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

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

3

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10

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

19

20 **Key words** wheat, *Thinopyrum intermedium*, stem rust resistance, genomic *in situ*  
21 hybridization

22

## 1 **Introduction**

2

3 Stem rust of wheat caused by the fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks. &  
4 Henn. (*Pgt*) is one of the most important threats to wheat production worldwide. For the  
5 last 30 years, stem rust epidemics have been controlled by the deployment of resistance  
6 genes and the removal of the alternate host, *Berberis vulgaris* L. (Singh et al. 2006,  
7 2008a, 2008b; Jin et al. 2006, 2009).

8         However, the emergence of a new stem rust race, Ug99, first detected in 1999  
9 from a Uganda *Pgt* collection threatens wheat production worldwide (Pretorius et al.  
10 2000; Wanyera et al. 2006; Jin et al. 2008a, b). Race Ug99 and other members of the  
11 Ug99 race complex are virulent to most of the resistance genes deployed in commercial  
12 cultivars rendering much of the world wheat crop susceptible (Singh et al. 2006, 2008a).  
13 Migration of Ug99 from East Africa to Sudan and Yemen in 2006 (Yin et al. 2008a) and  
14 to Iran in 2007 (Nazari et al. 2009) has increased the urgency of deploying resistant  
15 cultivars.

16 Thus, there is an urgent need to identify new and effective sources of resistance and use  
17 them in wheat improvement. Faris et al. (2008) reported a new source of resistance to  
18 Ug99 derived from *Aegilops speltoides* Tausch and chromosome engineering was used to  
19 shorten the *Ae. speltoides* segment in the *Sr39* transfer making this gene more useful in  
20 cultivar development (Mago et al. 2009; Niu et al. 2011). Another *Ae. speltoides*-derived  
21 Ug99 stem rust resistance gene was also transferred to durum wheat (Klindworth et al.  
22 2012). Qi et al. (2011) reported a new source of Ug99 resistance, designated as *Sr52*,

1 derived from *Dasypyrum villosum* (L.) Candargy that was transferred to wheat in the  
2 form of the Robertsonian translocation (RobT) T6AS·6V#3L. A second new gene for  
3 Ug99 resistance, designated as *Sr51*, was transferred to wheat from *Ae. searsii* Feldman  
4 & Kislev ex Hammer, in the form of the RobTs T3AL·3S<sup>S</sup>S, T3BL·3S<sup>S</sup>S, and T3DL·3S<sup>S</sup>S  
5 by Liu et al. (2011a). A third new gene for Ug99 resistance, *Sr53*, derived from *Ae.*  
6 *geniculata* Roth was transferred to wheat in the form of a T5DL·5M<sup>g</sup>L·5M<sup>g</sup>S  
7 recombinant chromosome and in the form of an interstitial translocation Ti5DS·5DL-  
8 5M<sup>g</sup>L·5DL (Liu et al. 2011b).

9         Cauderon et al. (1973) produced a partial wheat-*Thinopyrum intermedium* (Host)  
10 Barkworth & D.R. Dewey (2n=6x=42, JJJ<sup>S</sup>J<sup>S</sup>SS) amphiploid and six derived disomic  
11 chromosome addition lines in the French wheat cultivar ‘Vilmorin 27’ background  
12 (Friebe et al. 1992). The short arm of the *Th. intermedium* group-7 chromosome in this  
13 set, designated as 7Ai#1, conditions purple coleoptiles and harbors a gene conferring  
14 resistance to stem rust (*Sr44*) (Friebe et al. 1996), whereas the long arm has a gene  
15 conferring resistance to barley yellow dwarf virus (*Bdv2*) (Brettel et al. 1988; Banks et al.  
16 1995; Hohmann et al. 1996). Our previous studies revealed that stem rust resistance gene  
17 *SrAgi* (later designated as *Sr44*) on 7Ai#1S was also highly effective against stem rust  
18 race Ug99 (Xu et al. 2008). McIntosh (unpublished) used induced homoeologous  
19 recombination to transfer *Sr44*, from the group-7 *Th. intermedium* chromosome to wheat  
20 chromosome 7D. *Sr44* in the wheat germplasm 86.187 is present on a noncompensating  
21 wheat-*Th. intermedium* translocation consisting of part of the short arm of wheat  
22 chromosome 7D, part of the long arm of 7Ai#1L and the complete short arm of 7Ai#1S  
23 (T7DS-7Ai#1L·7Ai#1S) (Friebe et al. 1996). Noncompensating wheat-alien

1 translocations are involving nonhomoeologous chromosome arms with different gene  
2 content and gene order, and thus, lead to genomic duplications and deficiencies, which  
3 results in poor agronomic performance, and therefore, prohibit their direct use in wheat  
4 improvement.

5         One important step in the transfer of alien genes to wheat is the production of  
6 compensating RobTs. These can be produced for the targeted chromosomes by the  
7 centric breakage-fusion mechanism of univalents during the meiotic division (Sears  
8 1952). RobTs arise by centric misdivision of univalents during meiotic anaphase I  
9 followed by the fusion of the broken ends during interkinesis of the second meiotic  
10 division (Friebe et al. 2005). The present study was initiated to produce stem rust-  
11 resistant compensating wheat-*Th. intermedium* RobTs as a first step to exploit the *Sr44*  
12 gene in wheat improvement.

13

## 14 **Material and methods**

15

### 16 **Plant material**

17 The stocks used in the present analysis included the wheat-*Th. intermedium* disomic  
18 chromosome addition (DA) in Vilmorin 27 (VIL) background VILDA7Ai#1 (TA3647),  
19 and the derived ditelosomic addition lines (DtA) VILDtA7Ai#1S (TA3656) in Vilmorin  
20 27 background and CORDtA7Ai#1L (TA3659) in ‘Courtot’ (COR) background  
21 (Cauderon et al. 1973). The *Th. intermedium* 7Ai#1S arm is the physically longer arm but

1 is homoeologous to group 7 short arms, has a small distal C-band, conditions purple  
2 coleoptiles, and harbors a gene for stem rust resistance (*Sr44*), whereas the physically  
3 shorter 7Ai#1L arm is homeologous to group 7 long arms, has a small proximal C-band,  
4 and harbors a gene for barley yellow dwarf resistance (*Bdv2*) (Friebe et al. 1996). In  
5 addition, the *Sr44* resistant noncompensating CST7DS-7Ai#1L·Ai#1S translocation stock  
6 (TA5584) in ‘Chinese Spring’ (CS) background and the barley yellow dwarf resistant  
7 translocation stocks SNRT7DS-7Ai#1S·7Ai#1L (TC6, TA5546) and SNRT7DS·7DL-  
8 7Ai#1L in Sunstar (SNR) background (Hohmann et al. 1996) were included together with  
9 the recipient wheat cultivars Vilmorin 27, Courtot, Chinese Spring, Sunstar, and the (CS)  
10 monosomic stock CSM7D (TA3061) (2n=41, 20”+7D’), the ditelosomic stocks  
11 CSDt7DS (TA3130), CSDt7DL (TA3071), the ‘Canthach’ (CTH) ditelosomic stocks  
12 CTHDt7DS (TA3068) and CTHDt7DL (TA3069), and ditelosomic wheat-*Th.*  
13 *intermedium* stock CSDtA7S#3L (TA7700). All materials are maintained by the Wheat  
14 Genetic and Genomic Resources Center at Kansas State University, Manhattan, KS, USA  
15 (<http://www.ksu.edu.wgrc/>).

16

17 Marker development

18

19 For assaying 7Ai#1 and detecting wheat-*Th. intermedium* RobTs, three STS-EST PCR  
20 markers were developed by screening CS and VILDA7Ai#1 with primers designed on the  
21 sequences of 109 ESTs mapped to the short arms, and 119 ESTs mapped to the long arms  
22 of group-7 chromosomes ([http://wheat.pw.usda.gov/cgi-bin/westsql/map\\_locus.cgi](http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi)).



1 STS-PCR reactions were performed in 15  $\mu$ L of reaction mixture containing 1X PCR  
2 buffer (Bioline USA Inc., Taunton, MA, USA), 2 mM MgCl<sub>2</sub>, 0.25 mM dNTPs, 5 pmol  
3 forward and reverse primer, respectively, 0.02 unit/ $\mu$ l of Taq DNA polymerase (Bioline  
4 USA Inc., Taunton, MA, USA), and 90 ng of genomic DNA. PCR products were  
5 amplified with the program Touch-down 63 (Qi et al. 2007). STS-PCR-amplified  
6 products were digested with four-base cutter restriction enzymes (*Msp*I and *Hae*III). A  
7 total of 5  $\mu$ l of enzyme mixture composed of 3.25  $\mu$ l of ddH<sub>2</sub>O, 1.5  $\mu$ l of 10X NEB buffer  
8 4, 0.15  $\mu$ l of 100X BSA, 0.1  $\mu$ l of enzyme stock solution was added to 10  $\mu$ l PCR  
9 products and incubated for 2 hrs at 37°C. PCR products were resolved on 1.5% agarose  
10 gels and visualized by Ethidium bromide staining under UV light.

11 The chromosomal constitution of the wheat-*Th. intermedium* RobTs was  
12 confirmed by using 7D short arm markers BARC126, CFD31, CFD66, WMC463 and the  
13 7D long arm markers GDM46 and GWM428 (Somer et al. 2004).

14

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

16

17 In order to produce compensating RobTs involving the *Th. intermedium* chromosome  
18 7Ai#1, wheat chromosome 7D monosomics (CSM7D) were crossed as female with  
19 VILDA7Ai#1 (Fig. 1). F<sub>1</sub> plants with 2n=6x=42 chromosomes were double monosomic  
20 for chromosomes 7D and 7Ai#1 (20''+7D'+7Ai#1') and were allowed to self pollinate.  
21 F<sub>2</sub> progenies were screened for the presence of putative compensating RobTs first by  
22 using molecular markers and progenies with dissociation of the 7Ai#1S and 7Ai#1L

1 markers were further characterized by genomic *in situ* hybridization (GISH) and C-  
2 banding analysis.

3

4 Cytological procedures

5

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

7 Genomic DNA was extracted using a DNeasy Plant Mini Kit following the  
8 manufacturer's instructions (QIAGEN Inc. Valencia, CA, USA). Genomic *in situ*  
9 hybridization (GISH) was performed according to Zhang et al. (2001) using genomic  
10 DNA of *Th. intermedium* and *Pseudoroegneria spicata* (Pursh) Love ( $2n=2x=14$ , SS).  
11 The ratios of *Th. intermedium* and *Ps. spicata* probes to CS blocking DNA were 1:30-50  
12 and 1:70, respectively with some modifications. Squash preparations were made after  
13 staining with acetocarmine. After hybridization at 37°C overnight, the slides were  
14 washed in 2X SSC twice at room temperature for 5 min, twice at 42°C for 10 min and 5  
15 min each, and once at room temp for 5 min. A drop (25-30µl) of Vectashield mounting  
16 medium containing 1µg/ml of PI (Cat.No.H-1400, Vector laboratories Inc, Burlingame,  
17 CA, USA) was added to each slide after 15-20 min, then covered with a 24X30 mm glass  
18 cover slip. Images were captured with a SPOT2.1 charge-coupled device (CCD) camera  
19 (Diagnostic Instruments, Sterling Heights, MI, USA) using an epifluorescence Zeiss  
20 Axioplan 2 microscope. Images were processed with Adobe Photoshop CS3 (Version  
21 10.0.1) (Adobe Systems Incorporated, San Jose, CA, USA). C-banding and chromosome  
22 identification was according to Gill et al. (1991).

1 For fluorescence *in situ* hybridization (FISH), somatic chromosome preparations  
2 were made using the drop technique, and probe labeling and hybridization conditions  
3 were as described in (Kato et al. 2004, 2006). Three probes were used for FISH: for NOR  
4 labeling we used clone pTa71 containing a 9 kb *EcoRI* fragment of 45S rDNA that was  
5 isolated from bread wheat (Gerlach and Bedbrook 1979) and for tandem repeat labeling  
6 we used the oligonucleotide probes Cy-5(GAA)<sub>9</sub>, 6-FAM-(GAA)<sub>9</sub> and 6-FAM-pAs1  
7 (Danilova et al., in preparation). Clone pAs1 was isolated from *Aegilops tauschii* and  
8 inserted into the plasmid pUC8 (Rayburn and Gill 1986) that preferentially hybridized to  
9 D-genome chromosome. Images were captured with Zeiss Axioplan 2 microscope using a  
10 cooled charge-coupled device camera CoolSNAP HQ2 (Photometrics) and AxioVision  
11 4.8 software (Zeiss). Images were processed using the Photoshop software.

12

13 Stem rust resistance screening

14

15 Infection types were scored at 12-14 days post-inoculation. The infection type scale was  
16 originally developed by Stakman et al (1962) and modified by Roelfs and Martens (1988)  
17 to differentiate between resistance and susceptibility. Infection types of class 3 and 4  
18 were used to denote susceptibility and of classes 0, 0;, 1, and 2 to denote resistance. The  
19 primary distinguishing features separating resistance and susceptibility are size of  
20 uredinia and effects on plant tissue adjacent to the uredinia. Infection type 2 indicates  
21 round shaped (small to medium size) uredinia surrounded by plant tissue exhibiting the  
22 green island effect where plant tissue immediately adjacent to the uredinia is green and

1 surrounded by a border of chlorotic tissue. Infection type 3 indicates elongated uredinia  
2 (not round) without the green island effect. Plus and minus signs indicate variability of  
3 uredinia size within an infection type class. Disease reactions to Ug99 complex stem rust  
4 races TTKSK, TTKST, and TTTSK, along with TRTTF, were evaluated on homozygous  
5 translocation stocks together with the appropriate controls at the USDA-ARS Cereal  
6 Disease Laboratory, St. Paul, MN, USA, following procedures reported previously (Jin  
7 and Singh 2006). A total of five plants were phenotyped for reaction to each isolate of  
8 *Puccinia graminis* Pers. f. sp. *tritici*. Three isolates of the Ug99 lineage were assayed  
9 (races TTKSK, TTKST, and TTTSK).

10

## 11 **Results**

12

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

14

15 The *Th. intermedium* chromosome pair in TA3647 was previously shown to be  
16 homoeologous to group-7 chromosomes of wheat and was designated as 7Ai#1 (The and  
17 Baker 1970; Figueiras et al. 1986; Forster et al. 1987; Friebe et al. 1992). However, its  
18 genomic affinity remained to be determined. GISH using the diploid progenitor species  
19 *Ps. spicata* as a probe was shown previously to allow discrimination between the J-, J<sup>s</sup>-,  
20 and S-genome chromosomes of *Th. intermedium*. Whereas the S-genome chromosomes  
21 are labeled over their entire lengths, the J-genome chromosomes only have hybridization

1 sites at the telomeres and J<sup>s</sup>-genome chromosomes are labeled in their pericentromeric  
2 and telomeric regions (Chen et al. 1988a, b, 1999, 2003). GISH using total genomic *Ps.*  
3 *spicata* DNA as a probe labeled the *Th. intermedium* chromosomes in TA3647 at both  
4 telomeres, indicating that this chromosome belongs to the J genome of *Th. intermedium*  
5 and, thus, was re-designated as 7J#1 (Fig. 2).

6

7 Developing PCR-based markers specific to 7J#1

8

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

15

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

17

18 A total of 2402 F<sub>2</sub> plants and F<sub>2;3</sub> lines were screened for the presence of putative wheat-  
19 *Th. intermedium* RobTs. Three selection procedures were used. In the first selection  
20 procedure, F<sub>2</sub> progenies derived from double-monosomic plants were screened by the  
21 7J#1S markers *Xbe473884* and *Xbe404728* and 7J#1L marker *Xbe498418* and plants that

1 were lacking the long arm marker were further characterized by GISH. In the second  
2 selection scheme, only plants with purple coleoptiles conditioned by the 7J#1S arm were  
3 screened by the 7J#1 long-arm marker *Xbe498418*, and plants that were lacking this  
4 marker were further analyzed by GISH. In the third selection scheme, F<sub>2:3</sub> families were  
5 first screened by their coleoptile color and families that were either homozygous or  
6 heterozygous for purple coleoptiles were kept and further analyzed. Genomic DNA of  
7 these plants was pooled in each family and used to screen for the presence of 7J#1S and  
8 7J#1L markers. Plants that were positive for the 7J#1S and negative for the 7J#1L  
9 markers were further characterized by GISH.

10 A total of 1152 F<sub>2</sub> plants derived from double monosomic plants were screened  
11 with the three STS-PCR markers as outlined in the first selection scheme. Twenty-six  
12 plants were positive for both short-arm markers and were missing the long-arm marker,  
13 indicating that they had putative RobTs and were further characterized by GISH. Two  
14 plants (U6032-359, U6032-1444) had a wheat-*Th. intermedium* RobT (Fig. 4), whereas  
15 the remaining plants had either telosomes, isochromosomes, had no hybridization signals,  
16 or were unidentified (Table 2, Fig. 4).

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

1           A total of 410 F<sub>2,3</sub> families were screened using the third selection scheme  
2 outlined above and none of these families had either wheat-*Th. intermedium* dicentric  
3 chromosomes or RobTs (Table 3).

4

5 Selection and identification of homozygous RobT stocks

6

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

14           GISH of the F<sub>3</sub> offspring derived from the four plants with wheat-*Th. intermedium*  
15 RobTs showed that in the U6032-633 and U6032-1444 progenies the RobTs were  
16 stabilized as telosomes (Table 3, Fig. 4) suggesting that the parental plants mostly were  
17 undergoing breakage fusion bridge cycles that remained undetected. The progeny of  
18 U6032-637 was segregating in 7 plants with *Th. intermedium* telosomes and 11 plants  
19 with RobTs (Table 3, Fig. 4). A total of 14 F<sub>3</sub> seeds were harvested from U6032-359,  
20 which was heterozygous for a RobT. Twelve plants were positive with both short-arm  
21 markers and six of them were analyzed by GISH, two plants were heterozygous and four

1 plants were homozygous for wheat-*Th. intermedium* RobTs (Table 3, Fig. 4).  
2 Unfortunately, plant U6032-321 did not set any seeds and was completely sterile.

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

4

5 The F<sub>2</sub> plant U6032-359 had a wheat-*Th. intermedium* RobT that was stably transmitted  
6 to the offspring and four plants that were homozygous for this RobT (designated as  
7 TA5657) were recovered in F<sub>3</sub>, which set an average of 270 seeds per plant. C-banding  
8 analysis of U6032-359 revealed that this family was homozygous for the wheat-wheat  
9 RobTs T5BL·7BL and T5BS·7BS (Fig. 2) that were inherited from the French wheat  
10 cultivar Vilmorin 27 (Friebe et al. 1992). In addition, 7D of wheat was involved in a  
11 RobT where the short arm with a small distal C-band was derived from 7J#1S and the  
12 long arm with proximal and small interstitial C-bands were derived from 7DL of wheat  
13 (Fig. 2). This compensating RobT can be described as T7DL·7J#1S. The chromosomal  
14 composition of this line was further analyzed by FISH using probes pAs1, pTa71, and  
15 (GAA)<sub>9</sub>.

16 Chromosome 7D has prominent pAs1 FISH sites at the telomeres of both arms  
17 (Fig. 2). FISH using the (GAA)<sub>9</sub> probe detected a distinct distal GAA FISH site in the  
18 7DL arm and a minor pTa71 FISH site was observed in the distal region of the 7DS arm  
19 (Fig. 2). An identical FISH pattern using these probes was observed in the CSDt7DS and  
20 CSDt7DL stocks (Fig. 2) and in the corresponding ditelosomic 7DS and 7DL stocks in  
21 Canthatch background (data not shown). In U6032-359, chromosome 7D had only one  
22 pAs1 FISH site at the telomere of the 7DL arm in addition to a diagnostic distal GAA



1 FISH site, whereas the short arm of this chromosome had no hybridization signals (Fig.  
2 2), confirming the presence of a T7DL·7J#1S RobT in this line.

3 GISH of line U6032-637 confirmed that this line was homozygous for a wheat-*Th.*  
4 *intermedium* RobT (Fig.2). C-banding of this family revealed that this line was  
5 segregating for T5BL·7BL and 5B and had a wheat *Th. intermedium* RobT where the  
6 short arm with a centromeric and proximal C-band was derived from 7BS and the long  
7 arm was derived from 7J#1S (Fig. 2). FISH using (GAA)<sub>9</sub> and pAs1 as probes confirmed  
8 that line U6032-637 is segregating for T5BL·7BL and 5B and is homozygous for the  
9 wheat-*Th. intermedium* RobT where the wheat arm has a centromeric and proximal GAA  
10 FISH site and was derived from 7BS and the *Th. intermedium* chromosome arm had no  
11 hybridization signal (Fig. 2). Thus, line U6032-637 is homozygous for the non-  
12 compensating RobT T7BS·7J#1S.

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

21

22 Stem rust resistance screening

1

2 Screening of the disomic chromosome addition line DA7J#1 (TA3647) in Vilmorin 27  
3 background and the ditelosomic addition lines DtA7J#1S (TA3656) in Chinese Spring  
4 background and DtA7J#1L (TA3659) in Courtot background with the *Pgt* isolates  
5 TTKSK, TTSKT, and TTTSK showed that all three lines were resistant, whereas the  
6 recipient wheat cultivars Vilmorin 27, Courtot, and Chinese Spring were susceptible  
7 (Table 4, Fig. 6). These data suggest that not only the short *Th. intermedium* chromosome  
8 arm 7J#1S, but also the 7J#1L arm harbors a stem rust resistance gene that is effective  
9 against the three Ug99 complex races tested. Whereas the stem rust resistance gene in the  
10 7J#1S arm has been previously designated as *Sr44* (Friebe et al. 1996) the presence of a  
11 stem rust resistance gene in the 7J#1L arm was previously unknown. The non-  
12 compensating T7DS-7J#1L-7J#1S translocation (TA5584) in Chinese Spring background  
13 and the compensating RobT T7DL-7J#1S (TA5657) identified in the present study  
14 conferred resistance to the *Pgt* isolates TTKSK, TTSKT, and TTTSK, whereas only the  
15 former line and the ditelosomic addition for 7J#1L (TA3659) in Courtot background but  
16 not TA5657 was resistant to TRTTF (Table 4, Fig. 6). Because DtA7J#1L in Courtot  
17 background and T7DS-7J#1L-7J#1S in Chinese Spring background displayed resistance  
18 to the *Pgt* isolate TRTTF, and the stock with the complete 7J#1 chromosome in Vilmorin  
19 27 background was susceptible, these data suggest that Vilmorin 27 has a gene that  
20 suppresses the resistance of the unnamed stem rust resistance gene present in the 7J#1L  
21 arm.

22 We also evaluated the T7DS-7J#1S-7J#1L (TC6, TA5546) and T7DS-7DL-7J#1L  
23 (TC14, TA5551) stocks in Sunstar background that were derived from the same 7J#1 *Th.*

1 *intermedium* chromosome and harbor a resistance gene against barley yellow dwarf  
2 (*Bvd2*) together with their recipient wheat cultivar Sunstar against the *Pgt* isolates  
3 TRTTF and TTKSK. Whereas Sunstar, TC6, and TC14 were resistant to race TRTTF, all  
4 three lines were susceptible against TTKSK (Table 4, Fig. 6), suggesting that the stem  
5 rust resistance gene *Sr44* is located on the distal 7J#1S fragment that is replaced by 7DS  
6 in T7DS-7J#1S·7J#1L in TC6.

7

## 8 **Discussion**

9

10 In the present study we produced plants that were double monosomic for wheat  
11 chromosome 7D and the *Th. intermedium* chromosome 7J#1 and, thus, we targeted  
12 chromosomes 7D and 7J#1 to be involved in the formation of RobTs. In these plants  
13 chromosomes 7D and 7J#1 do not pair at meiotic metaphase I and can misdivide at the  
14 centromeres, which after fusion of the broken ends can give rise to the formation of  
15 wheat-*Th. intermedium* RobTs, (Sears 1952; Friebe et al. 2005). However we also  
16 identified three plants in the progeny of such double monosomic plants that had wheat-*Th.*  
17 *intermedium* dicentric chromosomes, one of which was stabilized as a *Th. intermedium*  
18 telosome, one as a wheat-*Th. intermedium* recombinant chromosome and one was  
19 stabilized as a wheat-*Th. intermedium* RobT. Dicentric chromosomes are known to  
20 undergo chromosome-type breakage-fusion-bridge (BFB) cycles and usually never enter  
21 the meiotic divisions (Friebe et al. 2001). In addition, in two of the four plants that had  
22 wheat-*Th. intermedium* RobTs, the translocations were stabilized as *Th. intermedium*

1 telosomes, indicating that the original plants also had dicentric chromosomes that were  
2 undergoing BFB cycles, which remained undetected. The mechanism leading to the  
3 formation of wheat-*Th. intermedium* dicentric chromosomes in progenies of plants  
4 double monosomic for a *Th. intermedium* and a homoeologous wheat chromosome is  
5 unknown. However, it appears that this process is not a very rare event. Previously we  
6 reported the recovery of a wheat-*Th. intermedium* T7BS·7S#3L RobT conferring  
7 resistance to wheat streak mosaic virus that was also derived from a wheat-*Th.*  
8 *intermedium* dicentric chromosome in the progeny of plants double monosomic for  
9 chromosomes 7D and 7S#3 (Liu et al. 2011c).

10         The compensating T7DL·7J#1S RobT identified in the present study harbors the  
11 stem rust resistance gene *Sr44*, which confers resistance to the Ug99 race complex  
12 including races TTKSK, TTSKT, and TTTSK and is located in the 7J#1S arm.  
13 Surprisingly, our data also showed that the *Th. intermedium* long arm 7J#1L harbors an  
14 unnamed stem rust resistance gene that confers resistance to all Ug99 isolates tested in  
15 the present study. However, our data further indicate that the expression of this gene is  
16 modified by the wheat background. Whereas the 7J#1L stem rust resistance gene confers  
17 resistance of the DtA7J#1L and T7DS-7J#1L·7J#1S stocks in Courtot and Chinese Spring  
18 background, the expression of this gene in the DA7J#1 stock with the complete *Th.*  
19 *intermedium* chromosome is suppressed in Vilmorin 27 background. It is well known that  
20 the expression of alien disease resistance genes when transferred to wheat can be  
21 modified and suppressed by wheat backgrounds. Suppressors of leaf rust resistance genes  
22 have been previously mapped to A- and B-genome chromosomes by Innes and Kerber  
23 (1994) and to D-genome chromosomes by Bai and Knott (1992). Similarly, the

1 expression of the leaf rust resistance gene *Lr23* was shown to be modified by suppressors  
2 present in the recipient wheat cultivars (McIntosh and Dyck 1975; Nelson et al. 1997).  
3 Recently, McIntosh and coworkers (2011) showed that the expression of the powdery  
4 mildew resistance gene *Pm8* was suppressed by the presence of the *Pm3* locus.

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

16

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- 17

## 1 **Legends of Figures**

2

3 Figure 1: Crossing scheme for producing compensating RobTs involving wheat  
4 chromosome 7D and the *Th. intermedium* chromosome 7Ai#1. The Chinese  
5 Spring stock monosomic for chromosome 7D is crossed as a female with the  
6 7Ai#1 disomic addition stock in Vilmorin 27 background. F<sub>1</sub> plants with  
7 2n=6x=42 chromosomes are selected that are double monosomic for 7D and  
8 7Ai#1, allowed to self pollinate and their progenies are screened for putative  
9 RobTs.

10 Figure 2: C-banding, GISH and FISH patterns of the critical chromosomes involved in  
11 the *Sr44* transfer. Upper panel from left to right: C-banding and GISH of *Th.*  
12 *intermedium* chromosomes and telosomes from VILDA7J#1 (TA3647),  
13 VILDtA7J#1S (TA3656) and CORDtA7J#1L (TA3659); C-banding of  
14 chromosome CS7D and the compensating RobT T7DL·7J#1S present in U6032-  
15 359; FISH of CS7D (TA3008), CSDt7DS (TA3130), CSDt7DL (TA3071) and  
16 FISH and GISH of the compensating RobT present in U6032-359. Lower panel:  
17 C-banding and FISH of the reciprocal RobTs T5BL·7BL and T5BS·7BS present  
18 in U6032-359 and U6032-637 and FISH and GISH of the noncompensating RobT  
19 T7BS·7J#1S present in U6032-637.

20 Figure 3: PCR patterns of Chinese Spring (CS), VILDA7J#1 (TA3647), CSDtA7S#3L  
21 (TA7700), CSM7D X TA3647 (U6032), VILDtA7J#1S (TA3656), and  
22 CORDtA7J#1L (TA3659) with specific markers for 7J#1 chromosome arms: a)

1 7J#1 short-arm marker *Xbe404728* (using *MspI*), b) 7J#1 short-arm marker  
2 *Xbe473884* (*MspI*), c) 7J#1 long-arm marker *Xbe498418* (*HaeIII*). Polymorphic  
3 fragments are marked by arrows.

4 Figure 4: Genomic *in situ* hybridization pattern using total genomic *Th. intermedium*  
5 DNA as a probe of putative wheat-*Th. intermedium* RobTs identified in U6032 F<sub>2</sub>  
6 plants and the derived chromosomal rearrangements recovered in F<sub>3</sub> progenies;  
7 note that plant # 176, 286, and 321 originally had a dicentric chromosome where  
8 the centromeres are marked by arrows.

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

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

18



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

Marker	Forward /Reverse primer 5'-3'	Location (deletion bin)	Enzyme for polymorphism	EST accession
<i>Xbe404728</i>	5' GGTGGTGCCTGTCAAGATT 3' 5' TTGATGGATCCTGGCTTAGG 3'	C-7BS1-0.27	<i>MspI</i>	BE404728
<i>Xbe473884</i>	5' GTTGACG TTCATAGCGAGCA' 5' CGAGCCACAGTCCTTCCTAC 3'	7AS1-0.89-1.00	<i>MspI</i>	BE473884
<i>Xbe498418</i>	5' GCAGATCTTGGGGATCAAAA 3' 5' CTCCATGAGAAGCCATAGCC 3'	C-7DL5-0.30	<i>HaeIII</i>	BE498418

4

5

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

3

No. of plants	Selection scheme 1	Selection scheme 2	Selection scheme 3
No. of plants planted	1152	840	410
No. of plants with purple coleoptile	N/A	572	281
No. of plants with positive <i>Xbe473884</i> (C-7BS1-0.27)	970	N/A	272
No. of plants with positive <i>Xbe404728</i> (7AS1-0.89-1.00)	970	N/A	272
No. of plants positive for <i>Xbe473884</i> and <i>Xbe404728</i> and negative for <i>Xbe498418</i> (C-7DL5-0.30)	26	N/A	9
No. of plants with purple coleoptiles that were negative for <i>Xbe498418</i>	N/A	13	N/A
No. of plants GISHed	26	13	9
No. of plants with no signal	3	1	-
No. of plants with 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	-	-

4

5

1 **Table 3:** GISH analysis of F<sub>3</sub> progenies derived from F<sub>2</sub> plants with dicentric and RobT  
 2 chromosomes

Rearrangement in F <sub>2</sub>	F <sub>3</sub> 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			-	-

3

4

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

3  
 4

Germplasm	Chromosomal constitution	Background	<i>Pgt</i> race TRTTF	<i>Pgt</i> race TTKSK	<i>Pgt</i> race TTSKT	<i>Pgt</i> race TTTSK
TA5584	T7DS-7J#1L-7J#1S	Chinese Spring	2-	2	22-	2-
TA5657	T7DL-7J#1S	Chinese Spring	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

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