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ORIGINAL ARTICLE

Characterization of recombinants of the *Aegilops peregrina*-derived *Lr59* translocation of common wheat

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Abstract

Key message **A compensating, recombined *Lr59* translocation with greatly reduced alien chromatin was identified. Microsatellite locus *Xdupw217* occurs within the remaining segment and can be used as a co-dominant marker for *Lr59*.**

Abstract In earlier studies, leaf rust (caused by *Puccinia triticina* Eriks.) resistance gene *Lr59* was transferred from *Aegilops peregrina* (Hackel) Maire et Weiler to chromosome arm 1AL of common wheat (*Triticum aestivum* L.). The resistance gene was then genetically mapped on the translocated chromosome segment following homoeologous pairing induction. Eight recombinants that retained the least alien chromatin apparently resulted from crossover within a terminal region of the translocation that was structurally different from 1AL. These recombinants could not be differentiated by size, and it was not clear whether they were compensating in nature. The present study determined that the distal part of the original translocation has group 6 chromosome homoeology and a 6BS telomere (with the constitution of the full translocation chromosome being 1AS·1L^{P}·6S^{P} ·6BS). During the allosyndetic pairing induction experiment to map and shorten the full size translocation, a low frequency of quadrivalents involving 1A, the 1A translocation, and two 6B chromosomes was likely formed. Crossover within such quadrivalents apparently produced comparatively small compensating alien chromatin inserts within the 6BS satellite region on chromosome 6B of seven of the eight recombinants. It appears that the *Gli-B2* storage protein locus on 6BS has not been affected by the recombination events, and the translocations are therefore not expected to affect baking quality. Simple sequence repeat marker results showed that *Lr59*-151 is the shortest recombinant, and it will therefore be used in breeding. Marker DUPW217 detects a homoeo-allele within the remaining alien chromatin that can be used for marker-assisted selection of *Lr59*.

Introduction

Translocation of desirable alien resistance genes to wheat inevitably results in the co-transfer of linked genes contained within the introgressed region. Co-introgressed genes may be without apparent effect, or they may harm or improve adaptation, yield, or end use quality. Many examples exist, such as the sticky dough trait (Martin and Stewart 1986) and yield increase (Carver and Rayburn 1994) associated with the *Sr31* translocation from rye; yellow endosperm pigmentation (Sharma and Knott 1966) and yield increase/decrease (Singh et al. 1998) associated with the *Lr19* translocation; a yield penalty associated with *Sr26* (The et al. 1988); yellow endosperm pigmentation associated with *Sr43* (Knott et al. 1977; Niu et al. 2014); poor agrotype associated with *Lr45* (McIntosh et al. 1995a, b); and gametocidal effects associated with *Lr66* (Marais et al.

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2010b). While associated traits may be due to accompanying alien genes (linkage drag), the deleterious effects of some alien translocations result from genic imbalances brought about by reduced or non-homoeology with the displaced wheat chromosome region e.g., *Lr25* (4B-2R translocation; McIntosh et al. 2013), *Lr38* (several non-homoeologous translocations; Friebe et al. 1993), and *Sr37* (limited homoeology; McIntosh and Gyarfas 1971; McIntosh et al. 1995a, b).

Commercial acceptance of new resistance genes derived from alien sources is generally very slow. The testing of new translocations for associated effects is a tedious process, requiring crosses and backcrosses to develop near-isogenic lines or bulk populations in adapted germplasm. Ideally, the contrasting populations will then be compared in replicated field trials over several seasons and environments. Such verification is seldom done, often causing newly developed resistance genes to remain unutilized for many years. Breeders may sometimes involve new translocations in cycles of pre-breeding and assess its utility by observing the general performance of progeny carrying it. Hence, potentially useful translocations do in time become part of the mainstream breeding material.

Alien translocations mostly involve large chromosome regions that are inherited as single linkage blocks. In the presence of *Ph1*, which suppresses homoeologous chromosome pairing in wheat, genes contained within the alien segment cannot be individually recombined with genes in homoeologous wheat chromatin, thus also limiting the formation of novel gene combinations in breeding applications. In view of the above, it is sensible to minimize the co-introgressed alien chromatin associated with a favorable gene carried on a new translocation. Chromosome engineering techniques pioneered by Sears (1981) not only made it possible to tailor introgressed chromosome regions, but to also assess and improve their compensating abilities.

Antonov and Marais (1996) crossed a leaf rust-resistant accession of *Aegilops peregrina* (genomes UUSS) with Chinese Spring (CS) and doubled the chromosomes of the hybrid. Resistant progeny was then backcrossed to wheat (Marais et al. 2003) generating a spontaneous translocation line with pedigree: *Ae. peregrina*–680/2*CS//5*W84-17 (Marais et al. 2008). The translocation contained a dominant resistance gene (*Lr59*) which provided resistance to all *Puccinia triticina* pathotypes known to occur in South Africa and western Canada at the time (Marais et al. 2008). The *Lr59* translocation was thought to have replaced the complete 1AL arm and did not pair with the normal wheat 1AL telosome during meiosis.

An allosyndetic mapping experiment (Marais et al. 2010a) subsequently located *Lr59* towards the distal end of the translocation. In the latter experiment, strong segregation distortion resulted in the recovery of an unexpectedly high number (160) of resistant recombinants. In 152 recombinant lines, crossover occurred proximally of the *Aegilops*-specific SCAR locus *Xs15-t3,* which mapped 69 cM distally from microsatellite locus *Xbarc83* in the second deletion bin (0.17–0.61) on 1AL (Sourdille et al. 2014). The relatively high recombination frequencies from the allosyndetic pairing induction experiment were indicative of extensive homoeology between the respective alien and 1AL wheat regions. The *Lr59* locus was mapped roughly 5 cM distally from *Xs15-t3.* The eight most promising recombined translocation lines that retained *Lr59* in association with the least alien chromatin appeared to have resulted from homoeologous crossover distal to the *Xs15-t3* locus. However, further characterization of the subset of eight recombinants with twelve *Ae. peregrina*-specific AFLP markers (mapped distally to *Xs15-t3*) produced data that could not be ordered into a physical map. The results hinted at the presence of an intercalary chromosome structural difference between the translocated segment and 1AL of the CS *ph1b* mutant line. Assuming that all of the translocated alien chromatin belonged to homoeologous group 1, the most likely explanation at the time was that an inversion was present between *Lr59* and the telomere on the alien segment. When crossover occurred within an inversion loop, dicentric translocation chromosomes and random breakage-fusion-bridge events could have resulted. These random events coupled with selection for the presence of *Lr59* provided a plausible explanation of the inconsistent AFLP marker data that were obtained (Marais et al. 2010a).

The aim of this study was to further investigate the structure of the *Lr59* full length translocation and eight smaller *Lr59* allosyndetic recombinants and to identify the most useful recombinants for wheat breeding purposes. Towards this end, we continued to characterize them in terms of their chromosome location and size, making use of physical mapping with microsatellite markers, genetic mapping with single nucleotide polymorphism (SNP) markers and genomic in situ hybridization (GISH).

Materials and methods

The *Lr59* translocations

Marais et al. (2010a) pollinated plants that were heterozygous for the full length *Lr59* translocation (*Lr59*-full) yet homozygous for the CS *ph1b* deletion with either CS nullisomic 1A tetrasomic 1B (CSN1AT1B) or CSN1AT1D plants. Resistant monosomic 1A testcross F_1 progeny were then characterized with markers to do genetic and physical mapping. Selfed F_2 and F_3 progeny of each resistant

testcross F_1 plant were therefore expected to include susceptible nullisomics and resistant mono- or disomics for the specific, recombined 1AL translocation chromosome arm. The eight seemingly most useful recombinants from the experiment were designated *Lr59*-10, -21, -25, -29, -36, -101, -144, and -151, respectively (Marais et al. 2010a) and had the general pedigree: *Ae. peregrina*-680/2*CS//5* W84-17/3/2* CS*ph1b*/4/CSN1AT1B (or CSN1AT1D).

Plants carrying an *Lr59* translocation chromosome were mostly identified by screening with *P. triticina* pathotype MFPS (isolate 05-69-1, Cereal Research Center, Agriculture and Agri-Food Canada (CRC-AAFC)) which is virulent on CS, W84-17, and Thatcher but avirulent on *Lr59* and Superb. In crosses involving Superb, pathotype TDBG (isolate 06-1-1, CRC-AAFC) was used instead (virulent on CS, W84-17, Thatcher and Superb, but avirulent on *Lr59*). The North American system for naming pathotypes of *P. triticina* has been explained by Long and Kolmer (1989).

Microsatellite analyses

Chromosome 1A microsatellite analyses employed the genotype panel: CS, W84-17, CSN1AT1B, CS ditelosomic 1AL (CSDT1AL), CSN1BT1D, CSDT1BL, CSN1DT1B, CSDT1DL, *Ae. peregrina*-680, *Lr59*-full, -10, -21, -25, -29, -36, -101, -144, and -151. These lines were tested for the presence of wheat chromosome 1A-specific microsatellite loci on two occasions. First, analyses were done at the CRC-AAFC in Winnipeg using the 1AL microsatellite loci *Xbarc17*, *Xbarc158*, *Xbarc287*, *Xgpw2010*, *Xgpw2083*, *Xgpw2224*, *Xgpw4166*, *Xgpw4311*, *Xgwm135*, *Xgwm357*, *Xgwm497*, *Xwmc59*, *Xwmc93*, and *Xwmc312*. Second, 14 1AL loci were analyzed at North Dakota State University (NDSU), Fargo, ND, including some of the latter loci plus *Xgpw3142* and *Xgwm99*. In addition, the *GluA1* locus (marker UMN19; Liu et al. 2008) on 1AL and 1AS microsatellite loci *Xgwm136, Xgpw4169, Xgpw3010, Xgpw2277*, and *Xgpw4410* were analyzed. The DNA used for the two sets of analyses was derived from different testcross F_3 plants.

When it became apparent that some or all of the eight *Lr59* recombinants were not associated with 1A, translocation homozygotes were identified for each. For this purpose individual, resistant F_2 or F_3 plants from specific crosses were harvested separately and 35–50 progeny of each were seedling screened to identify those families that contained only resistant plants. Wheat-specific markers were then tested on the translocation homozygotes plus controls CS, Superb, Thatcher, and CSN6BT6A. The markers included the 6BS microsatellite loci *Xdupw217*, *Xgpw1079*, *Xgpw4032*, *Xgpw4095*, *Xgpw4395*, *Xgwm508*, *Xgwm518*, *Xgwm613* and the 6BL locus, *Xgwm193*. In addition, 21 hard red winter wheat varieties were tested with the primer set DUPW217 to determine which alleles they possess.

At NDSU, lyophilized seedling leaves were used for DNA extraction following a modified Doyle and Doyle (1987) protocol described by Diversity Arrays Technology Pty Ltd (http://www.diversityarrays.com/sites/default/files/resources/DArT_DNA_isolation.pdf accessed August 2014). PCR reactions were performed in 20 µl volumes consisting of 4 µl (~100 ng) template DNA, 1X GoTaq® Flexi Buffer (Promega Corporation, Madison, WI), 2 mM MgCl2, 0.2 mM PCR Nucleotide Mix (Promega Corporation, Madison, WI), 10 pmol each of reverse and forward primer, and 1.25 u GoTaq® Flexi DNA Polymerase (Promega Corporation, Madison, WI). PCR amplification was performed in a BIORAD T100TM Thermal Cycler (Life Sciences, Hercules, CA). Primer sequences and polymerase chain reaction conditions were obtained from the Graingenes website (http://wheat.pw.usda.gov/GG2/index.shtml accessed August 2014). PCR products were separated in 3–4 % (w/v) AMRESCO® Agarose SFRTM agarose gel (AMRESCO, Solon, OH), treated with ethidium bromide and visualized using a ProteinSimple AlphaImager® HP System (ProteinSimple, Santa Clara, CA). At the CRC-AAFC in Winnipeg, DNA extraction, microsatellite amplification, and visualization followed the procedures described by Somers et al. (2004).

Linkage mapping

In an attempt to identify the most likely chromosome location of the eight translocation recombinants, an F_2 mapping population (90 individuals) was derived from the cross *Lr59*-25/Thatcher//Superb. Resistant plants (infection type = 0;) were distinguished from susceptible plants (infection type = 4) following inoculation with *P. triticina* pathotype TDBG. Selfed progeny ($F_{2:3}$) of the resistant plants were then progeny tested to identify the resistant homozygotes. The parents and F_2 progeny were genotyped at the USDA-ARS Genotyping laboratory at Fargo, ND using the Illumina wheat 9 K iSelect genotyping assay (Cavanagh et al. 2013). Markers that were monomorphic, did not conform to Mendelian segregation or had a call frequency less than 95 % were excluded from the analysis. Informative markers were analyzed with JMP genomics 6.1 (SAS Institute Inc., Cary, NC) software to generate 42 linkage groups using the 'Average' clustering option of the 'Automatic Hierarchical Clustering' linkage grouping method. Multiple hypothesis testing for all segregation ratios was conducted using the false discovery rate (FDR) method with *p* value set at 5 %. The information from the consensus map developed by Cavanagh et al. (2013) was used to assign the linkage groups obtained to the 21 wheat chromosomes. The linkage group that included *Lr59* was analyzed with JMP genomics v6.0 to generate a map. The 'Map order optimization' algorithm was used to order the

markers, while the Kosambi mapping function (Kosambi 1944) was used to determine the distance between markers.

Genomic in situ hybridization (GISH)

The original *Lr59* translocation and eight allosyndetic recombinants were used for GISH analyses employing the protocol of Cai et al. (1998). Root tips were cut and pretreated in ice water (0–4 °C) for 20 h. Following fixation in 3:1 ethanol:acetic acid, somatic chromosome mounts were prepared by squashing root tips in 45 % acetic acid. An Olympus phase contrast microscope (Olympus Corp., Tokyo, Japan) was used to select good chromosome spreads, which were then treated with RNase to remove RNA. Total genomic DNA was extracted from *Ae. peregrina* leaves, quantified, and labeled with Biotin-16-dUTP via nick translation (Bioprobe Nick Translation Kit, Enzo Life Sciences International, Inc., Plymouth Meeting, PA, USA) for use as a probe. Genomic DNA of CS was sheared in 0.4 M NaOH in boiling water for 40–50 min for use as blocking DNA. Fluorescein isothiocyanate conjugate (FITC-Avidin) was used with VECTA-SHIELD anti-fading medium to detect the signal and propidium iodide (Vector Laboratories Inc., Burlingame, CA, USA) was used to counterstain the chromatin. The GISH images were observed with an Olympus D72 fluorescence microscope (Olympus America, Inc., Center Valley, PA, USA) and images captured using a CCD camera (DP72, Olympus Corp., Tokyo, Japan).

Results

Allosyndetic recombination involving 1AL

The results obtained with the eight *Lr59* recombinants that were tested for resistance and the presence of 1A microsatellite loci are summarized in Table 1. For each recombinant, marker data were derived from two different resistant F_3 plants tested at the CRC-AAFC and NDSU, respectively. The markers were chosen to cover all of chromosome arm 1AL. With respect to six of the recombinants (*Lr59*-10, -21, -25, -29, -36 and -144), all of the 1AL wheat loci were present in most of the plants. However, two plants (recombinants *Lr59*-10-plant 1 and *Lr59*-25-plant 2) did not express any 1AL loci. Thus, the latter two plants were nullisomic for 1A yet had retained *Lr59* on a different chromosome.

Only recombinants *Lr59*-101 and -151 showed evidence of allosyndetic recombination between alien chromatin with group 1 homoeology and 1AL. In some of the F_3 plants tested, certain 1AL markers were absent, while others were present (Table 1). In recombinant *Lr59*-101, both F_3 plants showed that a distal 1AL wheat segment had been recovered with alien chromatin being present next to the centromere. In recombinant *Lr59*-151, plant 1 recovered a proximal 1AL wheat segment and retained an intercalary/terminal region of alien chromatin. However, plant 2 (resistant) did not express any 1A loci, suggesting that it too was nullisomic for 1A and that *Lr59* also occurred on a different chromosome in this recombinant. Thus, the data (Table 1) obtained for at least three of the recombinants clearly suggested that a different wheat chromosome(s) might have been involved in those allosyndetic crossovers and that *Lr59* got relocated to that unknown chromosome(s).

Genetic mapping of recombinant *Lr59*-25

In an attempt to identify the non-1AL chromosome(s) that was most likely involved in the allosyndetic recombination events, one of the seven recombinants that did not involve 1AL (*Lr59*-25) was genetically mapped. The mapping population of 90 F_2 plants was inoculated with pathotype TBDG and segregated 65 resistant:25 susceptible plants. The resistant F_2 were self-pollinated and the $F_{2:3}$ populations (minimum of 30 plants per family) tested for resistance to TDBG. Twenty two families were homozygous resistant and segregation conformed to a monogenic ratio ($P = 0.83$). The *Lr59* data were then combined with the SNP data and analyzed.

Analysis of the mapping population revealed 1755 polymorphic SNPs, of which only eight had missing data (with <2 % missing data for each of the affected SNPs). The polymorphic markers were distributed across 45 linkage groups and *Lr59* was assigned to a linkage group along with 171 SNP markers. Information from the consensus map developed by Cavanagh et al. (2013) was used to assign the latter linkage group to a wheat chromosome. 96 % of the markers were previously mapped on chromosome 6B of the consensus map, while two SNPs were mapped in chromosome 6A and the remaining four were unmapped. The total length of the linkage map produced for chromosome 6B was 106.5 cM. *Lr59* was mapped 0.5 cM distally from the co-segregating SNPs IWA1495, IWA6704, IWA2098, and IWA969 (Fig. 1). An additional 11 SNP loci mapped within an interval 0.5–6.5 cM proximally of *Lr59*.

Analysis of the eight recombinants with chromosome 6B microsatellite loci

Microsatellite analyses were done with homozygotes for each of the *Lr59* recombinants and the results are summarized in Table 2. The nine 6BS marker loci are

Table 1 Wheat chromosome 1A microsatellite marker data obtained for eight *Lr59* translocation recombinants and control lines

Control/ Translocation line	*Xgwm 136*	*Xgpw 4169*	*Xgpw 2277*	*Xgpw 3010*	*Xgpw 4410*	*Xgwm 497*	*Xbarc17*	*Xbarc 158*	*Xwmc59*	*Xgpw 2224*	*Xbarc 287*	*Xgpw 3142*	*Xgpw 4311*	*Xgpw 4166*	*Xgwm 135*	*Xgwm 357*	*GluA1*	*Xwmc 312*	*Xwmc 93*	*Xgwm 99*	*Xgpw 2010*	*Xgpw 2083*
Arm (Bin)[a]	S	S	S	S	S	L(I)	L(II)	L(II)							L(II)	L(II)				L(III)		
W84-17	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+
CS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CSN1AT1B	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
CSDT1AL	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lr59-full	+	+	+	+	+		−	−	−	−	−	−		−	−	−	−	−		−	−	
Lr59-10 (1st)[b]						−	−	−	−	−	−		−	−	−	−		−	−			−
Lr59-10 (2nd)						+	+	+	+	+	+				+	+		+				
Lr59-21 (1st)						+	+	+	+	+	+		+	+	+	+		+	+			+
Lr59-21 (2nd)						+	+	+	+	+	+				+	+		+				
Lr59-25 (1st)						+	+	+	+	+	+		+	+	+	+		+	+			+
Lr59-25 (2nd)						−	−	−	−	−	−				−	−		−				
Lr59-29 (1st)						+	+	+	+	+	+		+	+	+	+		+	+			+
Lr59-29 (2nd)	+	+	+	+		+	+	+	+	+	+	+			+	+	+	+	+	+	+	
Lr59-36 (1st)						+	+	+	+	+	+		+	+	+	+		+	+			+
Lr59-36 (2nd)						+	+	+	+	+	+				+	+		+				
Lr59-101 (1st)						−	−	−	−	−	−		−	−	−			+	+			+
Lr59-101 (2nd)	+	+	+	+	+	−	−	−	−	−	−	−			−	+	+	+	+	+	+	
Lr59-144 (1st)						+	+	+	+	+	+		+	+	+	+		+	+			+
Lr59-144 (2nd)							+	+	+	+	+				+	+		+			+	
Lr59-151 (1st)						+	+	+	+	+	+		−	−	−	−		−	−			−
Lr59-151 (2nd)	−	−	−	−	−	−	−	−	−	−	−	−			−	−	−	−	−	−		

The Presence (+) or absence (−) of the wheat-specific amplification product is indicated for those combinations that were tested

[a] Chromosome arm 1AL marker loci were separated into proximal and distal clusters based on recombination patterns in recombinants *Lr59*-101 and -151, as well as the deletion bin locations of six of the loci (Sourdille et al. 2014). *S* short arm, *L(I)* long-arm deletion bin C-1AL1-0.17, *L(II)* long-arm deletion bin 1AL1-0.17-0.61, *L(III)* long-arm deletion bin 1AL3-0.61-1.00

[b] Two different, resistant F_3 plants that were derived from a monosomic 1A testcross F_1 plant were analyzed for each of the eight recombinants. An F_3 plant could therefore be disomic, monosomic, or nullisomic with respect to chromosome 1A. The first plant was analyzed at the Cereal Research Center in Winnipeg in 2010, whereas the second plant was analyzed at NDSU in 2014

given in the consensus map order provided by Sourdille et al. (2014), except for *Xgwm613* that was mapped distally to *Xgpw4395*. In order to conform to the deletion data obtained with the eight recombinants in this paper, *Xgwm613* has to be located proximally to *Xgpw4095*.

Marker DUPW217 detected co-dominant wheat and alien-derived alleles (Fig. 2a). A larger *Ae. peregrina*-specific fragment was amplified in the alien species, the full length translocation and eight recombinants (Fig. 2a; Table 2) as compared to the control (parental) wheat varieties W84-17, CS, Thatcher, and Superb. Both wheat and alien-derived fragments occurred in the homozygous *Lr59*-full translocation line (Fig. 2a) and in translocation heterozygotes. Homozygotes for the full length translocation have normal 6B chromosomes and carry *Lr59* on two chromosome 1AL translocations, thus they have four copies of the *Xdupw217* locus. The DUPW217 marker can therefore be a very useful indicator of the presence of *Lr59* during breeding. When 21 hard red winter wheat varieties were tested, 12 produced the W84-17 polymorphism at the *Xdupw217* locus, whereas nine produced the CS polymorphism. The larger *Xdupw217* fragment that is associated with the introgressed resistance was absent in the cultivated varieties, suggesting that this marker allele could be diagnostic for the presence of *Lr59* in a wide range of genotypes. Marker locus *Xgpw4395* which had previously been mapped to the telomeric region of 6BS (Sourdille et al. 2014) was not detected in six of the translocation homozygotes and was therefore replaced by alien chromatin in those recombinants. The microsatellite Gpw4095 primers did not produce a clearly recognizable wheat-specific polymorphism with the detection protocol that was used, but did amplify three co-segregating *Ae. peregrina*-specific bands (Fig. 2b). The three species-specific bands were absent in four of the eight recombinants. Marker locus *Xgwm613* was detected in seven recombinants but not in recombinant *Lr59*-101, which may be a homozygote for the Thatcher null allele at this locus. The results of Table 2 therefore show that (1) a homoeo-allele of *Xdupw217* is associated with the *Lr59* alien insert and replaced the corresponding wheat allele in seven of the eight recombined translocation chromosomes. Homozygotes for *Lr59*-36 expressed both the alien and wheat alleles of *Xdupw217* which suggests that this recombinant does not occur on 6BS. (2) Using the results, it will be possible to determine which translocation retained the least alien chromatin. (3) The amplification of *Ae. peregrina*-specific fragments in the shortened translocations by the DUPW217 and GPW4095 primer sets (detect 6BS-specific SSR loci in wheat) strengthens the assumption of group 6 homoeology of alien chromatin surrounding the *Lr59* locus.

Genomic in situ hybridization

The full length *Lr59* translocation that was recovered by Marais et al. (2008) is shown in Fig. 3a and b. It clearly originated from two chromosome exchanges that involved an alien chromosome and one or two wheat chromosomes. One break occurred within the centromere, whereas a second sub-terminal exchange also occurred. Thus, *Lr59*-full is not a Robertsonian translocation as was believed earlier (Marais et al. 2008).

GISH pictures of several recombined translocation chromosomes are provided in Fig. 4. Small, sub-terminal translocations on 6BS that harbor *Lr59* in recombinants 29, 101, and 151 are shown. Also included, it is a small sub-terminal translocation to an unknown chromosome in recombinant 36. Each recombined chromosome contains a similarly sized, distal wheat chromosome segment. The terminal translocation break point in the full length and seven recombined translocations on 6BS is probably in the same position since the allosyndetic crossovers that gave rise to each of the seven recombinants had to have occurred proximally of *Lr59*. Considered with the SSR data of Table 2, this means that among the seven 6BS recombinants, *Lr59*-151 retained the least alien chromatin.

In addition to the small group 6 translocations containing *Lr59*, two larger (chromosome 1A) translocations were, respectively, present in some of the recombinant 101 and recombinant 151 plants (Fig. 4). In accordance with the microsatellite results of Table 1, the second translocation in recombinant 151 involved an interstitial region of alien chromatin that was located away from the centromere. The second (interstitial) translocation in recombinant 101 bordered on the centromere. These translocations were lost in subsequent crossing, backcrossing, and self-pollination events.

Discussion

Structure of the *Lr59*-full translocation

In order to understand how the eight recombinants were formed, it is necessary to first determine the structure of the full length translocation that was used in the allosyndetic pairing experiment done by Marais et al. (2010a). Such a reconstruction is provided in Fig. 3c. Marais et al. (2010a) used resistant testcross F_1 from their allosyndetic pairing experiment to map the translocation. One hundred and sixty recombinants were obtained, of which 152 had breakpoints between the centromere and the *Ae. peregrina*-specific locus *Xs15-t3*. This confirmed that the proximal section of the translocation has high homoeology with 1AL.

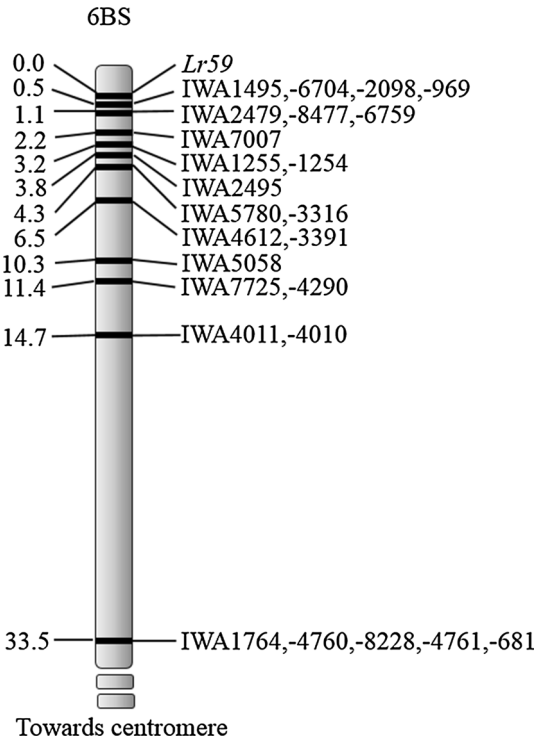

Fig. 1 Genetic map of the chromosome 6BS telomeric region in recombinant *Lr59*-25

The *Lr59* locus mapped distally from *Xs15-t3* and only eight recombinants had retained *Lr59*, yet had lost *Xs15-t3*. The SSR and genetic mapping results of the present study showed that in seven of the eight recombination events, *Lr59* got relocated to chromosome arm 6BS. Since pairing of this terminal region of *Lr59*-full with 6BS could only have been initiated from the telomere, the result implies that the telomere and sub-telomeric chromatin of the translocated alien segment has homoeology to the group 6 chromosomes of wheat. The GISH data of Fig. 3a confirmed that the distal part of *Lr59*-full is of wheat origin. Marais et al. (2008) showed that the long arm of *Lr59*-full never paired with 1AL of wheat in monotelo 1AL-disomic plants, suggesting that the telomere comes from a different chromosome, which has to be 6BS (since seven of the eight homoeologous exchanges involved 6BS of wheat).

Thus, *Lr59*-full is a non-compensating translocation with the composition: $1AS{\cdot}1L^{P}{\cdot}6S^{P}{\cdot}6BS$. The translocation occurred spontaneously in the course of crosses to produce the common wheat/*Ae. peregrina* hybrid and the subsequent backcrosses to wheat. Since the alien chromosome had a terminal region with group 6 homoeology, a homoeologous recombination event that involved 6BS may have occurred during backcrossing. Marais et al. (2008) provided C-band patterns of the addition chromosome and a long-arm telosome of the *Lr59*-full translocation. The C-banding patterns of the telomeric and sub-telomeric regions of both the addition chromosome and telosome are similar to the corresponding 6BS region. While this does not prove its origin, the result does not argue against the assumption that the telomeric region derives from 6BS. Thus, the terminal exchange with 6BS may have happened first. While allosyndetic recombination in the presence of *Ph1* is normally unlikely, it is well known that some wild species carry genes that enhance allosyndetic pairing in their hybrids with wheat. The genomes of various wild species including *Ae. umbellulata* (UU) and *Ae. speltoides* (SS) have been shown to suppress the effect of *Ph1* in their hybrids with wheat and genotypes of a species were found to vary in their ability to do so (Dvorak 1972; Sears 1976; Knott 1989). In hybrids of *Ae. speltoides* and common wheat, at least two independently segregating major genes plus an unknown number of minor genes are involved in the suppression of *Ph1* (Chen and Dvorak 1984).

It is also possible that a second (Robertsonian) translocation occurred in the later stages of backcrossing. Backcrossing coupled with selection for the resistance would have resulted in an unpaired alien chromosome being present during meiosis, which would have facilitated centromeric break and fusion events (Sears 1981).

Meiotic pairing structures that most likely produced the translocation recombinants

In the allosyndetic pairing induction experiment of Marais et al. (2010a), the majority of recombination events occurred within the region of group 1 homoeology, and most likely within heteromorphic bivalents. These crossovers would have reduced the amounts of alien chromatin, yet would not have changed the non-compensating nature of the full translocation (Fig. 5). The data furthermore suggest that the 152 proximal allosyndetic crossovers on 1AL resulted after meiotic pairing was initiated from the 1AS telomere and centromere and extended into the long arm (since the structure of the translocation chromosome precluded pairing from the 1AL telomere).

Seven of the eight shortest recombinants most likely resulted from quadrivalents whose formation would have been facilitated by the presence on the translocation of a sub-terminal region with group 6 homoeology and a 6BS telomere. Clearly, chromosome 1A heteromorphic bivalents and chromosome 6B regular bivalents would have formed in most meiocytes. The presence of an unpaired chromosome 6BS telomere on each 1A heteromorphic bivalent would, however, have increased the likelihood of a quadrivalent involving chromosome 6B being formed. Such a quadrivalent is shown in Fig. 6 and could have been the source of recombinants *Lr59*-10, -21, -25, -29, -101, -144 and -151.

Table 2 Chromosome 6BS microsatellite markers tested on eight *Lr59* recombined translocation homozygotes

Relative location on chromosome 6B[a]	SSR locus	*Lr59*-10/2*Tc	*Lr59*-21/2*Tc	*Lr59*-25/2*Tc	*Lr59*-29/2*Tc	*Lr59*-36/2*Tc	*Lr59*-101/Tc//Su	*Lr59*-144/2*Tc	*Lr59*-151	CS	Thatcher	CSN6BT6A	Superb
6BS Telomere													
III (u)	*Xdupw217*[b]	p	p	p	p	p+	p	p	p	+	+	–	+
III (c)	*Xgpw4395*	–	–	–	–	+	–	–	+	+	+	–	+
III (c)	*Xgpw4095*[c]			p	p	p	p						
III (c)	*Xgwm613*	+	+	+	+	+	–[d]	+	+	+	–	–	+
III (c)	*Xgpw4032*	+	+	+	+	+	+	+	+	+	+	–	+
III (p)	*Xgpw1079*	+	+	+	+	+	+	+	+	+	+	–	+
III (p)	*Xgwm518*	+	+	+	+	+	+	+	+	+	+	–	+
II (p)	*Xgwm508*	+	+	+	+	+	+	+	+	+	+	–	+
Centromere													
6BL (p)	*Xgwm193*	+	+	+	+	+	+	+	+	+	+	–	+

Symbols "+" and "−" indicate that a wheat-specific band was amplified or not amplified, respectively. Symbol "p" indicates that an *Aegilops peregrina*-specific band was amplified

[a] Relative map positions of marker loci on chromosome 6B according to a physical map posted by Sourdille et al. (2014). Chromosome arm 6BS deletion bins are indicated as follows: II = 6BS5-0.76-1.05 and III = 6BS-Sat. The bracketed symbol indicates that the locus position was based on a physical map (p); based on an integrated consensus map (c); not mapped (u)

[b] A co-dominant alien-derived allele, different from that produced in the parents CS, Thatcher and Superb was detected by DUPW217 in each *Lr59*-carrying genotype

[c] A clear, wheat 6BS-specific amplification product could not be identified; however, three *Aegilops peregrina*-specific bands were detected

[d] The absence of an amplification product could be due to either the Thacher null allele or the presence of alien chromatin

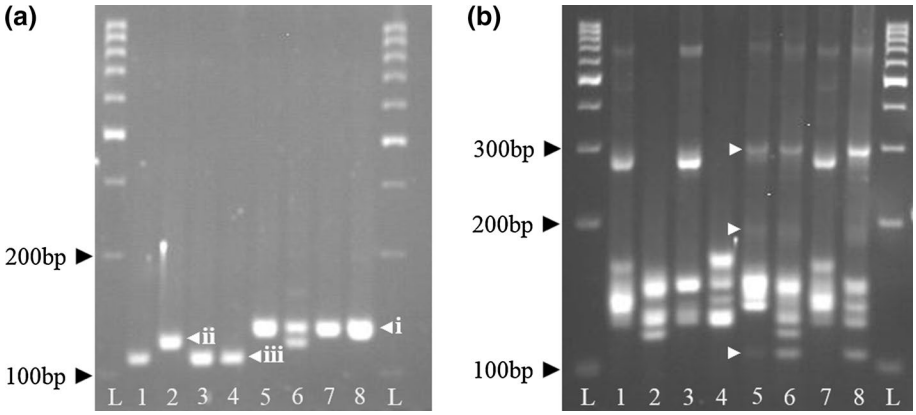

Fig. 2 *Aegilops peregrina*-specific bands amplified with two microsatellite primer sets: **a** DUPW217 amplified co-dominant bands associated with the alien (i) and wheat loci (ii & iii), respectively. **b** Gpw4095 amplified three characteristic bands (*arrows*) that were associated with the alien chromatin in four of the recombinants. The lanes were as follows: *L* size ladder, *1* Chinese Spring, *2* W84-17, *3* Superb, *4* Thatcher, *5 Aegilops peregrina* 680, *6 Lr59*-full homozygote, *7 Lr59*-144 homozygote, *8 Lr59*-151 homozygote

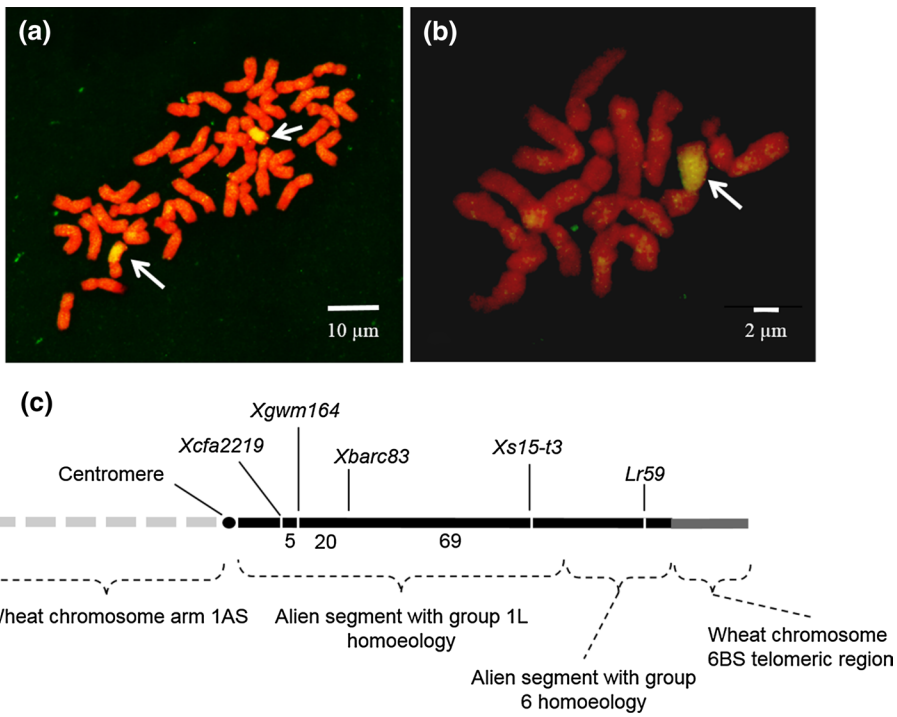

Fig. 3 **a**, **b** Genomic in situ hybridization pictures of the *Lr59*-full translocation that show its intercalary nature and the presence of a wheat-derived telomere. **c** The structure of the *Lr59*-full translocation was deduced from the mapping results of Marais et al. (2010a) and the data of the present study. Distances are not to scale

Crossover within the alien chromatin region with chromosome 6 homoeology needs to have occurred proximally to *Lr59* (position (i) in Fig. 6) in order to relocate the resistance gene to chromosome 6B in recombinants *Lr59*-10, -21, -25, -29, -101, -144, and -151. However, in some quadrivalents simultaneous crossover could also have occurred within the alien chromatin region with chromosome 1 homoeology [positions (ii) and (iii)]. The specific combination of a chromosome 6B and a chromosome 1A that ended up in a megaspore would therefore have depended upon (1) whether crossover occurred distally or proximally from *Lr59* in the alien region with group 6 homoeology; (2) whether a simultaneous crossover occurred within the alien region with group 1 homoeology; (3) whether simultaneous crossovers (when they occurred) involved the same or the alternative chromatids of the translocated chromosome; and (4) whether adjacent or alternate segregation of the centromeres occurred. Thus, gametes that were very different with respect to the chromosomes 1A and 6B that they received (recombined or non-recombined plus pattern of recombination) would have been produced. These variations can explain the recombined chromosomes 1A that occurred

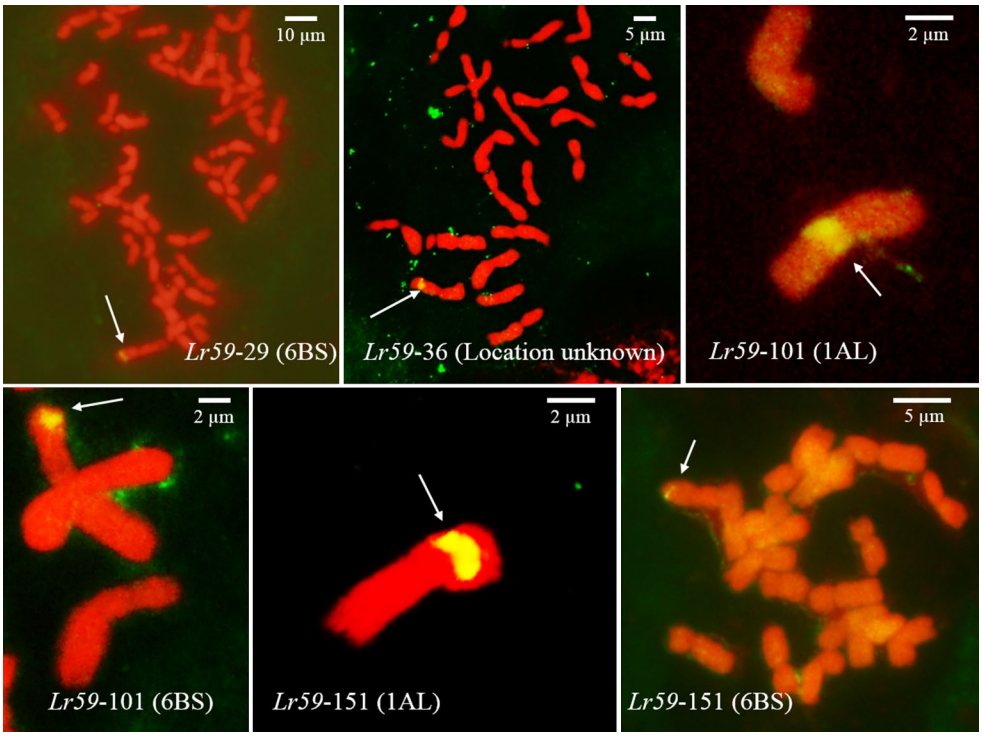

Fig. 4 Genomic in situ hybridization of mitotic metaphase chromosomes to show the *Aegilops peregrina*-derived chromosome segments harboring *Lr59* on chromosome arm 6BS in four shortened versions of the translocation. Also shown are non-*Lr59* carrying translocations on chromosome arm 1AL in two of the recombined lines

in addition to the recombined 6B chromosomes with *Lr59* (Table 1; recombinants *Lr59*-101 and -151; Fig. 4).

In recombinant *Lr59*-36, the alien segment has most likely been relocated to either a group 1 or a group 6 homoeologue. If it resulted from crossover between an alien region with group 1 homoeology and either of chromosome arms 1BL or 1DL, the recombinant will remain non-compensating and less useful. If it was produced within a quadrivalent that involved chromosome 1A, the full translocation on 1A and two copies of either of chromosomes 6A or 6D, an exchange within the alien chromatin with group 6 homoeology would have resulted in a recombined chromosome of constitution 6AL·6AS·$6S^{P}$·6BS or 6DL·6DS·$6S^{P}$·6BS. Such structural defects will detract from the potential usefulness of a recombination product. In addition, the data of Table 2 suggest that the alien segment in recombinant 36 could be larger than that in recombinants 10, 21, 144, and 151. In view of this, recombinant 36 was not pursued further.

Utility of the 6BS translocations

The alien insert in each of the seven 6B recombinants has group 6 homoeology and appears to be fully compensating. The reduced translocations are comparatively small and recombinant *Lr59*-151 retained the least alien chromatin. While *Lr59* provided resistance to all South African and Canadian pathotypes that it was tested with in the past (Marais et al. 2008), it has not been widely tested in the US.

According to a durum wheat consensus map produced by Marone et al. (2012), the *Gli-B2* locus maps proximally to *Xgwm132* within the 6BS satellite. On the map of Sourdille et al. (2014), *Xgwm132* is located proximally from *Xgpw1079* (Table 2), while all recombinants mapped distally to *Xgpw1079*. Thus, the *Gli-B2* locus would not have been affected in the recombinants. Similarly, Gadaleta et al. (2009) mapped *Gli-B2* proximally of *Xgwm613* in the durum wheat Svevo/Siccio cross. The *Xgwm613* locus was present in seven recombinants, while its absence in recombinant *Lr59*-101 could be the result of the Thatcher null allele. The recombinants are therefore not expected to change the storage protein profile and affect processing quality.

The *Ae. peregrina* allele of *Xdupw217* occurs within the alien insert in each recombinant and produces a slightly larger amplification product than the two fragment sizes amplified from the corresponding wheat alleles in 21 winter wheat varieties grown in the Great Plains. Thus, this may be a very useful marker for tracing *Lr59* in the segregating progenies of a breeding program.

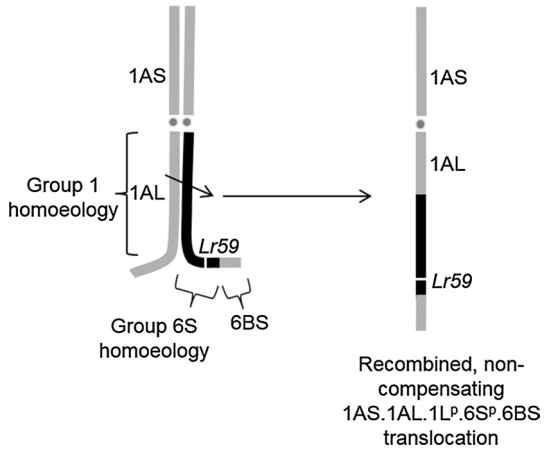

Fig. 5 Allosyndetic recombination within a heteromorphic bivalent involving a normal chromosome 1A and the *Lr59*-full translocation. *Lr59*-full is a non-compensating, intercalary translocation. Its distal (sub-telomeric) end contains alien chromatin with group 6S homoeology that harbors *Lr59*, whereas the proximal alien region has chromosome 1 homoeology. The telomere derives from 6BS. Crossover within the proximal alien region with group 1 homoeology will reduce the amount of alien chromatin, yet will not change the non-compensating nature of resistance carrying translocations

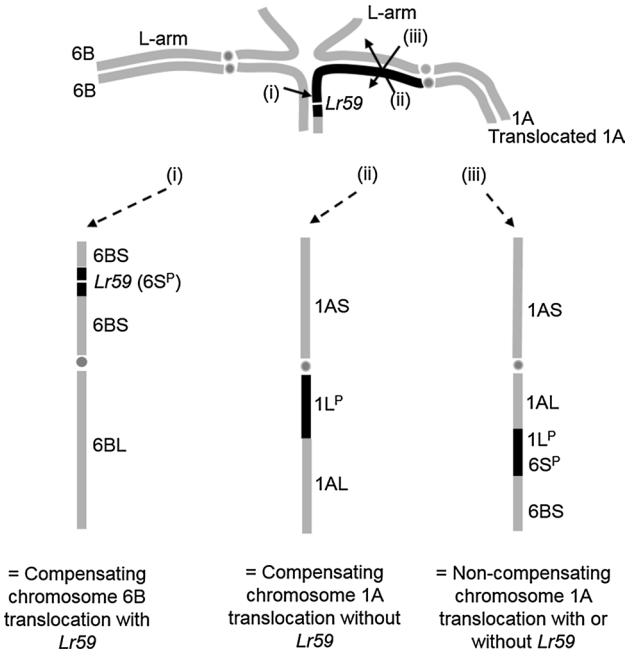

Fig. 6 Allosyndetic recombination within a quadrivalent involving two normal chromosomes 6B, a normal chromosome 1A, and the *Lr59*-full translocation most probably resulted in seven of eight recombined versions of the translocation. Crossover within alien chromatin with group 6 homoeology (position i) could have produced the recombined 6B chromosomes in *Lr59*-10, -21, -25, -29, -101, -144, and -151. Simultaneous crossover within alien chromatin with group 1 homoeology (position ii) could have produced the second 1A translocation chromosome that occurred in line *Lr59*-101 (Table 1), whereas crossover in (position iii) could have produced the second non-compensating, recombined 1A chromosome that occurred in line *Lr59*-151

Author contribution statement SMP performed the molecular marker analyses that were done in Fargo; MS performed the GISH analyses under the guidance of XC; RSP did the F_2 mapping analysis with one of the recombinants; BM, BS and TF provided oversight and resources for the molecular marker analyses that were done in Winnipeg; SC performed the SNP genotyping whereas FM initiated and supervised the project, provided the plant genetic materials and research funding. All authors provided comments and participated in the revision of the manuscript.

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Compliance with ethical standards

Conflict of interest All authors have no conflict of interest.

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