table 3; Fig. 4).

GISH of the F_3 offspring derived from the four plants with wheat-*Th. intermedium* RobTs showed that in the U6032-633 and U6032-1444 progenies the RobTs were stabilized as telosomes (Table 3; Fig. 4), suggesting that the parental plants mostly were undergoing breakage-fusion bridge cycles that remained undetected. The progeny of U6032-637 was segregating in 7 plants with *Th. intermedium* telosomes and 11 plants with RobTs (Table 3; Fig. 4). A total of 14 F_3 seeds were harvested from U6032-359, which was heterozygous for a RobT. Twelve plants were positive with both short-arm markers and six of them were analyzed by GISH, two plants were heterozygous and four plants were homozygous for wheat-*Th. intermedium* RobTs (Table 3; Fig. 4). Unfortunately, plant U6032-321 did not set any seeds and was completely sterile.

Characterization of the wheat-*Th. intermedium* chromosomal rearrangements

The F_2 plant U6032-359 had a wheat-*Th. intermedium* RobT that was stably transmitted to the offspring and four plants that were homozygous for this RobT (designated as TA5657) were recovered in F_3, which set an average of 270 seeds per plant. C-banding analysis of U6032-359 revealed that this family was homozygous for the wheat–wheat

RobTs T5BL•7BL and T5BS•7BS (Fig. 2) that were inherited from the French wheat cultivar Vilmorin 27 (Friebe et al. 1992). In addition, 7D of wheat was involved in a RobT where the short arm with a small distal C-band was derived from 7J#1S and the long arm with proximal and small interstitial C-bands was derived from 7DL of wheat (Fig. 2). This compensating RobT can be described as T7DL•7J#1S. The chromosomal composition of this line was further analyzed by FISH using probes pAs1, pTa71, and $(GAA)_9$.

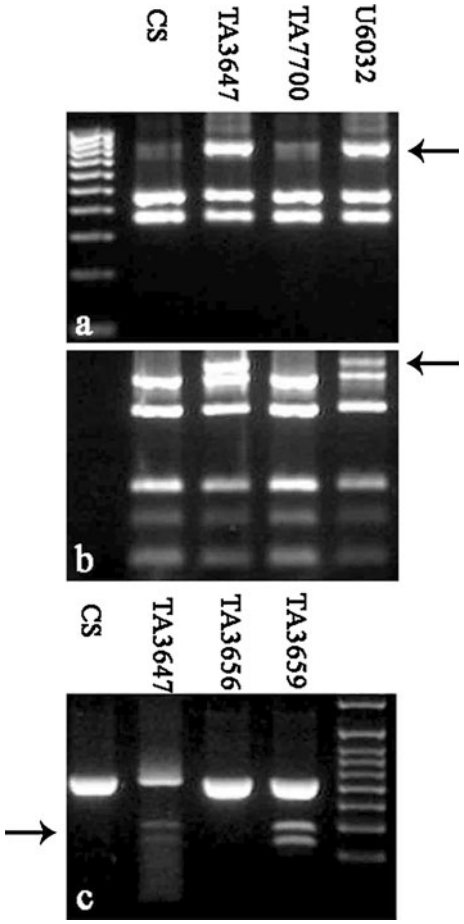

Fig. 3 PCR patterns of Chinese Spring (CS), VILDA7J#1 (TA3647), CSDtA7S#3L (TA7700), CSM7D X TA3647 (U6032), VILDtA7J#1S (TA3656), and CORDtA7J#1L (TA3659) with specific markers for 7J#1 chromosome arms: **a** 7J#1 short-arm marker *Xbe404728* (using *Msp*I), **b** 7J#1 short-arm marker *Xbe473884* (*Msp*I), **c** 7J#1 long-arm marker *Xbe498418* (*Hae*III). Polymorphic fragments are marked by *arrows*

Chromosome 7D has prominent pAs1 FISH sites at the telomeres of both arms (Fig. 2). FISH using the $(GAA)_9$ probe detected a distinct distal GAA FISH site in the 7DL arm and a minor pTa71 FISH site was observed in the distal region of the 7DS arm (Fig. 2). An identical FISH pattern using these probes was observed in the CSDt7DS and CSDt7DL stocks (Fig. 2) and in the corresponding ditelosomic 7DS and 7DL stocks in Canthatch background (data not shown). In U6032-359, chromosome 7D had only one pAs1 FISH site at the telomere of the 7DL arm in addition to a diagnostic distal GAA FISH site, whereas the short arm of this chromosome had no hybridization signals (Fig. 2), confirming the presence of a T7DL•7J#1S RobT in this line.

GISH of line U6032-637 confirmed that this line was homozygous for a wheat-*Th. intermedium* RobT (Fig. 2). C-banding of this family revealed that this line was segregating for T5BL•7BL and 5B and had a wheat-*Th. intermedium* RobT where the short arm with a centromeric and proximal C-band was derived from 7BS and the long arm was derived from 7J#1S (Fig. 2). FISH using $(GAA)_9$ and pAs1 as probes confirmed that line U6032-637 is segregating for T5BL•7BL and 5B and is homozygous for the wheat-*Th. intermedium* RobT, where the wheat arm has a centromeric and proximal GAA FISH site and was derived from 7BS and the *Th. intermedium* chromosome arm had no hybridization signal (Fig. 2). Thus, line U6032-637 is homozygous for the non-compensating RobT T7BS•7J#1S.

The identity of the RobT in line U6032-359 was further confirmed using genetically or chromosome bin-mapped SSR markers. The 7D short-arm markers BARC126, CFD31, CFD66, and WMC463 detected polymorphic fragments in CSDt7DS, CTHDt7DS, U6032-637, and in CS, whereas the 7D long-arm markers GDM46 and GWM428 detected polymorphic fragments in CSDt7DL, CTHDt7DL, U6032-637, U6032-359, and in CS (Fig. 5) and, thus, confirming that the RobT in U6032-359 consists of the 7DL arm translocated to 7J#1S, resulting in the compensating RobT T7DL•7J#1S.

Table 1 Primer sequences of *Th. intermedium* 7J#1-specific STS-PCR markers on wheat group-7 chromosomes and primer/enzyme combinations producing 7J#1 polymorphism

Marker	Forward/Reverse primer 5′–3′	Location (deletion bin)	Enzyme for polymorphism	EST accession
Xbe404728	5′ GGTGGTGCCTGTCAAGATT 3′	C-7BS1-0.27	*Msp*I	BE404728
	5′ TTGATGGATCCTGGCTTAGG 3′			
Xbe473884	5′ GTTGACGTTCATAGCGAGCA 3′	7AS1-0.89-1.00	*Msp*I	BE473884
	5′ CGAGCCACAGTCCTTCCTAC 3′			
Xbe498418	5′ GCAGATCTTGGGGATCAAAA 3′	C-7DL5-0.30	*Hae*III	BE498418
	5′ CTCCATGAGAAGCCATAGCC 3′			

Stem rust resistance screening

Screening of the disomic chromosome addition line DA7J#1 (TA3647) in Vilmorin 27 background and the ditelosomic addition lines DtA7J#1S (TA3656) in Chinese Spring background and DtA7J#1L (TA3659) in Courtot background with the *Pgt* isolates TTKSK, TTSKT, and TTTSK showed that all three lines were resistant, whereas the recipient wheat cultivars Vilmorin 27, Courtot, and Chinese Spring were susceptible (Table 4; Fig. 6). These

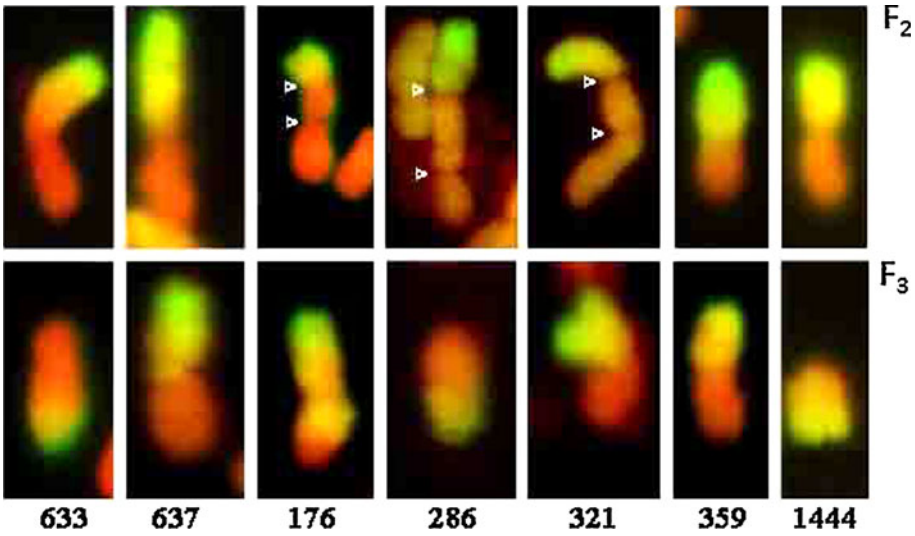

Fig. 4 Genomic in situ hybridization pattern using total genomic *Th. intermedium* DNA as a probe of putative wheat-*Th. intermedium* RobTs identified in U6032 F_2 plants and the derived chromosomal rearrangements recovered in F_3 progenies; note that plant # 176, 286, and 321 originally had a dicentric chromosome where the centromeres are marked by *arrows*

Table 2 Marker and GISH analyses of progenies derived from plants double-monosomic for chromosomes 7D and 7J#1

No. of plants	Selection scheme 1	Selection scheme 2	Selection scheme 3
No. of plants planted	1,152	840	410
No. of plants with purple coleoptile	N/A	572	281
No. of plants with positive *Xbe473884* (C-7BS1-0.27)	970	N/A	272
No. of plants with positive *Xbe404728* (7AS1-0.89-1.00)	970	N/A	272
No.of plants positive for *Xbe473884* and *Xbe404728* and negative for Xbe498418 (C-7DL5-0.30)	26	N/A	9
No. of plants with purple coleoptiles that were negative for *Xbe498418*	N/A	13	N/A
No. of plants GISHed	26	13	9
No. of plants with no signal	3	1	–
No. of plants wit 7J#1 telosomes	14	5	5
No of plants with 7J#1 isochromosome	5	2	4
No. of plants with 7Ai#1 dicentric chromosome	–	3	–
No. of plants with 7Ai#1 Robertsonian translocation	2	2	–
No. of plants unidentified	2	–	–

Table 3 GISH analysis of F_3 progenies derived from F_2 plants with dicentric and RobT chromosomes

Rearrangement in F_2	F_3 lines	No hybridization signal	Telosomes	Het. Rec	Hom. Rec	Het. RobT	Hom. RobTs
RobT	U6032-633	27	13			–	–
RobT	U6032-637	7	7			9	2
Dicentric	U6032-176	12	–	9	4		
Dicentric	U6032-286	18	2			–	–
Dicentric	U6032-321	1	–			–	1
RobT	U6032-359	0	–			2	4
RobT	U6032-1444	18	2			–	–

data suggest that not only the short *Th. intermedium* chromosome arm 7J#1S, but also the 7J#1L arm harbors a stem rust resistance gene that is effective against the three Ug99 complex races tested. Whereas the stem rust resistance gene in the 7J#1S arm has been previously designated as *Sr44* (Friebe et al. 1996), the presence of a stem rust resistance gene in the 7J#1L arm was previously unknown. The non-compensating T7DS-7J#1L•7J#1S translocation (TA5584) in Chinese Spring background and the compensating RobT T7DL•7J#1S (TA5657) identified in the present study conferred resistance to the *Pgt* isolates TTKSK, TTSKT, and TTTSK. Lines T7DS-7J#1L•7J#1S and DtA 7J#1S, but not T7DL•7J#1S were resistant to isolate TRTTF (Table 4; Fig. 6). Because DtA7J#1L in Courtot background and T7DS-7J#1L•7J#1S in Chinese Spring background displayed resistance to the *Pgt* isolate TRTTF, and the stock with the complete 7J#1 chromosome in Vilmorin 27 background was susceptible, these data suggest that Vilmorin 27 has a gene that suppresses the resistance of the unnamed stem rust resistance gene present in the 7J#1L arm.

We also evaluated the T7DS-7J#1S•7J#1L (TC6, TA5546) and T7DS•7DL-7J#1L (TC14, TA5551) stocks in Sunstar background that were derived from the same 7J#1 *Th. intermedium* chromosome and harbor a resistance gene against barley yellow dwarf (*Bvd2*) together with their recipient wheat cultivar Sunstar against the *Pgt* isolates TRTTF and TTKSK. Whereas Sunstar, TC6, and TC14 were resistant to race TRTTF, all three lines were susceptible against TTKSK (Table 4; Fig. 6), suggesting that the stem rust resistance gene *Sr44* is located on the distal 7J#1S fragment that is replaced by 7DS in T7DS-7J#1S•7J#1L in TC6.

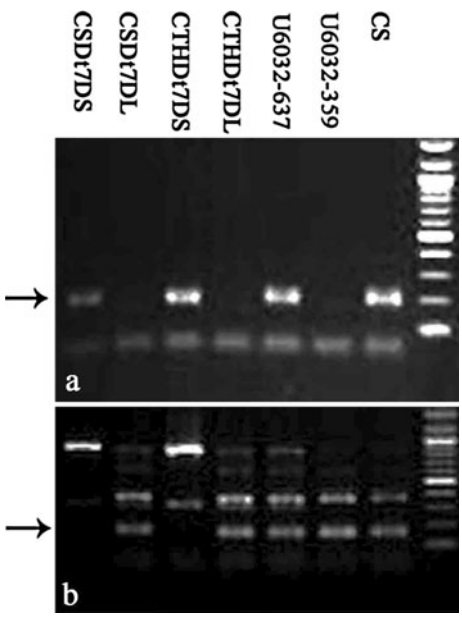

Fig. 5 PCR pattern of Chinese Spring, the ditelosomic 7DS and 7DL stocks in Chinese Spring (CS) and Canthatch (CTH) background, and the wheat-*Th. intermedium* RobT stocks U6032-637 and U6032-359: **a** 7D short-arm marker CFD66 detected a 202 bp polymorphic fragment and **b** 7D long-arm marker GDM46 detected a 163 bp polymorphic fragment

Discussion

In the present study, we produced plants that were double monosomic for wheat chromosome 7D and the *Th. intermedium* chromosome 7J#1 and, thus, we targeted chromosomes 7D and 7J#1 to be involved in the formation of RobTs. In these plants, chromosomes 7D and 7J#1 do not pair at meiotic metaphase I and can misdivide at the centromeres, which after fusion of the broken ends can give rise to the formation of wheat-*Th. intermedium* RobTs (Sears 1952; Friebe et al. 2005). However, we also identified three plants in the progeny of such double-monosomic plants that had wheat-*Th. intermedium* dicentric chromosomes, one of which was stabilized as a *Th. intermedium* telosome, one as a wheat-*Th. intermedium* recombinant chromosome and one was stabilized as a wheat-*Th. intermedium* RobT. Dicentric chromosomes are known to undergo chromosome-type breakage-fusion-bridge (BFB) cycles and usually never enter the meiotic divisions (Friebe et al. 2001). In addition, in two of the four plants that had wheat-*Th. intermedium* RobTs, the translocations were stabilized as *Th. intermedium* telosomes, indicating that the original plants also had dicentric chromosomes that were undergoing BFB cycles, which remained undetected. The mechanism leading to the formation of wheat-*Th. intermedium* dicentric chromosomes in progenies of plants double monosomic for a *Th. intermedium* and a homoeologous wheat chromosome is unknown. However, it appears that this process is not a very rare event. Previously, we reported the recovery of a wheat-*Th. intermedium* T7BS•7S#3L RobT conferring resistance to wheat streak mosaic virus that was also derived from a wheat-*Th. intermedium* dicentric chromosome in the progeny of plants double monosomic for chromosomes 7D and 7S#3 (Liu et al. 2011c).

The compensating T7DL•7J#1S RobT identified in the present study harbors the stem rust resistance gene *Sr44*, which confers resistance to the Ug99 race complex including races TTKSK, TTSKT, and TTTSK and is located in the 7J#1S arm. Surprisingly, our data also showed that the *Th. intermedium* long-arm 7J#1L harbors an unnamed stem rust resistance gene that confers resistance to all Ug99 isolates tested in the present study. However, our data further indicate that the expression of this gene is modified by the wheat background. Whereas the 7J#1L stem rust resistance gene confers resistance of

Table 4 Infection types of wheat-*Th. intermedium* introgression lines 16 days after inoculation with *Pgt* races TRTTF, TTKSK, TTSKT, and TTTSK

Germplasm	Chromosomal constitution	Background	*Pgt* race TRTTF	*Pgt* race TTKSK	*Pgt* race TTSKT	*Pgt* race TTTSK
TA5584	T7DS-7J#1L•7J#1S	Chinese Spring	2–	2	22–	2–
TA5657	T7DL•7J#1S	Chinese Spring/ Vilmorin 27	4	2	2–	2–
TA3647	DA7J#1	Vilmorin 27	4	22–	2–	2–
TA3656	DtA7J#1S	Vilmorin 27	4	2–	2–	2–
TA3659	DtA7J#1L	Courtot	2	22+	2	22+
TA5546 (TC6)	T7DS-7J#1S•7J#1L	Sunstar	2	3+	–	–
TA5551 (TC14)	T7DS•7DL-7J#1L	Sunstar	2	32+	–	–
TA2912	Sunstar	Sunstar	2	3	–	–
T3014	Courtot	Courtot	3+	3+	–	–
TA3997	Vilmorin 27	Vilmorin 27	4	4	4	4
TA3008	Chinese Spring	Chinese Spring	4	4	4	4

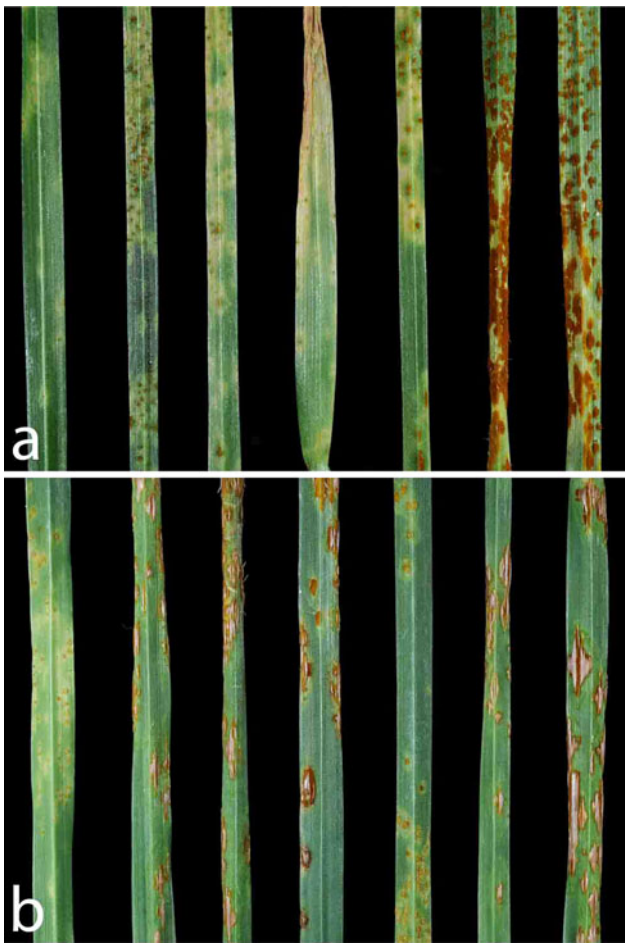

Fig. 6 Infection types 16 days after inoculation with *Pgt* cultures **a** TTTSK and **b** TRTTF, from *left* to *right*: TA5584 (T7DS-7J#1L•7J#1S), TA5657 (T7DL•7J#1S), TA3647 (VILDA7J#1), TA3656 (VILDtA7J#1S), TA3659 (CORDtA7J#1L), TA3997 (Vilmorin 27), TA3008 (Chinese Spring)

the DtA7J#1L and T7DS-7J#1L•7J#1S stocks in Courtot and Chinese Spring background, the expression of this gene in the DA7J#1 stock with the complete *Th. intermedium* chromosome is suppressed in Vilmorin 27 background. It is well known that the expression of alien disease resistance genes when transferred to wheat can be modified and suppressed by wheat backgrounds. Suppressors of leaf rust resistance genes have been previously mapped to A- and B-genome chromosomes by Innes and Kerber (1994) and to D-genome chromosomes by Bai and Knott (1992). Similarly, the expression of the leaf rust resistance gene *Lr23* was shown to be modified by suppressors present in the recipient wheat cultivars (McIntosh and Dyck 1975; Nelson et al. 1997). Recently, McIntosh et al. (2011) showed that the expression of the powdery mildew resistance gene *Pm8* was suppressed by the presence of the *Pm3* locus.

The production of a compensating Robertsonian T7DL•7J#1S translocation stock with *Sr44* resistance is the first step for utilizing this gene in wheat improvement. Further chromosome engineering is underway aimed at shortening the *Th. intermedium* segment using *ph1b*-induced homoeologous recombination. The present study also revealed the presence of a stem rust resistance gene that is effective against Ug99 isolates in the 7J#1L arm. The distal part of this arm is present in the TC14 T7DS•7DL-7J#1L translocation that confers resistance to barley yellow dwarf (*Bvd2*), which has been widely used in wheat improvement. If the stem rust resistance gene in the 7J#1L arm is located on the *Th. intermedium* segment in the TC14 translocation, these translocations stocks may also express Ug99 resistance depending on the presence of modifiers in the recurrent wheat cultivars.

Acknowledgments This research was part of the project "Durable Rust Resistance in Wheat" conducted by Cornell University and supported by the Bill and Melinda Gates Foundation and by grants from the Kansas Wheat Commission and the Kansas Crop Improvement Association. We thank W. John Raupp for critical editorial review of the manuscript and Shuangye Wu for her technical assistance. This is contribution number 12-451-J from the Kansas

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