Derivation and Characterization of Recombinants of the *Lr54/Yr37* Translocation in Common Wheat

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Abstract: The wild relatives are an important source of new genes for the genetic improvement of wheat. Leaf and stripe rust resistance genes *Lr54* and *Yr37* occur on an *Aegilops kotschyi*-derived chromosomal translocation that had apparently replaced wheat chromosome arm 2DL. The alien chromatin also includes the locus of a gene for reduced plant height (*H*), which appears to be different from *Rht8* on chromosome arm 2DS. The introgressed genes were mapped relative to homoeologous wheat marker loci following the induction of chromosome pairing in translocation heterozygotes that lacked the *Ph1* locus. Ten recombined *Lr54/Yr37* translocation chromosomes were derived and characterized with microsatellite, AFLP and SCAR markers. The data suggested that there was significant homoeology between the full-length translocated segment and the wheat 2DL chromosome arm. The recombined translocations apparently resulted from single crossovers during which the distal end of the long arm of the translocation chromosome was replaced with wheat chromatin. Recombinant (*Lr54/Yr37*-74) retained the least alien chromatin and both resistance genes, yet had lost the reduced plant height gene. A polymorphic AFLP fragment was converted into a dominant SCAR marker to detect rec. #74. In addition three wheat microsatellite loci that map to the introgressed region provide a useful recessive marker system to detect *Lr54/Yr37*. The shortened translocation could be useful in breeding and may be used for continued, closer mapping of the resistance genes.

Keywords: Chromosome engineering; leaf rust resistance; *Ph1*; stripe rust resistance, *Triticum aestivum* L.

INTRODUCTION

Leaf rust (*Puccinia triticina = P. recondita* Roberge ex Desmaz. f. sp. *tritici*) and stripe rust (*P. striiformis* f. sp. *tritici* Westend) resistance genes *Lr54* and *Yr37* were transferred to common wheat (*Triticum aestivum* L.) from *Aegilops kotschyi* Boiss. [1]. The translocation derives from a plant that was double monosomic for 2D and an unknown *Ae. kotschyi* group 2 chromosome and it was probably formed following centric breaking and subsequent fusion of an *Ae. kotschyi* chromosome 2L arm with 2DS of wheat. The stripe rust resistance proved to be effective against prevailing pathotypes in South Africa and Australia [2], whereas *Lr54* provided effective resistance against all the South African and western Canadian *P. triticina* pathotypes [1]. The introgression appeared to include an uncharacterized gene for reduced plant height that could limit its use in breeding. The translocated *Ae. kotschyi* chromosome arm also failed to pair with its common wheat 2DL homoeologue during meiosis causing the alien genes to be inherited as a single, large linkage block. Such linkage blocks can be broken up by inducing synapsis and crossing over between the alien and homoeologous wheat chromosome segments [3-6]. Even though homoeologous crossover frequencies are considerably reduced compared to homologous crossover frequencies, and also vary with the evolutionary divergence of the wheat and alien chromatin [7], the methodology simultaneously allows for genetic/physical mapping of the introgressed region [8]. The use of an appropriate wheat nulli tetrasomic or ditelosomic stock [3, 9] as the testcross parent produces testcross progeny that are hemizygous for the chromosome region involved in the translocation. As a result, both wheat specific and alien chromatin specific genetic markers can be employed for analysis of the testcross progeny. Well developed and publicly available wheat genetic maps [10-12] provide adequate markers that are evenly spread across the region affected by the introgression. The extent and distribution of recombination will reflect the degree of homoeology and compensating ability of the translocation. Chromosome structural differences that occur within otherwise homoeologous regions may, however, cause aberrant meiotic pairing structures and formation of single crossover products that appear to result from complex multiple crossovers [8, 13]. Interpretation of these products may be difficult yet could provide an indication of the type of structural difference that was involved. Such information could help with deciding which recombination products are most likely to be useful. Chromosome structural differences that are encountered may be the result of evolutionary divergence of the alien and corresponding common wheat chromosome regions [14]; may be introduced through the involvement of the Chinese Spring homoeologous pairing locus deletion line (CS*ph1b*) which is chromosomally unstable [15]; or it may be due to natural karyotype variation within wheat itself [16-18].

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This study aimed, firstly, to confirm the presence of a height reducing gene (here designated *H*) on the *Lr54/Yr37* translocation. Secondly, homoeologous pairing was induced between the translocated chromosome region and the normal wheat 2DL chromosome arm (through deletion of the *Ph1* locus) and the progeny analyzed to find recombinants for the translocated region. The recombinants were used as indicator of homoeology between the foreign and wheat DNA; to determine the relative locations of the *Lr54*, *Yr37* and *H* loci and to identify recombinants in which the height reducing gene had been separated from the resistance genes.

MATERIALS AND METHODOLOGY

Plant Material and Seedling Resistance Screening

Line CS-*Lr54/Yr37* (pedigree = CS monosomic 2D// *Ae. kotschyi*-617/ 4*CS/3/ CS-S) was the source material used in the study; it is a near-isogenic line of the common wheat, Chinese Spring (CS) and carries a 2DL translocation from *Ae. kotschyi*. In the above pedigree, parent CS-S (pedigree = Inia 66/ 7*CS) is a semi-dwarf near-isogenic line of CS that has acquired the reduced height gene, *Rht-B1b* (from Inia 66) on chromosome 4BS.

The presence of *Lr54* in segregating populations was detected by employing seedling leaf rust resistance tests. This was done with the use of *Puccinia triticina* pathotype UVPrt8 which is virulent on the common wheat genotypes used in the study (CS, CS-S and W84-17) yet is avirulent on *Lr54* (infection type = ;1). Line W84-17 is a semi-dwarf spring wheat (pedigree 'Inia 66'/5/ 'El Gaucho'/ Son 64/4/ Tg/3/ Son 64// Tzpp/ Nai 60). Seedling screening tests to detect *Yr37* relied on the use of *P. striiformis* f. sp. *tritici* pathotype 6E22A-. The wheat background genotypes are susceptible to pathotype 6E22A- whereas *Yr37* provides resistance (infection type ;c-;1^n). Seedling resistance tests were done as described earlier [1].

Confirmation of a Gene for Reduced Height on the *Lr54* Translocation

The effect of the translocation on plant height was investigated by making two sets of crosses. Firstly, CS (tall phenotype; *Rht-B1aRht-B1a hh*) was crossed with W84-17 (semi-dwarf phenotype; *Rht-B1bRht-B1b hh*). The F_1 was backcrossed reciprocally to the parents and F_2 seeds were harvested. The material served as control populations to provide an indication of the variation in height resulting from the segregation of *Rht* genes in the CS and W84-17 backgrounds. Secondly, experimental populations segregating for the *Lr54* translocation were produced. CS was crossed with resistant (UVPrt8) plants selected from the W84-17 near-isogenic line F_1:0514 (CS-*Lr54-Yr37*/4*W84-17; *Rht-B1bRht-B1b Hh*). Leaf rust resistant F_1 plants were selected and backcrossed to the parents. The parents, F_1 and F_2 progeny together with the respective backcrosses of both groups were raised in a greenhouse. As it was necessary to screen the plants in the experimental group for the presence of *Lr54*, all the plants in both the experimental and control groups were subjected to leaf rust seedling tests and were then transplanted to pots in a greenhouse. In the greenhouse, four plants were grown per 2L plastic pot and seedlings were randomly allocated to pots and positions within pots. The pots were randomly placed in a water-cooled greenhouse with automated irrigation. Commercially available nutrient mixtures were applied following the manufacturer's guidelines. At maturity, plant height (cm) was measured from the base of the plant to the tip of the ear on the primary spike. Standard errors, confidence intervals and Student's t-test statistic (one-tailed) were calculated (Microsoft Excel) to compare the mean plant heights of populations.

Derivation and Characterization of Translocation Recombinants

The CS-*Lr54/Yr37* translocation line was crossed with, and the F_1 backcrossed to, the CS*ph1b* mutant. The F_1 plants were screened for seedling resistance (*Lr54*) to *P. triticina* pathotype UVPrt8 and absence of the *Ph1* locus (using a PCR marker [19]). Plants that were *ph1b* deletion homozygotes and at the same time heterozygous for *Lr54* were selected and testcrossed with the leaf rust susceptible breeding line, W84-17. A total of 468 TF_1 (04M144) seeds were produced (this will be referred to as the primary testcross population). Initially, an attempt was made to characterize the primary testcross population with the use of chromosome 2DL restriction fragment length polymorphism loci. However, when suitable markers of this type could not be found it was decided to use wheat chromosome 2DL microsatellite markers instead. In order to apply the microsatellite markers (present in wheat but absent in *Ae. kotschyi* chromatin) it was necessary to first derive testcross plants monosomic for the translocated chromosome 2D. To achieve this, resistant TF_1: 04M144 plants were used to pollinate CS nullisomic 2D tetrasomic 2A (CSN2DT2A) or CS nullisomic 2D tetrasomic 2B (CSN2DT2B) plants. DNA extracts [20] were made of the resistant progeny from the crosses (secondary testcross population) and used for microsatellite analysis.

Eight 2DL microsatellite markers (loci *Xbarc228*, *Xwmc41*, *Xcfd233*, *Xgwm539*, *Xgwm157*, *Xcfd50*, *Xwmc167* and *Xgdm6*) and four 2DS microsatellite markers (loci *Xbarc124*, *Xgwm261*, *Xgwm484* and *Xcfd116*) were used in the study. Microsatellite amplification products were obtained and visualized by silver staining [21, 22]. Relevant map detail and primer sequences were obtained from the Graingenes website [23]. The microsatellite markers were confirmed to be specific for the respective chromosome arms and to be polymorphic (present in CS and W84-17 but absent within the *Lr54* translocated chromosome region). For this purpose they were tested on a genotype panel consisting of: *Ae. kotschyi* accession 617, CS, W84-17, CSN2AT2B, CSN2BT2A, CSN2DT2A, CS ditelosomic 2DS (CSDT2DS), CSDT2DL and a hemizygote for the segment of translocated *Ae. kotshyi* chromatin (= CS-*Lr54/Yr37* hemizygote; 2n = 41 + t = monotelodisomic 2DS plus $2D^{Lr54/Yr37\ translocation}$).

Further Characterization of the Translocation Recombinants

When allosyndetic recombinants were identified, these were characterized with additional sequence characterized amplified region (SCAR) and amplified fragment length polymorphism (AFLP) markers. The SCAR markers included primer set Ust2b [24] which amplifies a 2DL wheat-

specific locus and primer set Sopw7 which amplifies a fragment of the same length in several alien translocations yet not in wheat [8].

The genotype panel that was used to confirm the microsatellite loci was also used in a search for polymorphic AFLP markers associated with 2DL. Fluorescence-based semi-automated AFLP analysis was done as outlined in the 'AFLP Plant Mapping Kit' of PE/Applied Biosystems Inc. (850 Lincoln Centre Drive, Foster City, CA 94404, USA). The final amplification step utilized five *Mse*I selective primers (-CAT, -CTC, -CAG, -CTA and –CTG) which were used in all possible combinations with fluorescently labelled *Eco*RI–ACA (6-FAM™), *Eco*RI–AAC (NED™) and *Eco*RI– AGG (JOE™) selective primers. The amplification products were detected with an Applied Biosystems 3130XL Genetic Analyzer (Foster City, California, USA) operated by the Central Analytical Facility (http://academic.sun.ac.za/saf/) at Stellenbosch University. "GeneMapper" software [25] was used to visualize and analyze the results.

Development of a Dominant Marker

An AFLP polymorphism that was specific for the shortest recombinant was converted into a SCAR marker. Following unlabeled selective AFLP amplification, the polymerase chain reaction (PCR) product was size separated on a 6% (w/v) denaturing poly-acrylamide sequencing gel and silver stained. The region containing the polymorphic band was excised from the *Ae. kotschyi* and CS (negative control) lanes; the DNA was eluted with a GenElute gel extraction kit (Sigma-Aldrich Chemie Gmbh, Munich, Germany), re-amplified and separated on 2% agarose gel. Methodology and kits from Promega Corporation (2800 Woods Hollow Road, Madison, WI 53711-5399, USA) were then used to transform and clone the fragment. Clones were sequenced at the Central Analytical Facility of Stellenbosch University. Sequence analysis was done using the program BioEdit [26] and primers were designed using OligoAnalyzer [27].

The newly designed primers were evaluated on the ten translocation recombinants as well as panels of resistant and susceptible F_2 (20) and F_3 (31) plants derived from the first backcross of the shortest recombinant to W84-17. The PCR reaction mixture consisted of 200 ng of template DNA, 1 X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 µM of each primer, 1 U of *Taq* DNA polymerase and dH_2O to a final volume of 25 µl. A thermal cycler (Applied Biosystems) was programmed as follows: 5 min at 94°C, 35 cycles consisting of 1 min at 94°C, 1 min at 61°C and 1 min at 72°C followed by a final extension step at 72°C for 7 min and a soak temperature of 4°C. Following PCR amplification the PCR product was size separated on a 1.5% agarose gel (Ethidium bromide, 1 X Tris/Borate/EDTA) together with a molecular size marker.

RESULTS AND DISCUSSION

Gene for Reduced Plant Height

The distribution of plant height measurements in the parental lines, F_1, backcross and F_2 populations of the control and experimental groups of crosses are shown in Fig. **1**. The parental lines, 0514, W84-17 and CS, formed distinct height groups (95% confidence level) with average heights of 71 ± 6.9 cm (*Rht-B1bRht-B1b Hh*), 95 ± 2.0 cm (*Rht-B1bRht-B1b hh*) and 149 ± 3.9 cm (*Rht-B1aRht-B1a hh*), respectively.

The height data of the control progeny reflected the segregation of the single, major height reducing gene, *Rht-B1b*. The F_1 (*Rht-B1aRht-B1b hh*) distribution was similar to that of CS confirming that the *Rht-B1a* allele is dominant. The height distributions of the F_2 and the two BF_1 populations fell within the ranges spanned by the parental lines. With the exception of one backcross plant, the plants in all the groups were taller than 80 cm. Only one backcross plant was taller than 180 cm. The average height of F_1 plants in the experimental group was eight centimetres less than in the control group. The experimental group showed much wider distributions of individual plant heights for both backcrosses and the F_2. As in the control group, plants were never taller than 180 cm; however, many plants were shorter than 80 cm. For backcrosses to CS, the mean height of the experimental group (134 cm) was lower (P=0.016) than that of the control group (145 cm). A comparable difference (P<0.0001) was seen for backcrosses to the shorter parent. In the latter, the mean height of the experimental group was 72 cm compared to 104 cm in the control group. With respect to the F_2, t-test comparison showed that the experimental group (110 cm) was on average significantly (P<0.0001) shorter than the control group (131 cm). Similarly, resistant F_2 plants within the experimental group (average height = 106.6 cm) were significantly shorter than the susceptible plants (average height = 128.9 cm) (P<0.01); thus confirming the presence of a height-reducing gene. The shorter stature (71 cm) of the resistant, near-isogenic 0514 F_1 plants (*Rht-B1bRht-B1b Hh*) as compared to the recurrent parent, W84-17 (95 cm; *Rht-B1bRht-B1b hh*) (P<0.01) and that of the experimental group F_1 (138 cm; *Rht-B1aRht-B1b Hh*) as compared to the control F_1 (146 cm; *Rht-B1aRht-B1b hh*) (P=0.087) suggested that *H* is a dominant gene. Furthermore, the height reducing effect of *H* was much less pronounced in the presence of *Rht-B1a*, ie *H* is hypostatic to *Rht-B1a*.

The height reducing genes *Rht4*, *Rht7* and *Rht8* occur on homoeologous group 2 chromosomes and produce semi-dwarf phenotypes. Unlike the GA-insensitive *Rht-B1b* and *Rht-D1b* genes they do not affect coleoptile and early plant growth [28]. Gene *Rht7* (chromosome 2A) was induced through EMS treatment and is associated with a detrimental effect on yield [29]. *Rht4* is another induced mutation that occurs on 2BL [30]. Only *Rht8* has been widely used in breeding. It was derived from the Japanese landrace Akakomugi in the 1920s and was mapped close to the microsatellite locus, *Xgwm261* on 2DS [30, 31]. The dwarfing gene, *H*, described here is associated with 2DL and its relationship (or not) with the group 2 *Rht* genes is unclear. While *H* has not been evaluated for its potential use in breeding, it will be useful to have recombinants of the *Lr54/Yr37* translocation with and without the height reducing gene.

Identification of *Lr54/Yr37* Translocation Recombinants

One hundred and nine resistant plants from the primary testcross population were used to pollinate CSN2DT2A or CSN2DT2B plants. The F_1 (hemizygous 2D) of each secondary testcross was seedling screened for the presence of *Lr54* and a resistant plant from each population was used for DNA extraction and microsatellite analysis. Based on the micro-

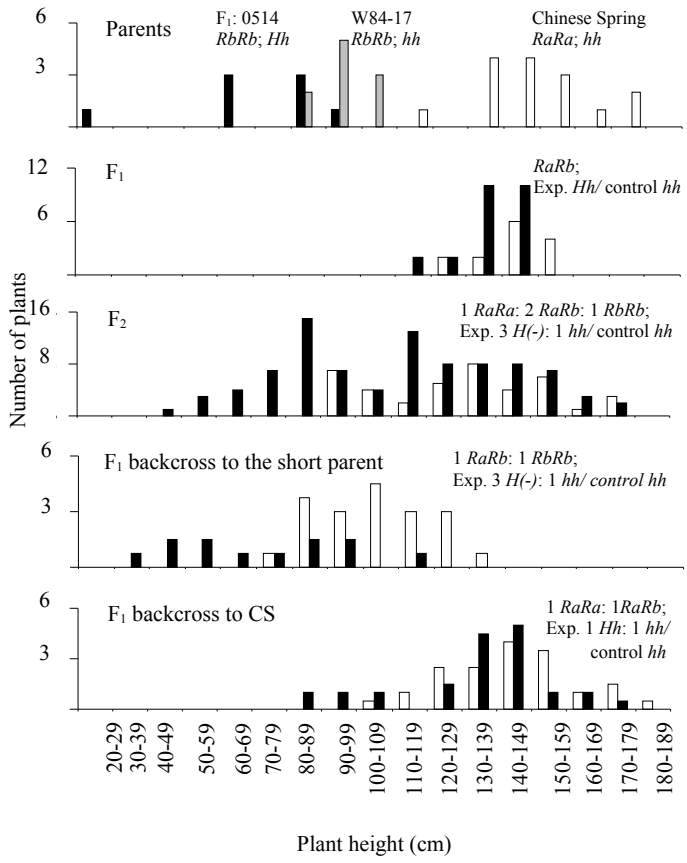

Fig. (1). Plant height distributions in the parental lines, F_1, F_2 and backcross generations of a control cross (W84-17 X CS) and an experimental cross involving the *Lr54/Yr37* translocation (F_1: 0514 X CS). Colour codes used for the parental lines are white (CS), grey (W84-17) and black (F_1: 0514 = W84-17-*Lr54/Yr37*). Progeny distributions in the control group are shown with white bars and that in the experimental group with black bars. Also indicated are the respective height genotypes with *Ra* = *Rht-B1a*; *Rb* = *Rht-B1b* and *H* = unknown height reducing gene associated with the *Lr54/Yr37* translocation.

satellite data, ten translocation recombinants could be identified, the results of which are shown in Table **1**. The order of the eight chromosome arm 2DL microsatellite loci [12] is shown in Table **1**; however, the relative location of *Xgdm6* with respect to *Xwmc167* is uncertain. Conventional linkage analysis was not done in view of the relatively small number of recombinants that were obtained. Additional, unmapped AFLP and SCAR loci were subsequently employed for the continued characterization of the ten recombinants and were ordered relative to the mapped loci by following a physical mapping approach (Table **1**). Six polymorphic AFLP loci were discovered following an initial (duplicate) screen of the control genotypes and ten recombinants. The productive selective primer pairs and the sizes of the polymorphic PCR fragments are given in Table **1**. Each locus could be amplified in *Ae. kotschyi* and the *Lr54/Yr37* translocation yet was absent in the wheat parents. The results were confirmed on a further independent panel of 16 B_1F_1 (CS-*Lr54/Yr37* translocation /2*CS*ph1b* mutant) plants, eight with (resistant) and eight without (susceptible) the *Lr54/Yr37* translocation.

The recombined translocation chromosomes that are described in Table **1** are the results of two meiotic events: Firstly, in the absence of *Ph1* crossover occurred between the alien segment and 2D of the CS*ph1b* line to produce the ten primary wheat-alien recombined chromosomes. A normal, second meiosis then occurred in the primary testcross F_1 which involved each recombined translocation chromosome and 2D of the testcross parent (W84-17).

Homoeologous Recombination with 2DL

The molecular marker data of Table **1** reveal the following: (1) Allosyndetic recombination between the CS-*Lr54/Yr37* translocated segment and chromosome arm 2DL of CS*ph1b* (Fig. **2A**) produced ten recombinants which were resolved into three size categories by the marker loci. In the formation of each recombinant, crossover occurred proximally of the *Xcfd50* locus and CS*ph1b* chromatin replaced a distal region of alien chromatin. The modified primary translocations will have acquired CS*ph1b* 2DL telomeres and all the wheat microsatellite loci that were restored would have expressed the CS-specific alleles (Fig. **2A**). (2) The primary recombined wheat-alien 2DL arms then paired and recombined regularly with 2DL of W84-17 during the second meiosis. This follows from the fact that the W84-17 allele of locus *Xcfd50* occurred in nine of the ten secondary recombinants. (3) Each of recombinants #74 and #265 expressed both the W84-17 and CS alleles of *Xcfd50* (but only one of the two *Xgdm6* alleles) and therefore contained duplicated regions. The duplications were most likely introduced during

Table 1. Data Obtained Following the Characterization of Ten Allosyndetic Recombinants of the CS-*Lr54/Yr37* Translocation and Controls with Molecular Markers. Symbols: "S" and "W" Respectively Indicate Absence or Presence of Wheat Specific Marker Fragments Associated with the Microsatellite and SCAR *Xust2-IIJ$_1^d$* loci. In the Case of the AFLP, SCAR 410 and SCAR *Xsopw7* loci, which Amplify *Ae. Kotschyi*-Specific Fragments, "S" and "W" Respectively Indicate Presence and Absence of the Alien Marker Fragments. Symbol "-" Indicates Chromosome Regions that Appear to be Deleted

	Xgwm261[1]	*Xgwm484*[1]	*Xbarc124*[1]	*Xcfd116*[1]	Centromere	*Xbarc228* *Xwmc41* *Xcfd233* AFLP[2] -410 *Lr54* *Yr37*	*Xgwm539*[1]	*Xgwm157*	AFLP[2] -306 -360 -416 SCAR *Xust2-IIJ$_1^d$* SCAR *Xsopw7*	AFLP[2] -212 -228	*Xcfd50*[1]	*Xwmc167*	*Xgdm6*[1]	Cross-over[3]
	2DS					2DL								
Ae. kotschyi	S	S	S	S		S	S	S	S	S	S*	S	S	
Lr54/Yr37-hemizygote	W^{CS}	W^{CS}	W^{CS}	W^{CS}		S	S	S	S	S	S	S	S	
CS	W^{CS}	W^{CS}	W^{CS}	W^{CS}		W	W^{CS}	W	W	W	W^{CS}	W	W^{CS}	
W84-17	W^{W}	W^{W}	W^{W}	W^{W}		W	W^{W}	W	W	W	W^{W}	W	W^{W}	
Recombinant #74	-	-	-	W^{CS}		S	W^{CS}	W	W	W	$W^{CS\ \&\ W}$	W	W^{W}	(iii)
Recombinant #37	W^{W}	W^{W}	W^{W}	W^{CS}		S	S	S	S	W	W^{W}	W	W^{W}	(i)
Recombinant #205	W^{CS}	W^{CS}	W^{CS}	W^{CS}		S	S	S	S	W	W^{W}	W	-	(ii)
Recombinant #256	W^{W}	W^{W}	W^{W}	W^{CS}		S	S	S	S	S	W^{CS}	W	W^{CS}	(iv)
Recombinant #265	W^{CS}	W^{CS}	W^{CS}	W^{CS}		S	S	S	S	S	$W^{CS\ \&\ W}$	W	W^{CS}	(v)
Recombinant #119	W^{W}	W^{W}	W^{CS}	W^{CS}		S	S	S	S	S	W^{W}	W	-	(ii)
Recombinant #148	W^{W}	W^{CS}	W^{CS}	W^{CS}		S	S	S	S	S	W^{W}	W	-	(ii)
Recombinant #247	W^{W}	W^{CS}	W^{CS}	W^{CS}		S	S	S	S	S	W^{W}	W	-	(ii)
Recombinant #273	W^{CS}	W^{CS}	W^{W}	W^{CS}		S	S	S	S	S	W^{W}	W	-	(ii)
Recombinant #25	W^{W}	W^{CS}	W^{CS}	W^{CS}		S	S	S	S	S	W^{W}	W	-	(ii)

[1]These microsatellite markers produced distinct CS and W84-17 alleles which are indicated with the superscripts CS and W, respectively. Superscripts CS & W indicate duplicated loci.
[2]Primer combinations that detected the respective AFLP loci (fragment size in brackets): *Eco*RI–AGG/*Mse*I–CAG (212 bp); *Eco*RI–AGG/*Mse*I–CAG (228 bp); *Eco*RI–AAC/*Mse*I–CAT (410 bp); *Eco*RI–ACA/*Mse*I–CTG (306 bp), *Eco*RI–AGG/*Mse*I–CTG (360 bp); *Eco*RI–AAC/*Mse*I–CTG (416 bp).
[3]Possible meiotic pairing structure and position on 2DL where crossover could have occurred (Fig. **2**) to produce the particular recombinant.

the second meiosis as a result of a structural (intra-chromosomal translocation?) difference between the CS*ph1b* and W84-17 sub-telomeric regions. (4) Six recombinants appeared to carry a deletion of the *Xgdm6* locus that probably resulted from the same aberration. Subsequent back-crossing of rec. #74 to W84-17 also resulted in segregates (Table **2**) that had the *Xgdm6* locus deleted and thus confirmed the presence of the chromosome structural difference.

The second meiosis occurred in testcross F_1 plants that carried a primary recombined wheat-alien translocation chromosome plus a complete chromosome 2D from W84-17 (Fig. **2B**). As will be shown below, the data of Tables **1** & **2** imply that (compared to the sequence in CS*ph1b* and published map [12]) the relative locations of *Xcfd50* and *Xgdm6* are switched in W84-17 chromosome 2D. Such a difference could have arisen following an intra chromosome arm translocation of either locus. The precise nature and extent of the structural difference cannot be deduced from the limited data; however, the recombination products obtained are consistent with a translocation rather than a duplicated or inverted region.

Inter-variety chromosome structural differences appear to occur frequently in common wheat. Non-homologous, reciprocal translocations were detected (mostly through meiotic analyses) in studies involving both wheat varieties and species [17]. Yasumuro and co-workers found that multivalent configurations that are typical of reciprocal translocation differences occurred in 81% of 466 F_1 cross combinations. Furthermore, sixty two of 117 Japanese common wheat land races and varieties differed from CS by 1-2 reciprocal translocations involving at least three different pairs of chromosomes [32]. C-band studies [18] revealed translocations, deletions, duplications and inversions within a large and diverse collection (499 accessions) of tetraploid and hexaploid wheat as well as triticale. One hundred and fourty of these had structurally different chromosomes. Certain aberrations

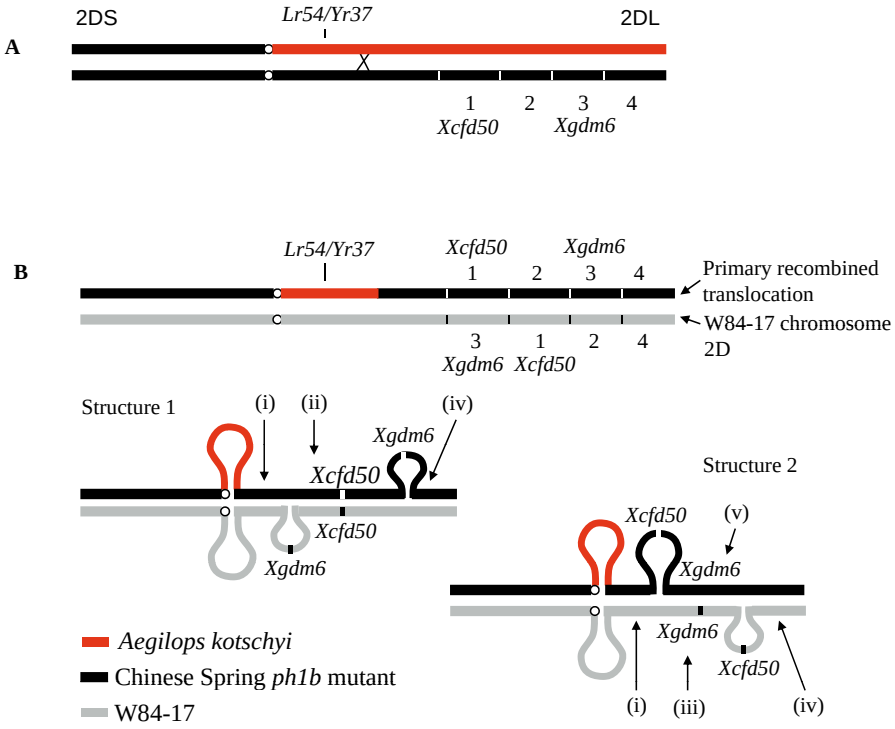

Fig. (2). Sketches showing the probable origin of ten secondary recombined translocation chromosomes of which the marker data are summarized in Table **1**. (**A**) During a first (allosyndetic) meiosis, 2DL of CS*ph1b* recombined with the homoeologous *Ae. kotschyi*-derived chromosome segment to produce the respective primary translocation recombinants. (**B**) During the second meiosis (*Ph1* present) the translocated chromosomes 2DL recombined with 2DL of W84-17 which has a structurally different sub-telomeric region. In the 2DL arm of W84-17, a chromosome region surrounding *Xgdm6* occurs proximally, rather than distally from *Xcfd50*. This structural difference results in two possible aberrant pairing structures being formed. Crossover within the two pairing configurations may result in formation of normal gametes, gametes carrying an *Xcfd50* duplication or an *Xgdm6* deletion.

were widespread while others occurred less frequently. While C-banding proved useful for the detection of large aberrations, smaller structural differences or those involving chromosome regions with few bands would have remained undetected. The latter can only be reliably exposed by employing more laborious genetic and aneuploid mapping procedures [18].

Table 2. Results Obtained Following Screening for the Presence of Microsatellite loci *Xgdm6* and *Xcfd50* in 15 BC_1F_2: Recombinant #74/2* W84-17 Plants

Number of BC_1F_2 Plants:	***Xgdm6***	***Xcfd50***
Eight resistant	W^W	W^W
One resistant	-	W^W
Five susceptible	W^W	W^W
One susceptible	-	W^W

Heterozygosity for the *Xcfd50 – Xgdm6* structural difference resulted in aberrant pairing during the second meiosis. Possible meiotic pairing structures that could have been produced are shown in Fig. (**2B**). The relative positions within the pairing structures where crossovers could have occurred to produce the respective secondary recombinant chromosomes are shown in Fig. (**2B**) and given in Table **1**. Recombinant #74 was subsequently used in backcrosses to W84-17. Since recombinant chromosome #74 carried an *Xcfd50* duplication ($\#74^{(\text{dupl } Xcfd50)\text{-}Xgdm6}$); aberrant pairing structures (containing compensation loops) could once again form in plants that were either heterozygous or homozygous for this specific translocation. Crossing over within such structures could have resulted in translocation chromosomes without *Xgdm6* ($\#74^{Xcfd50\text{-}(\text{del } Xgdm6)}$). Characterization of 15 BC_1F_2 plants for the presence of microsatellite loci *Xcfd50* and *Xgdm6* (Table **2**) showed that two of the plants did in fact lack *Xgdm6*. Thus, in a cross involving genotypes that differ structurally for the *Xcfd50-Xgdm6* region, rec. #74 progeny may be one of four types, i.e. $\#74^{Xcfd50\text{-}Xgdm6}$, $\#74^{Xgdm6\text{-}Xcfd50}$, $\#74^{Xcfd50\text{-}(\text{del } Xgdm6)}$ or $\#74^{(\text{dupl } Xcfd50)\text{-}Xgdm6}$. During subsequent inbreeding a percentage of the homozygous derivatives could therefore be agronomically inferior. However, as nine allosyndetic crossovers occurred between the translocation #74 breakpoint and the *Xcfd50*/*Xgdm6* loci (Table **1**), there is sufficient genetic recombination distance to readily recover plants that combine the resistance with either of the *Xcfd50-Xgdm6* sequences.

The ten primary allosyndetic recombinants apparently resulted from single crossovers involving the *Ae. kotschyi* and homoeologous wheat (CS*ph1b*) chromatin. Although limited in extent, the data suggest that significant homoeology existed between the two regions, however, the observed allosyndetic recombination frequency (= 0.092) between *Lr54* and *Xcfd50*/*Xgdm6* is still low as could be expected [7]. The recombinants appear to have regained functional 2DL telomeres and probably restored significant portions of the

wheat loci on this arm. The allosyndetic crossover that produced rec. #74 occurred within the most proximal of three chromosome arm 2DL deletion bins [12] (Table **1**). Only 25% of 185 EST-loci on 2DL were found to occur within the most proximal deletion bin, which accounts for 49% of the chromosome arm length [33]. This would suggest that more than 75% of the 2DL common wheat loci could have been restored in rec. #74.

Recombination with 2DS

Marker loci *Xgwm261*, *Xgwm484* and *Xcfd116* were mapped to the most proximal deletion bin on chromosome arm 2DS whereas *Xbarc124* was mapped to the most distal deletion bin [12]. The full-length translocation and primary recombinants would have expressed the CS allele of each locus. During the second meiosis, crossover occurred between the CS and W84-17 derived 2DS chromosome arms. Since recombination frequencies increase towards the telomeres, it can be expected that in the secondary recombinants, CS alleles would predominate towards the centromere (and alien chromatin) whereas W84-17 alleles would predominate towards the telomere. This was used as basis for ordering the four loci on chromosome arm 2DS (Table **1**) and suggested that *Xbarc124* may actually have a more proximal location, in-between *Xgwm484* and *Xcfd116.* Furthermore, three of the markers detected null alleles in rec. #74 which would suggest the presence of a large deletion distally from *Xcfd116*. Depending on its size and the genes involved, it could detrimentally affect plant phenotype. Since the aberration is also distant from the alien introgressed region, it should be possible to restore a complete 2DS arm through homologous crossover. This can for example be achieved between a rec. #74 and a rec. #37 chromosome. Tertiary recombinant progeny of the desired type can then be selected with the use of marker loci *Xsopw7* (specific for the longer translocation #37) and *Xgwm484* (occurs within the deleted area).

Thus, although a high frequency of deletions and duplications occurred among the secondary recombinants, these were brought about by chromosome structural differences inherent to the wheat parental genotypes, and involved chromosome regions that are well-removed from the introgressed alien chromatin regions. These aberrations should not impact on the utility of the translocation.

Recombinant #74

In *Rht-B1b* homozygotes the added presence of gene *H* resulted in a dwarf phenotype. When resistant secondary TF_1, TF_2 and TF_3 progeny (segregated for *Rht-B1a* and *Rht-B1b*) of the ten recombinant lines were evaluated for plant height, the progeny of four recombinants (#119, #205, #247 and #256) included dwarfs, which suggested that they had retained *H*. The progeny of the shortest recombinant (#74) did not include any dwarfs. To confirm that it had lost *H*, resistant (20 plants) and susceptible (11 plants) BCF_3 (rec. #74/ 2*W84-17) progeny were evaluated for plant height. When the average plant height of the susceptible and resistant groups was compared, the resistant group was only two cm shorter, whereas the distribution of plant heights was similar for the two groups. This suggested that *H* is no longer associated with rec. #74 and allowed for determining its approximate map location (Table **1**). Seedling inoculation with *P. striiformis* pathotype 6E22A- showed that *Yr37* had been retained in each of the ten recombinants. Near-isogenic lines of rec. #74 are being developed in adapted varieties in order to fully evaluate the utility of the *Lr54*/*Yr37* genes.

Marker Development

One of the six AFLP loci (detected with *Eco*RI–AAC/*Mse*I–CAT (410bp)) was retained in the shortest recombinant (#74) and was used as basis for deriving a SCAR marker. The critical band was excised from the *Ae. kotschyi* lane, purified and cloned. A clone with the correct insert size was identified and sequenced. From the sequence, forward (5'**:** ACCAATTCAACTTGCCAAGAG) and reverse (5'**:** GAGTAACATGCAGAAAACGACA) primer**s** were derived and validated on the ten recombinant and parental lines as well as on two additional panels of resistant and susceptible BCF_2 and BCF_3 (respectively, 20 and 31 plants derived from the cross: Rec. #74/2* W84-17) segregating for the presence of recombinant chromosome #74. The new primer set consistently amplified a 410 bp band in *Ae. kotschyi*, the *Lr54/Yr37* translocation lines and resistant BCF_2 and BCF_3 plants but not in susceptible control plants.

In addition to the newly-developed, dominant SCAR marker of rec. #74, it can also be detected using wheat microsatellite loci. Since the *Lr54*/*Yr37*–74 recombined translocation chromosome has null-alleles for the microsatellite loci *Xbarc228*, *Xwmc41* and *Xcfd233*, the latter may be used as recessive haplotype markers for its detection. For each of the three marker loci, the polymorphisms can readily be observed by separating the PCR product on 2% agarose gels, thus providing a rapid and inexpensive assay. As null alleles may also occur in normal wheat germplasm, simultaneous testing for the presence of two, or all three of the loci in a multiplex PCR reaction, should provide for more accurate labelling of rec. #74. When used in conjunction with the dominant SCAR S14-74 marker, it will therefore be possible to distinguish translocation homo- and heterozygotes.

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