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ORIGINAL PAPER

A novel Robertsonian translocation event leads to transfer of a stem rust resistance gene (*Sr52*) effective against race Ug99 from *Dasypyrum villosum* into bread wheat

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Abstract Stem rust (*Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn.) (the causal agent of wheat stem rust) race Ug99 (also designated TTKSK) and its derivatives have defeated several important stem rust resistance genes widely used in wheat (*Triticum aestivum* L.) production, rendering much of the worldwide wheat acreage susceptible. In order to identify new resistance sources, a large collection of wheat relatives and genetic stocks maintained at the Wheat Genetic and Genomic Resources Center was screened. The results revealed that most accessions of the diploid relative *Dasypyrum villosum* (L.) Candargy were highly resistant. The screening of a set of wheat–*D. villosum* chromosome addition lines revealed that the wheat–*D. villosum* disomic addition line DA6V#3 was moderately resistant to race Ug99. The objective of the present study was to produce and characterize compensating wheat–*D. villosum* whole arm Robertsonian translocations (RobTs) involving chromosomes 6D of wheat and 6V#3 of *D. villosum* through the mechanism of centric breakage-fusion. Seven 6V#3-specific EST–STS markers were developed for screening F_2 progeny derived from plants double-monosomic for chromosomes 6D and 6V#3. Surprisingly, although 6D was the target chromosome, all recovered RobTs involved chromosome 6A implying a novel mechanism for the origin of RobTs. Homozygous translocations (T6AS·6V#3L and T6AL·6V#3S) with good plant vigor and full fertility were selected from F_3 families. A stem rust resistance gene was mapped to the long arm 6V#3L in T6AS·6V#3L and was designated as *Sr52*. *Sr52* is temperature-sensitive and is most effective at 16°C, partially effective at 24°C, and ineffective at 28°C. The T6AS·6V#3L stock is a new source of resistance to Ug99, is cytogenetically stable, and may be useful in wheat improvement.

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Introduction

Triticeae relatives of bread wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) are an important source of genetic variation for biotic and abiotic stress tolerance and have been widely utilized in wheat improvement (for review, see Friebe et al. 1996; Gill et al. 2006). The specific approach for transferring alien genes to wheat depends on the evolutionary relationships of the species involved. Species belonging to the tertiary gene pool of bread wheat

have homoeologous genomes. Homoeologous chromosomes have similar gene content and collinearity and can replace each other in compensating nullisomic–tetrasomic combinations (Sears 1952a, 1966). Various strategies for transferring genes from the tertiary gene pool have been recently reviewed (Qi et al. 2007).

One important step in alien transfer is the production of compensating wheat–alien translocations. These can be readily produced for the targeted chromosomes through the mechanism of centric breakage–fusion (Sears 1952b). Friebe et al. (2005) investigated the mechanism of origin of Robertsonian translocations (RobTs) in plants double-monosomic for 1A of wheat and $1H^t$ of *Elymus trachycaulus* (Link) Gould ex Shinners by genomic in situ hybridization (GISH). This study revealed that RobTs arise by centric misdivision of univalents at anaphase I followed by the rejoining of the broken chromosome ends during interkinesis of meiosis II. The formation of compensating translocations in a double-monosomic plant requires that the derived telocentric chromosomes from opposite arms of homoeologous chromosomes in ana-/telophase I migrate to the same spindle pole followed by the fusion of the broken ends during the interkinesis. Zhang et al. (2001) showed that centric breakage–fusion can occur at different positions within the primary constriction without affecting the behavior of wheat–rye RobTs in mitosis or meiosis. Depending on the chromosomes involved and environmental conditions, the desired compensating wheat–alien RobTs can be recovered at fairly high frequencies (Lukaszewski and Gustafson 1983; Davies et al. 1985; Lukaszewski 1993, 1994, 1997; Friebe et al. 2005).

Dasypyrum villosum (L.) Candargy (syn. *Haynaldia villosa* (L.) Schur. is a diploid wild relative ($2n = 2x = 14$, VV) of bread wheat native to the Mediterranean region. Genes transferred from *D. villosum* to wheat include powdery mildew resistance gene *Pm21* in the form of a T6AL·6VS translocation (Qi et al. 1993; Chen et al. 1995) and wheat spindle streak mosaic virus resistance gene *Wss1* in the form of a T4DL·4VS translocation (Zhang et al. 2005). T6AL·6VS also has a gene conferring resistance to wheat curl mite colonization (Chen et al. 1996). In addition, resistance to cereal eyespot caused by *Tapesia* spp. was reported in a chromosome 4V addition line and later mapped to the long arm of 4V (Murray et al. 1994; Yildirim et al. 1998). Among the various transfers, T6AL·6VS carrying *Pm21* has been exploited in production agriculture.

Race Ug99 (also designated TTKSK) of the wheat stem rust pathogen, *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn. (*Pgt*) and its derivatives have defeated several important stem rust resistance genes widely used in wheat, rendering much of the worldwide wheat crop acreage susceptible (Singh et al. 2008; Jin et al. 2007, 2008, 2009). In order to identify additional sources of resistance to Ug99, we screened the Wheat Genetic and Genomic Resources Center (WGGRC) collection of *D. villosum* (95 accessions) with North American stem rust races (a mixture of TPMKC, RKQQC, and QFCSC). All accessions were found to be nearly immune with infection types ranging from "0" to "0;" (Roelfs and Martens 1988)]. Among the designated stem rust (*Sr*) resistance genes (McIntosh et al. 1995; http://shigen.lab.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp), none were transferred from *D. villosum*. These observations prompted us to characterize an existing set of *D. villosum* disomic addition stocks in the background of susceptible cultivar 'Chinese Spring', which revealed that chromosome 6V (in accession TA7682, $21'' + 1''$[DA6V#3]) harbors at least one stem rust resistance gene, designated as *Sr52*. *Sr52* conferred resistance to TTKSK in replicated seedling tests with ITs of;1+ to 2 based on testing of TA7682 (Pumphrey et al. 2008). The present study describes the production of compensating wheat–*D. villosum* RobTs involving chromosomes 6A and 6V and the localization of *Sr52* on the long arm of T6AS·6V#3L.

Materials and methods

Plant material

The lines used in this study included *T. aestivum* cv. Chinese Spring (CS), *D. villosum*, CS monosomic stock CSM6D, three nulli-tetrasomic (N6AT6B, N6BT6A, and N6DT6B) and six ditelosomic (Dt6AS, Dt6AL, Dt6BS, Dt6BL, Dt6DS, and Dt6DL) stocks (Table 1). A complete set of wheat–*D. villosum* disomic chromosome addition lines (DA) in CS background, in which a pair of chromosomes from *D. villosum* is added to the wheat complement, was provided by Dr. A.J. Lukaszewski, University of California Riverside. All materials are maintained by the WGGRC at Kansas State University, Manhattan, KS, USA (http://www.k-state.edu/wgrc/).

In order to produce compensating RobTs involving chromosomes 6D of wheat and 6V#3 of *D. villosum,* crosses between M6D ($20'' + 6D'$) and DA6V#3 ($21W'' + 6V\#3''$) were made. F_1 plants with 42 chromosomes that were double-monosomic for chromosomes 6D/6V#3 were identified cytologically and self-pollinated. The F_2 progeny were genotyped by molecular markers for putative RobTs.

EST selection and STS primer design

The short and long arms of chromosome 6V#3 have similar C-banding patterns making it difficult to distinguish them by C-banding analysis (Friebe et al. 1987). We developed

Table 1 Genetic stocks used in the study

WGGRC accession number	Genetic stocks	Description	Reference
TA3808	CS	*Triticum aestivum* cv. Chinese Spring	
TA3060	M6D	CS Monosomic 6D	
TA3152	N6AT6B	CS nullisomic6A tetrasomic 6B	Sears (1954)
TA3154	N6BT6A	CS nullisomic 6B tetrasomic 6A	
TA3157	N6DT6B	CS nullisomic 6D tetrasomic 6B	
TA3108	Dt6AS	CS ditelosomic 6AS	Sears and Sears (1978)
TA3109	Dt6AL	CS ditelosomic 6AL	
TA3119	Dt6BS	CS ditelosomic 6BS	
TA3120	Dt6BL	CS ditelosomic 6BL	
TA3128	Dt6DS	CS ditelosomic 6DS	
TA3129	Dt6DL	CS ditelosomic 6DL	
TA7677	DA1V	CS–*D. villosum disomic* addition 1V#3	Lukaszewski, unpublished data
TA7678	DA2V	CS–*D. villosum disomic* addition 2V#3	
TA7679	DA3V	CS–*D. villosum disomic* addition 3V#3	
TA7680	DA4V	CS–*D. villosum disomic* addition 4V#3	
TA7681	DA5V	CS–*D. villosum disomic* addition 5V#3	
TA7682	DA6V	CS–*D. villosum disomic* addition 6V#3	
TA7683	DA7V	CS–*D. villosum disomic* addition 7V#3	
TA10220	*D. villosum*	*D. villosum*	

PCR-based STS markers for screening large numbers of progeny to expedite the identification of rare compensating RobT chromosomes. Chromosome bin-mapped wheat ESTs (expressed sequence tags) are an invaluable resource for marker development, especially for distantly related wild species, where SSR markers are not available. Bin-mapped ESTs for each arm of wheat group-6 chromosomes from two chromosome bins were selected, a proximal bin close to the centromere and a distal bin close to the telomere. The molecular markers developed from these four regions provide representative coverage of chromosome 6V#3. A sample of 258 wheat ESTs, 162 from the short arm and 96 from the long arm of wheat group-6 chromosomes, were selected from the wheat EST mapping project (http://wheat.pw.usda.gov/NSF/project/mapping_data.html). The primers were designed using the software Primer 3 with manual selection (Rozen and Skaletsky 2000).

Genomic DNA of genetic stocks and progenies was isolated from leaves harvested from 2- to 4-week-old seedlings according to the protocol of Qi et al. (2003). The 258 primer pairs were screened for amplification length polymorphisms between CS and DA6V#3 using methods described by Qi et al. (2007). The PCR products were digested with the four-base recognition restriction enzymes *Alu*I, *Hae*III, *Msp*I, and *Rsa*I to increase the likelihood of polymorphism detection before size separation in a 1% agarose gel. The PCR products for microsatellites (SSRs) were separated in 2.3% agarose gels and visualized under UV light (Röder et al. 1998).

Cytogenetic analysis

C-banding and chromosome identification were according to Gill et al. (1991) and GISH followed the protocol of Qi et al. (2008). Fluorescence in situ hybridization (FISH) was according to Zhang et al. (2001).

Stem rust evaluation

Seedlings were grown in 20 × 20 × 5 cm aluminum pans in Metromix 360 (Hummert International, Earth City, MO) peat/perlite/bark ash growing mix at 20 ± 1°C and light intensity of 300–500 μmol m^{-2} s^{-1} in plant growth chambers at Manhattan, KS. Desiccated urediniospores of *Pgt* race RKQQC [virulence/avirulence formula: *Sr5*, *6*, *7b*, *8a*, *9a*, *9b*, *9d*, *9g*, *21*, *36*, *McN*/*Sr9e*, *10*, *11*, *17*, *24*, *30*, *31*, *38*, *Tmp* (Roelfs and Martens 1988; Jin et al. 2008)] were stored in glass vials in liquid nitrogen or in cryotubes in an ultralow freezer at −80°C. They were heat-shocked for 6 min in a water bath at 42°C prior to use. Spores were suspended in Soltrol 170 light oil (Chevron Phillips Chemical Company, The Woodlands, TX) and sprayed onto seedlings at the 2–3 leaf stage. The oil was allowed to evaporate for 10 min and then plants were placed in a dew

chamber at 22 ± 2°C with 100% relative humidity in the dark for 16 h. For routine screening, plants were moved to a greenhouse for symptom development at temperatures ranging from 18 to 24°C. Natural light was supplemented with high-pressure sodium lights to obtain a photoperiod of 16 h.

For the temperature study, plants were moved to reach-in plant growth chambers set at 16, 20, 24, or 28 ± 1°C with a 15 h photoperiod and light intensity of approximately 500 μmol m^{-2} s^{-1}. Infection types (ITs) were recorded for the second leaf using a 0–4 scale (Roelfs and Martens 1988) when susceptible controls showed fully developed symptoms. Six seedlings per line were scored and a consensus rating was determined. The experiment was repeated once. Seedling reactions to *Pgt* race TTKSK (virulence/avirulence formula: *Sr5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 30, 31, 38, McN/Sr24*, *36*, *Tmp*) were tested in greenhouse facilities at the USDA-ARS Cereal Disease Laboratory, St. Paul, MN, at 18 ± 2°C using procedures reported previously (Jin et al. 2007).

Results

Development of chromosome 6V#3 arm-specific STS markers

Seven out of 258 STS primer pairs (2.7%) displayed polymorphism between CS and DA6V#3, four on the short arm (6V#3S) and three on the long arm (6V#3L) (Table 2). The 6V#3S-specific STS markers cover ~55% of the distal region. Two of the 6V#3L-specific STS markers are located in the centromeric bin, and one is located in the distal bin. To assign specific alleles to homoeologous chromosomes and arms of wheat group-6, the seven polymorphic markers were amplified in group-6 nullisomic/tetrasomic and ditelosomic stocks. All wheat group-6 fragments co-migrated and were indistinguishable, but those derived from 6V#3 were polymorphic (Fig. 1).

Identification of wheat–*D. villosum* compensating RobTs

A total of 280 F_2 plants derived from F_1 plants double-monosomic for chromosomes 6D/6V#3 were screened with BF201083-STS marker for 6V#3S and BE497099-STS marker for 6V#3L. The 6V#3-specific polymorphic markers (for both S and L arms) were observed in 87 plants. Twenty plants positive for the 6V#3S marker only had either a 6V#3S telocentric chromosome (t6V#3S), a 6V#3S isochromosome (i6V#3S) or a wheat-6V#3S RobT. Nine plants had only 6V#3L marker indicating that all plants had misdivision products involving 6V#3L.

GISH analysis using *D. villosum* genomic DNA as probe on the 20 plants positive for the 6V#3S marker only revealed that five plants (1.8%) had wheat-6V#3S RobTs (progenies 103, 157, 164, 212, and 216), five had t6V#3S (1.8%), and 10 most probably were i6V#3S (3.8%). GISH analysis of the nine plants, which were positive for 6V#3L but negative for 6V#3S markers showed that two plants (0.7%) had wheat-6V#3L RobTs (progenies 60 and 197), two had t6V#3L (0.7%), and five had i6V#3L (1.8%). The total recovery rate of RobTs was 2.5% (1.8% for wheat-6V#3S plus 0.7% for wheat-6 V#3L). The overall centromeric misdivision recovery rate for 6V#3 was 8.1% (2.5% for telosomics plus 5.6% for isochromosomes).

C-banding analysis was used to identify the wheat chromosome arms involved in these translocations. Surprisingly, none of the RobTs involved the target wheat chromosome 6D but instead all seven involved 6A of

Table 2 Primer sequences of the STS markers derived from wheat ESTs on the wheat group-6 chromosomes and primer/enzyme Combinations producing polymorphic PCR products

Marker	EST	EST bin location[a]	Forward primer 5′-3′	Reverse primer 5′-3′	Annealing temperature (°C)	Enzyme producing polymorphic PCR product
BF201083-STS	BF201083	6S-0.99-1.00	CGACACACCTGGAGTCCATC	GGTTGGGGGAGTCAATGTAA	61	*Hae*III
BE497623-STS	BE497623	6DS4-0.79-0.99	ATTTTGTGGCCTTTTGCTTG	CGAGGAGCTCAAGAAAGCAT	60	*Msp*I
BE424523-STS	BE424523	6S-0.79-0.99	AGCAAACGAAAACCAAGCAC	GCTTGATGCAGAAAAAGTTGG	60	*Rsa*I
BE490365-STS	BE490365	6S-0.45-0.79	GTGCTCCTGCTGGTGATCT	TGACCTCATTATCCGGCTTC	55	*Msp*I
BE518064-STS	BE518064	C-6L-0.29	TCCACCGAAGAATCACCAAT	ACCTTTGTGGATGTGGCAAT	60	*Alu*I, *Hae*III, *Msp*I, *Rsa*I
BE422631-STS	BE422631	6L-0.29-0.36	CCCGCACAGTTCACAATAGA	GCAGTTGCACCGTTTTATGA	59	*Alu*I, *Hae*III, *Msp*I, *Rsa*I
BE497099-STS	BE497099	6L-0.90-1.00	TTCGCTCCACCAGGAGTCTA	GTGTCTCGCCATGGAAGG	60	*Msp*I

[a] The consensus bin location was used for those ESTs which mapped to two or three chromosomes of wheat group 6 (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi)

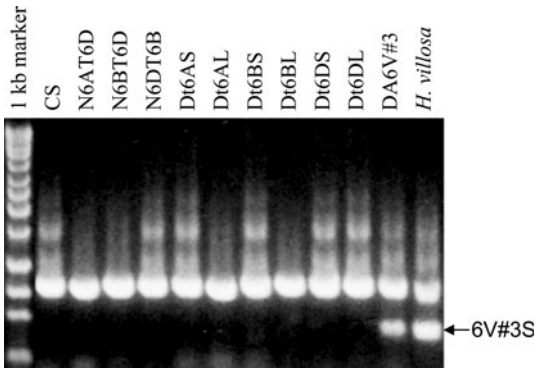

Fig. 1 PCR pattern of nullisomic–tetrasomic (NT) and ditelosomic (Dt) lines of wheat group-6 chromosomes, wheat–*Dasypyrum villosum* disomic addition line DA6V#3 and *D. villosum* amplified by BF201083-STS primers. The EST BF201083 was previously mapped to the short arm of wheat group-6 chromosomes (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). PCR products were digested with the enzyme *Hae*III. The wheat group-6 fragments co-migrated and could not be differentiated by NT lines. *Arrow* indicates the 6V#3S-specific fragment

wheat and both compensating T6AS·6V#3L and T6AL·6V#3S translocations were recovered (Fig. 2c). The possibility that the M6D stock used in the production of the double-monosomic 6D/6V#3 plants may have been misidentified was ruled out. The C-banding analysis on mitotic metaphase chromosomes of root tip meristems and on pollen mother cells (PMCs) at meiotic metaphase I and FISH on PMCs with a D-genome specific probe pAs1 (Rayburn and Gill 1986) confirmed that the monosomic chromosome and the univalent in the parental M6D stock is in fact 6D of wheat, and not 6A.

The STS markers developed in the present study are dominant and cannot be used to distinguish between homozygous and heterozygous wheat–alien translocation lines as previously described (Qi et al. 2008). However, combined use of 6V-specific markers and 6A-specific SSR markers allowed us to select homozygous translocations. Markers GWM334 and BARC3 for chromosome 6A short arm and BARC171 and GWM571 for 6A long arm were used to select homozygous T6AS·6V#3L and T6AL·6 V#3S translocations (Fig. 3).

Stem rust testing on the primary translocations T6AS·6V#3L and T6AL·6V#3S showed that the stem rust resistance gene *Sr52* resides in the long arm of chromosome 6V (data not shown). Therefore, homozygous plants for T6AL·6V#3S were identified based on marker and cytological analysis only. Fifty F_3 plants from each putative hemizygous (T6AL·6V#3S/−) or heterozygous (T6AL·6V#3S/6A) translocation F_2 plants 103, 157, 164, 212, and 216 were screened with STS markers BF201083 and BE490365, 6AS-specific SSR marker GWM334, and 6AL-specific SSR marker BARC171. Surprisingly, all progenies from 103, 157, and 212 only segregated for the translocation chromosome T6AL·6V#3S. They were all homozygous or hemizygous for T6AL·6V#3S. GISH observations were consistent with the marker data indicating that primary translocations in lines 103, 157, and 212 were hemizygous for T6AL·6V#3S and were lacking chromosome 6A. The gametes with 20 wheat chromosomes plus T6AL·6V#3S from these plants were preferentially transmitted to the progeny. However, the progenies of F_2 plants 164 and 216 segregated for T6AL·6V#3S and 6A; T6AL·6V#3S was transmitted to 58–62% of the progenies. Only one homozygous T6AL·6V#3S was recovered among 50 F_3 plants derived from the F_2 plant 164. The progeny from 216 was not analyzed.

Stem rust testing, marker and GISH analyses were used to identify plants homozygous for T6AS·6V#3L. Fifty plants each from F_2-60 (derived F_3: 09-17) and F_2-197 (derived F_3: 09-18) containing chromosome T6AS·6 V#3L were screened for resistance to *Pgt* RKQQC. Seventeen resistant plants from the two families were selected for further DNA marker and GISH analyses; seven homozygous T6AS·6V#3L plants were recovered (Fig. 3).

Characterization of resistance in homozygous translocation lines

Previous screening of a set of *D. villosum* disomic addition stocks in Chinese Spring revealed that the disomic addition line DA6V#3 was resistant to the three North American *Pgt* races RKQQC, TPMKC, and QFCSC, and race TTKSK (Pumphrey et al. 2008). Homozygous lines of T6AS·6V#3L (09-17-5, 09-18-4 and 09-18-11), T6AL·6V#3S (TA5618) along with resistant DA6V#3 (TA7682), and susceptible CS were tested for their reaction to race RKQQC at four different temperatures (Table 3). Resistance was ineffective at 28°C as indicated by a high IT (33+), partially effective at 24°C, and most effective at 16 or 20°C. DA6V#3 exhibited lowest IT (2−;) at 16°C, lower than the derived line T6AS·6V#3L, but the differences in ITs were not evident at higher incubation temperatures. With race TTKSK, DA6V#3 again showed lower IT than T6AS·6V#3L (Table 3). In all cases, T6AL·6V#3S exhibited susceptible ITs similar to the susceptible parent, CS.

Discussion

Sr52 is the first stem rust gene discovered in *D. villosum* that is effective against *Pgt* race Ug99. The integrated use of cytogenetic stocks, molecular resources, and combined molecular marker, C-banding, and GISH analyses, allowed to introgress gene *Sr52* from *D. villosum* into bread wheat by developing compensating RobTs T6AS·6V#3L and

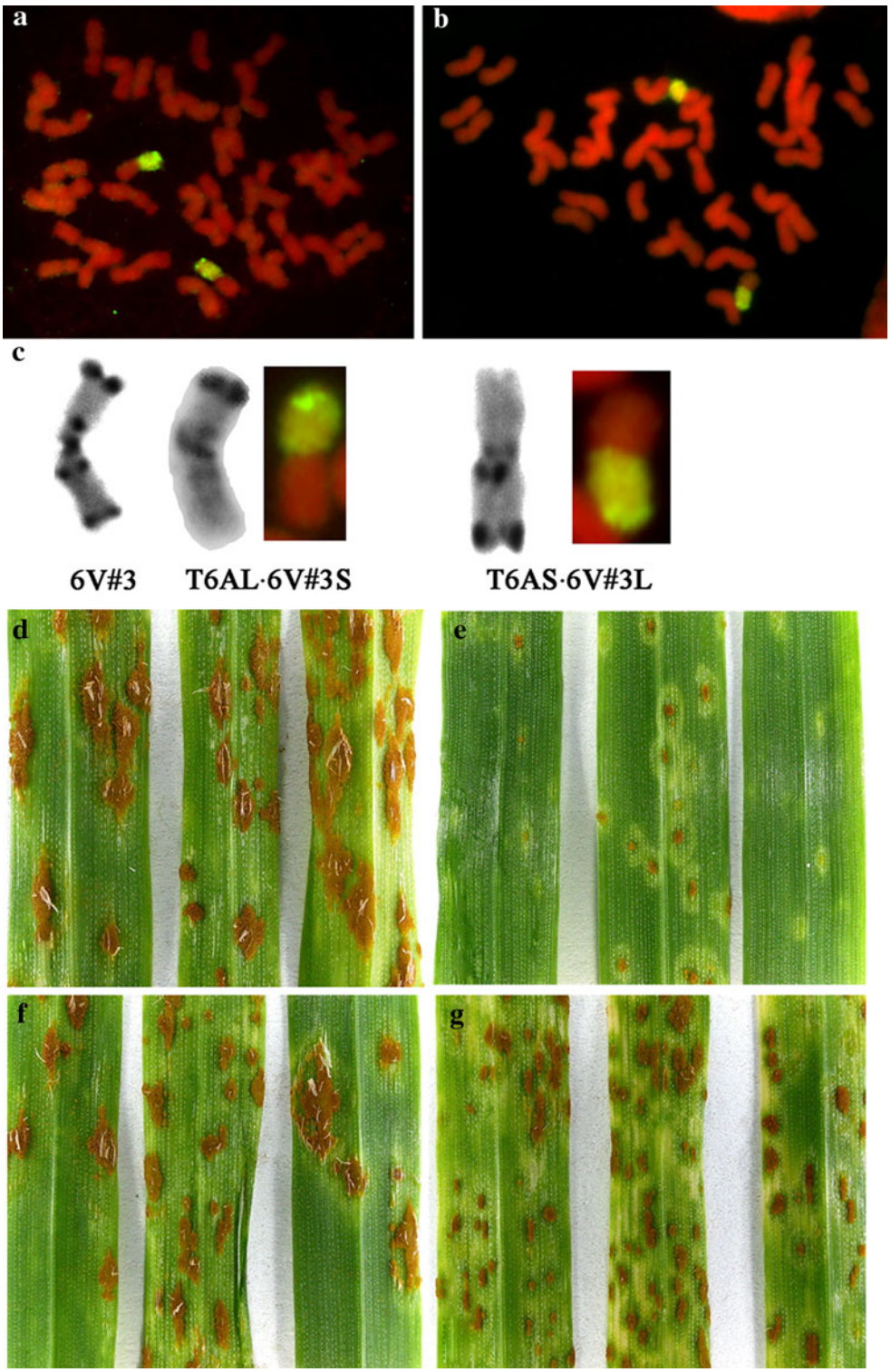

Fig. 2 C-banding and GISH patterns and infection types (ITs) of wheat–*Dasypyrum villosum* compensating translocations T6AL·6V#3S and T6AS·6V#3L; **a** GISH pattern of the T6AL·6V#3S translocation stock; **b** GISH pattern of the T6AS·6V#3L translocation stock; **c** from left to right: C-banding pattern of 6V; C-banding and GISH patterns of T6AL·6V#3S; C-banding and GISH patterns of T6AS·6V#3L; the translocation chromosomes were visualized by *yellow-green* FITC fluorescence and wheat chromosomes were counterstained with propidium iodide and fluoresced *red*; **d** TA3808 (Chinese Spring), **e** TA7682-3 (DA6V#3), **f** TA5618 (T6AL·6V#3S), **g** 09-17-5 (T6AS·6V#3L) infection types 17 days after inoculation with *Pgt* culture RKQQC incubated at 16°C

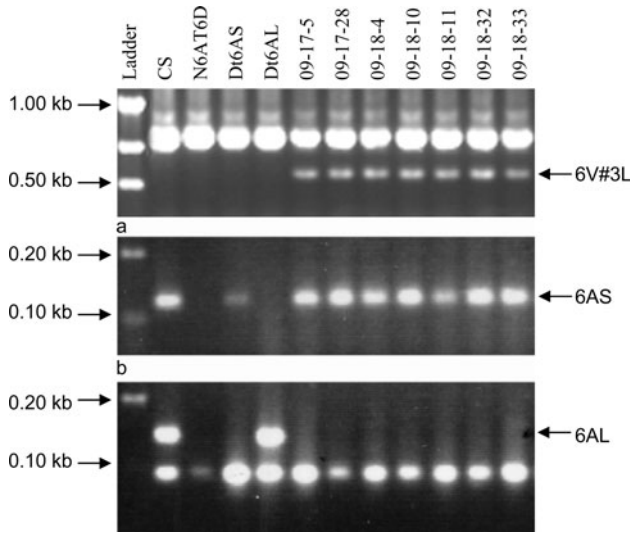

Fig. 3 PCR patterns of markers in CS, nullisomic 6A–tetrasomic 6D (N6AT6D), ditelosomic 6AS (Dt6AS), ditelosomic 6AL (Dt6AL), and homozygous translocation T6AS·6V#3L lines (*lanes 6–12*); **a** PCR patterns of BE497099-STS. The EST BE497099 was previously mapped to the long arm of wheat group-6 chromosomes (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). PCR products were digested with the enzyme *Msp*I, *arrow* indicates the 6VL-specific fragment; **b** PCR pattern of SSR marker GWM334, *arrow* indicates the 6AS-specific fragment; **c** PCR pattern of SSR marker GWM570, *arrow* indicates the 6AL-specific fragment. All T6AS·6V#3L lines harbor the 6VL- and 6AS-specific fragments, but are missing the 6AL-specific fragment, indicating they are homozygous for T6AS·6V#3L

Table 3 Infection types of 6V#3 introgression lines inoculated with races RKQQC and TTKSK of *P. graminis* f. sp. *tritici*

Line	Description	RKQQC[a]				TTKSK (Ug99)
		16°C	20°C	24°C	28°C	18°C
TA3808	Chinese Spring	3 + 3[b]	33+	33−	33+	4
TA7682-3	DA6V#3	2−;	23−	3 − 2+	33+	2
09-17-5	T6AS·6V#3L	23−	23−	3−	33+	2+
09-18-4	T6AS·6V#3L	23−	23−	3−	33+	2 + 3−
09-18-11	T6AS·6V#3L	23−	2+3−	3−	33+	2 + 3−
TA5618	T6AL·6V#3S	3+3	33+	33+	33+	3 + 3

[a] Race nomenclature described by Roelfs and Martens (1988) and modified by Jin et al. (2008)

[b] Infection types based on 0–4 Stakman scale described in Roelfs and Martens (1988)

mapped this gene to the long arm of chromosome 6V#3. RobTs facilitate gene mapping to chromosome arms, are a starting material for further chromosome engineering, and can be used for evaluating the agronomic performance of the introgressed segment (Qi et al. 2007).

The utility of resistance in translocation lines carrying *Sr52* is not yet clear. Although the original disomic addition line, DA6V#3, had a seedling IT of 2 to *Pgt* race TTKSK, the resistance of three independent long arm translocation lines appeared not as effective as the parental line, with ITs ranging from 2+ to 2 + 3− (Table 3). A similar pattern was seen for RKQQC at 16°C. This suggests that additional resistance genes might reside on the 6V short arm. However, the short arm translocation was fully susceptible, so that is unlikely. It appears that there is a weaker allele or another gene on 6AL that contributes to resistance in the DA6V#3. The substitution of 6AL by 6V#3L in T6AS·6V#3L stock leads to a reduced level of resistance. In fact, there may be several genes, both in *D. villosum* and wheat background that interact with *Sr52*. As mentioned before, the parental *D. villosum* line had IT of 0 or 0; whereas only DA6V#3 among the seven *D. villosum* DAs in CS wheat showed resistance but at a reduced level. Therefore, as proposed by Qi et al. (2007), whole genome chromosome engineering coupled with selection for optimum resistance may be a better approach for simultaneous transfer of all of resistance factors.

Sr52 in both the disomic addition line and the long arm translocation lines was temperature-sensitive, being most effective at 16°C and completely ineffective at 28°C. Similar temperature sensitivity has been noted for *Sr6*, *Sr12*, *Sr15*, and *Sr17* (McIntosh et al. 1995). Temperature sensitivity could reduce the utility of the gene because stem rust is more active in warmer weather. Studies of field performance in adult plants are needed to ascertain the level and environmental stability of resistance conferred by *Sr52*. The original *D. villosum* accession was not tested for the temperature sensitivity of the resistance and, thus, it is not known if this is a property of the gene in its native state or is caused by wheat background effect.

Classical genetic and molecular data show that genes conferring disease resistance in plants are frequently clustered in the genome, so RobTs often confer multiple desirable resistance traits. The most successful RobTs used in wheat improvement are the T1BL·1RS and T1AL·1RS translocations (Mettin et al. 1973; Zeller 1973). Translocation T1BL·1R#1S carrying *Sr31* also has additional genes that confer resistance to other important diseases such as leaf rust (*Lr26*), yellow rust (*Yr9*), and powdery mildew (*Pm8*) (Friebe et al. 1996). RobTs T6AL·6VS derived from *D. villosum* conferred resistance to powdery mildew and wheat curl mite colonization (Qi et al. 1993; Chen et al. 1996). This translocation has been extensively used in wheat breeding programs and has provided an immune reaction to powdery mildew disease for many years (Chen et al. 2008). Newly developed translocation T6AS·6V#3L may prove to be a valuable source of resistance to other biotic or abiotic stresses in addition to stem rust.

By producing plants double-monosomic for chromosomes 6D of wheat and 6V#3 of *D. villosum* we targeted

the formation of RobTs between these two chromosomes. The same approach was used to produce RobTs involving wheat chromosomes 1D, 2D, 3D, 4D, 5D, 7D and *D. villosum* 1V#3, 2V#3, 3V#3, 4V#3, 5V#3, and 7V#3 (Friebe et al. unpublished). In the present study seven RobTs were identified among the 280 progenies screened, corresponding to a frequency of 2.5%. Surprisingly, all translocations recovered in the present study involved chromosomes 6A and 6V#3, although the cross was certainly CSM6D/DA6V#3 (see "Results"). Univalent shift is known to occur in monosomic plants, which can change the chromosomal constitution of these stocks (Person 1956), but this was ruled out in this study.

Cereal plant centromeres consist of retrotransposon-like elements that are highly conserved (Argon-Alcaide et al. 1996; Jiang et al. 1996; Miller et al. 1998; Francki 2001). Sequence homology between the centromeres of homoeologous chromosomes can provide the structural basis for chromosome pairing and recombination, which may also result in formation of RobTs. This hypothesis was tested previously but no evidence of recombination events in the centromeres of plants double-monosomic for chromosomes 1A of wheat and $1H^t$ of *E. trachycaulus* was obtained (Friebe et al. 2005). However, the centromere structure of chromosome 6A might be unique and may allow crossover events between homoeologous centromeres to occur.

The described results suggest another possible explanation. The RobTs arising from breakage–fusion–bridge cycle come from a random event and are of both compensation and non-compensating type. However, the fact that all RobTs involving 6A and 6V#3 were non-random and of compensating type indicates close association between 6A and 6V homoeologues, including prealigment, but recombination may be restricted to the centromeric region. We have previously described an extreme example of a similar phenomenon including possible prealignment and recombination between non-homologous chromosomes producing a new chromosome accompanied by the loss of one centromere and two telomeres (Zhang et al. 2008).

The compensating translocation T6AS·6V#3L present in the stock TA5617 has a stem resistance gene, *Sr52,* which may be useful in wheat improvement. The effect of this chromosome on yield and quality-related traits is unknown at present. As a preemptive measure, we are pursuing further directed chromosome engineering aimed at shortening the *D. villosum* segment in the T6AS·6V#3L translocation, but retaining the stem rust resistance gene. As discussed in Qi et al. (2007), for the primary screening of recombinants, two (one proximal and one distal) informative molecular markers are sufficient to recover desirable recombinants. PCR markers developed in these two regions in the present study will not only facilitate the selection of RobTs, but also will be useful for screening *ph1b*-induced recombinants.

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