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Extension and use of a physical map of the *Thinopyrum*-derived *Lr19* translocation

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Abstract Twenty-nine deletion mutant lines were used to extend a physical map of the *Lr19* translocated chromosome segment. One hundred and forty-four *Sse*8387I/*Mse*I and 32 *Eco*RI/*Mse*I primer combinations were used to obtain 95 *Thinopyrum*-specific AFLP markers. The physical map confirmed that terminal deletions had mostly occurred, however, it appears that intercalary deletions and primer or restriction site mutations were also induced. The markers allowed for grouping of the deletion mutant lines into 19 clusters, with 7 AFLP markers mapping in the same marker bin as *Lr19*. Primary and secondary *Lr19* allosyndetic recombinants were subsequently physically mapped employing AFLP, RFLP, SCAR and microsatellite markers and the data integrated with the deletion map. A further shortened, tertiary *Lr19* recombinant was derived following homologous recombination between the proximally shortest secondary recombinant, *Lr19*-149-299, and distally shortest recombinant, *Lr19*-149-478. The tertiary recombinant could be confirmed employing the mapped markers and it was possible to identify new markers on this recombinant that can be used to reduce the translocation still further.

Key words Deletion mapping · Gamma irradiation · Homoeologous pairing · Allosyndetic pairing

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Introduction

Leaf rust (*Puccinia triticina* Eriks.) resistance gene *Lr19* is regarded worldwide as highly effective (Winzeler et al. 1995), although virulence has been reported by Huerta-Espino and Singh (1994) and Sibikeev et al. (1996) Transferred from *Thinopyrum ponticum* (Podp.) Barkworth & DR Dewey (= *Agropyron elongatum* Host.) to wheat by Sharma and Knott (1966), the *Lr19* translocation is homoeologous to 7DL (Dvořák and Knott 1977) and is associated with genes for stem rust resistance (*Sr25*), segregation distortion (*Sd1*), yellow endosperm (*Y*) and water-soluble protein (*Wsp-D1*); but shows null conditions for the endopeptidase (*Ep-D1*) and α-amylase (*α-Amy-D2*) loci (Marais 1992a; McMillin et al. 1993; Winzeler et al. 1995) In the presence of *Ph1*, the translocation does not recombine with homoeologous regions of the wheat genome and is therefore inherited as a single large linkage block (Knott 1980; Marais and Marais 1990)

Since the translocated region does not pair and recombine with homoeologous 7DL chromatin during meiosis it cannot be mapped using crossover frequencies (Marais 1992a), and physical mapping had to be attempted. Marais (1992a) irradiated spikes of the translocation line 'Indis' with gamma rays and pollinated these with 'Inia 66' pollen. Testcross progenies were screened for translocation mutants, using the leaf rust resistance and yellow endosperm pigmentation genes as markers. The EP-D1 null condition was used to recover mutant translocation homozygotes. Among the testcross progeny, 29 deletion mutants were identified and characterized for the presence or absence of the *Sr25*, *Y*, *Sd1* and *Wsp-D1c* loci to construct a first physical map. Prins et al. (1996) expanded the map using RFLP markers and assigned the mutant lines to seven deletion size groups. Most mutations appeared to be terminal and only two intercalary deletions were identified (Marais 1992a; Prins et al. 1996)

Marais (1992b) and Marais et al. (2001) employed the mapped markers in two successive attempts to shorten the translocation through homoeologous pairing induction. Primary recombinant *Lr19*-149 (Marais 1992b) apparently resulted from a double crossover event during which *Lr19* was translocated from chromosome arm 7DL to 7BL. During the exchange the terminally situated yellow pigment (*Y*) and *Sr25* loci were presumably replaced with 7BL chromatin. Marais et al. (2001) subsequently produced four secondary recombinants from *Lr19*-149. These included three that resulted from single, proximal exchanges (*Lr19*-149-252, -299 and -462) and one that was produced by a single, terminal exchange during which the terminally located *Wsp-D1c* gene was removed. *Lr19*-149-299 is the proximally shortest secondary recombinant (Marais et al. 2001) and had lost the two RFLP loci ($Xpsr129\text{-}7el_1$ and $XcsIH81\text{-}1\text{-}7el_1$) previously used to characterize the proximal end of the segment. Further attempts to shorten the translocation would therefore require extension of the existing physical map, especially in the area surrounding *Lr19*.

The aims of the present study were to (1) identify AFLP markers on the *Lr19* translocation in order to improve the resolution of the physical map of Prins et al. (1996); (2) use the proximally shortest (*Lr19*-149-299) and the distally shortest (*Lr19*-149-478) secondary recombinants to derive a still shorter tertiary recombinant through homologous recombination; (3) confirm the secondary and tertiary recombinants using existing and newly mapped markers; and (4) identify useful markers close to *Lr19* that may be employed in marker-assisted selection or further attempts to shorten the translocation.

Materials and methods

Extension of the *Lr19* deletion map

The physical map of the *Lr19* translocation developed by Prins et al. (1996) was extended by using 29 homozygous deletion lines (Marais 1992a), wheat accession 'Indis' containing the complete *Lr19* translocation and the susceptible cultivar 'Inia 66' to identify AFLP markers associated with the translocation. Total genomic DNA was extracted from plants grown in a greenhouse, using the procedure of Doyle and Doyle (1990) with slight modifications as described by Prins et al. (1996) DNA concentrations were determined on a Hoefer DyNA Quant 200 fluorometer according to the manufacturer's instructions. A core group of genotypes consisting of 'Indis', 'Inia 66', four mutants carrying the smallest deletions (87M23-225, 89M2-426, 89M1-18 and 87M23-266) and two mutants with the largest deletions (87M23-145 and 87M23-198) were used for preliminary polymorphism screening. In an attempt to find more markers for specific physical marker bins, a further set of 17 mutants plus the controls, 'Inia 66' and 'Indis', were subsequently used for additional screening. The AFLP protocol of Vos et al. (1995), as modified by Donini et al. (1997) and Groenewald et al. (2003), was used to screen the lines with *Sse*8387I and *Mse*I selective primers. Primer sequences and codes were taken from the standard list for AFLP primer nomenclature (http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html). For screening the lines with the restriction endonuclease combination *Eco*RI and *Mse*I, the AFLP Analysis System I (GIBCO BRL, PO Box 9418, Gaithersburg, MD, USA) was used according to the manufacturer's instructions. Fragments were scored manually for the absence and presence of an amplification product. Fragments present as a 'doublet' were scored as a single marker. Potentially useful polymorphisms were fragments that were amplified from 'Indis' and from one or more of the mutants with smaller deletions, but not from 'Inia 66' or any of the mutants with the largest deletions. Potentially useful polymorphisms were tested on all the 29 deletion mutants and the data were integrated with an existing physical map in order to determine the positions of the loci.

Derivation of a recombined, tertiary *Lr19* translocation

To confirm that four secondary *Lr19* translocations produced by Marais et al. (2001) still occur on 7B, monosomic analyses were done. Homozygotes of each secondary recombinant in Chinese Spring ('CS') genetic background were crossed to the 'CS' monosomics for chromosomes 7A, 7B and 7D. Resistant monosomic F_1 progeny were selected and planted. F_2 seedlings from each population were inoculated with leaf rust pathotype UVPrt8, which is virulent on 'CS' but avirulent on *Lr19* carriers. Two of the secondary allosyndetic recombinants, *Lr19*-149-299 (proximally shortest) and *Lr19*-149-478 (terminally shortest), were then used to derive a shorter, tertiary recombinant. Recombinant *Lr19*-149-299 expresses the wheat allele for *Xpsr129* (proximal side of *Lr19*) but the *Thinopyrum* allele, *Wsp-D1c* on the distal end. Conversely, recombinant *Lr19*-149-478 has the *Thinopyrum* allele for *Xpsr129* but lacks the *Thinopyrum* allele, *Wsp-D1c* (Marais et al. 2001). The two secondary recombinants were crossed and the F_1 testcrossed with the UVPrt8-susceptible breeding line W84-17 in an attempt to recover tertiary recombinants. The testcross (00M96) F_1 seeds were halved and the endosperm halves were kept to extract and separate water-soluble proteins at a later stage in order to test for the presence of *Wsp-D1c* (Marais 1992a). The embryo halves were planted and the seedlings screened for resistance to UVPrt8. Resistant plants (194) were transplanted to a greenhouse. Only those resistant plants that did not express *Wsp-D1c* were subsequently used for DNA extraction and CAPS analysis employing an *Xpsr129* derived primer set (Marais et al. 2001).

Physical mapping of molecular markers to the area surrounding *Lr19*

The putative tertiary recombinants obtained were characterized by employing 12 of the easier-to-score AFLP markers that occur closest to *Lr19*. In a further attempt to confirm these putative tertiary recombinants and to find useful RFLP, microsatellite or SCAR markers that map close to *Lr19*, near-isogenic lines in 'CS' of the complete *Lr19* translocation (chromosome 7D) as well as each primary, secondary and tertiary recombinants (chromosome 7B) were used. 'CS' and 'CS' nullisomic 7B were employed as controls. In addition a subset of the deletion lines that defines the area around *Lr19*, namely 89M2-40, 87M23-103, 89M2-245, 89M2-426, 87M23-225, 89M1-18 and 87M23-266 (in the order of deletion size with 89M2-40 carrying the largest deletion and 87M23-266 the smallest), were typed. 'Indis' and 'Inia 66' were included as controls with the deletion lines. The various genotypes were analysed for polymorphisms detected by the RFLP probes, TtksuE018 and PSR687, and the $STSLr19_{130}$ marker derived by Prins et al. (2001) from an AFLP fragment designated as $XustSS13M12_{174}$ in the present study. For RFLP analyses approximately 10 µg of DNA of each line was digested with *Hind*III (Roche Diagnostics GmbH, Roche Molecular Biochemicals, 68298 Mannheim, Germany), separated on a 0.8% agarose gel in 1X TBE buffer, and alkaline blotted overnight onto a positively charged nylon membrane (Roche Diagnostics GmbH) following the recommendations of the manufacturer. The probe was DIG-labelled, hybridized to the membranes and detected using the methodology outlined in the DIG Application Manual for Filter Hybridization (Roche Diagnostics GmbH). Probe PSR687 was obtained from Dr. M.D. Gale (John Innes Centre, Colney, Norwich, UK) whereas the TtksuE018 sequence was obtained from Dr. J. Raupp (Wheat Genetics Resource Centre, Kansas State University, Manhattan, KS, USA). The recombinants were also tested for amplification of the wheat 7B microsatellite loci *Xgwm146*, *Xgwm611*, *Xgwm577* (Röder et al. 1998) and *Xwmc276* (Gupta et al. 2002). Microsatellite amplification and high-resolution size separation were done as described by Groenewald et al. (2003) and the gels were silver-stained according to Beidler et al. (1982). Endopeptidase markers were analysed as outlined by Marais and Marais (1990). Data obtained using both the recombinants and deletion mutants were integrated with the existing physical map data to determine the relative position of each marker.

Results

Extension of the *Lr19* deletion map

Two *Sse*8387I +1, two *Sse*8387I +2, four *Sse*8387I +3, twelve *Mse*I +2 and six *Mse*I +3 AFLP primers in a total of 144 primer combinations were used in a preliminary screen of a core group of genotypes to identify *Lr19*-specific fragments (Table 1). Four *Eco*RI +3 and eight *Mse*I +3 AFLP primers in a total of 32 primer combinations were used in an extended screening of 'Indis', 'Inia 66' and 17 deletion mutants (Table 1). Potentially useful polymorphisms were tested on all 29 mutant lines. Fifty-one (75%) of the 68 useful *Sse*8387I/*Mse*I primer combinations yielded a single useful polymorphism, 16 (23.5%) yielded 2 useful polymorphisms and only 1 (1.5%) combination resulted in 3 useful polymorphisms. If all 144 combinations are considered, 0.60 useful polymorphisms per combination were observed. Three (50%) of the six useful *Eco*RI/*Mse*I primer combinations yielded a single useful polymorphism, while the other half yielded two useful polymorphisms. If all 32 combinations are taken into account, an average of 0.28 useful polymorphisms per combination was found. Two *Eco*RI and four *Mse*I selective primers did not reveal any useful polymorphisms when used in combination with *Mse*I and *Eco*RI selective primers, respectively, whereas all *Sse*8387I and *Mse*I selective primer combinations yielded at least one useful polymorphism. However, this may be a peculiarity of the specific primer combinations tested and the specific chromosome segment that was studied. Mutant lines 87M23-175 and 89M2-416 were found to produce inconsistent results, which may have been due to heterogeneous seed stocks and were not used in subsequent mapping.

A total of 95 useful translocation-specific polymorphisms were amplified by the 74 primer combinations and were integrated with the physical map of Prins et al. (1996) The 7 marker bins of the previous map were reorganized into 19 new bins and the revised map is shown as a line drawing in Fig. 1. It was not possible to size the mutants in some bins, whereas other bins contained only one mutant. Prins et al. (1996) reported intercalary deletions in two mutants, namely 87M23-175 and 89M2-416. As these were the two lines that produced inconsistent results and that were subsequently excluded, this was not confirmed. In several other mutants, evidence of intercalary deletions was found. Mutants 87M23-273 and 87M23-3 may each have an intercalary deletion spanning four AFLP markers (in bins 3 and 9, respectively), while mutants 89M2-39 (bin 10) and 87M23-103 (bin 1, bins 7/8) appear to have intercalary deletions involving two AFLP markers each. In mutant 89M2-225 (bins 16/17), two AFLP markers and the *Wsp-D1c* gene were deleted. In other intervals, single markers were deleted, for example in mutant 87M23-1 (bin 2, bin 3), mutant 87M23-314 (bin 6), mutant 87M23-3 (bin 6) and mutant 87M23-27 (bin 12). Absence of these markers may be due to mutations in the restriction site or at positions in the selective AFLP primers' binding site. However, it is also possible that some of these may be due to small intercalary deletions. Marker $XustSS29M19_{>330}$ (bin 2) was absent in four deletion lines which may be reflective of a mutational hotspot or poor repeatability. Overall, both *Sse*8387I/*Mse*I and

Table 1 Results obtained for the 32 *Eco*RI/*Mse*I and 144 *Sse*8387I/*Mse*I primer combinations tested

*Mse*I selective primers	*Eco*RI and *Sse*8387I selective primers												Polymorphism number
	E32 (AAC)	E33 (AAG)	E35 (ACA)	E36 (ACC)	S02 (C)	S04 (T)	S12 (AC)	S13 (AG)	S27 (AGA)	S28 (AGC)	S29 (AGG)	S30 (AGT)	
M11 (AA)					1	1					2		0.50
M12 (AC)						1		1	1		1		0.50
M13 (AG)						2		1		1		1	0.63
M14 (AT)					1	1	2	1					0.63
M15 (CA)						2	1	1	2		1	1	1.00
M16 (CC)								1			1		0.25
M17 (CG)						1	1		1	1		2	0.75
M18 (CT)					1		1			1			0.38
M19 (GA)					2	1	2	1			2		1.00
M20 (GC)						1		1			2	1	0.63
M21 (GG)					3		1	1	2	1		1	1.13
M22 (GT)								1				1	0.25
M32 (AAC)					1	2		2		1			0.75
M47 (CAA)													0.00
M48 (CAC)			1										0.25
M49 (CAG)	1		2										0.75
M50 (CAT)			1										0.25
M53 (CCG)						1		1			1		0.38
M59 (CTA)													0.00
M60 (CTC)	2		2										1.00
M61 (CTG)													0.00
M62 (CTT)													0.00
M64 (GAC)									1				0.13
M74 (GGT)						1				2			0.38
M88 (TGC)					1	1		1		1	1	1	0.75
M90 (TGT)					2	2		1			1		0.75
Polymorphism number	0.38	0.00	0.75	0.00	0.67	0.94	0.44	0.78	0.39	0.44	0.67	0.44	0.60 / 0.28 / 1.54[a]

The specific nucleotide(s) for each primer is indicated in brackets after the primer name. The numbers in the body of the table represent the number of useful polymorphisms amplified by the respective primer combinations. The polymorphism number is calculated by dividing the number of useful polymorphisms in the column or row by the number of primer combinations in that column or row

[a]Polymorphism numbers calculated for all 144 *Sse*8387I/*Mse*I, all 32 *Eco*RI/*Mse*I and all possible 176 primer combinations, respectively

*Eco*RI/*Mse*I markers were evenly distributed throughout the map.

Derivation of a recombined, tertiary *Lr19* recombinant

Monosomic analysis of the four secondary *Lr19* recombinants (Table 2) showed that *Lr19* is still located on chromosome 7B in each. Proximal recombinants *Lr19*-149-252, -299 and -462 therefore resulted from exchanges with wheat chromosome 7B. In the case of recombinant *Lr19*-149-478 (distal exchange), the data do not provide information about the origin of the telomeric section of chromosome arm 7BL. While the telomeric ends of the four translocations are likely to be derived from 7B, the possibility that it resulted from exchanges with other chromosomes cannot be ruled out. In each data set there is evidence of self-elimination, which agree with the results of Marais et al. (2001) and which would confirm that the *Sd2* locus is still present in each recombinant.

One hundred and ninety-four testcross F_1: 'CS' *Lr19*-149-299/'CS' *Lr19*-149-478//W84-17 seedlings were resistant, of which 74 lost the *Wsp-D1c* gene. Seventeen plants lacked both *Thinopyrum* genes (*Xpsr129-7el*$_1$ and *Wsp-D1c*) and were therefore putative tertiary recombinants. Four of the latter plants were selected for further characterization. The testcross F_2 showed an average segregation of 66% resistant plants: 34% susceptible, as would be expected if the tertiary recombinants retained *Sd2*. The plants were subsequently characterized for the various markers.

Physical mapping of molecular markers to the area surrounding *Lr19*

Integration of the marker data obtained in the AFLP mapping experiment and data generated in analyses of

Table 2 Percentages of resistance to susceptible F_2 progeny (total tested in brackets) observed following monosomic analyses of four secondary *Lr19*-149 recombinants

Recombinant *Lr19*-149-	Monosomic chromosome		
	7A	7B	7D
252	64:36 (231)	98:2 (145)	60:40 (139)
299	51:49 (156)	97:3 (291)	74:26 (258)
462	65:35 (218)	98:2 (253)	76:24 (187)
478	68:32 (173)	95:5 (190)	64:36 (266)

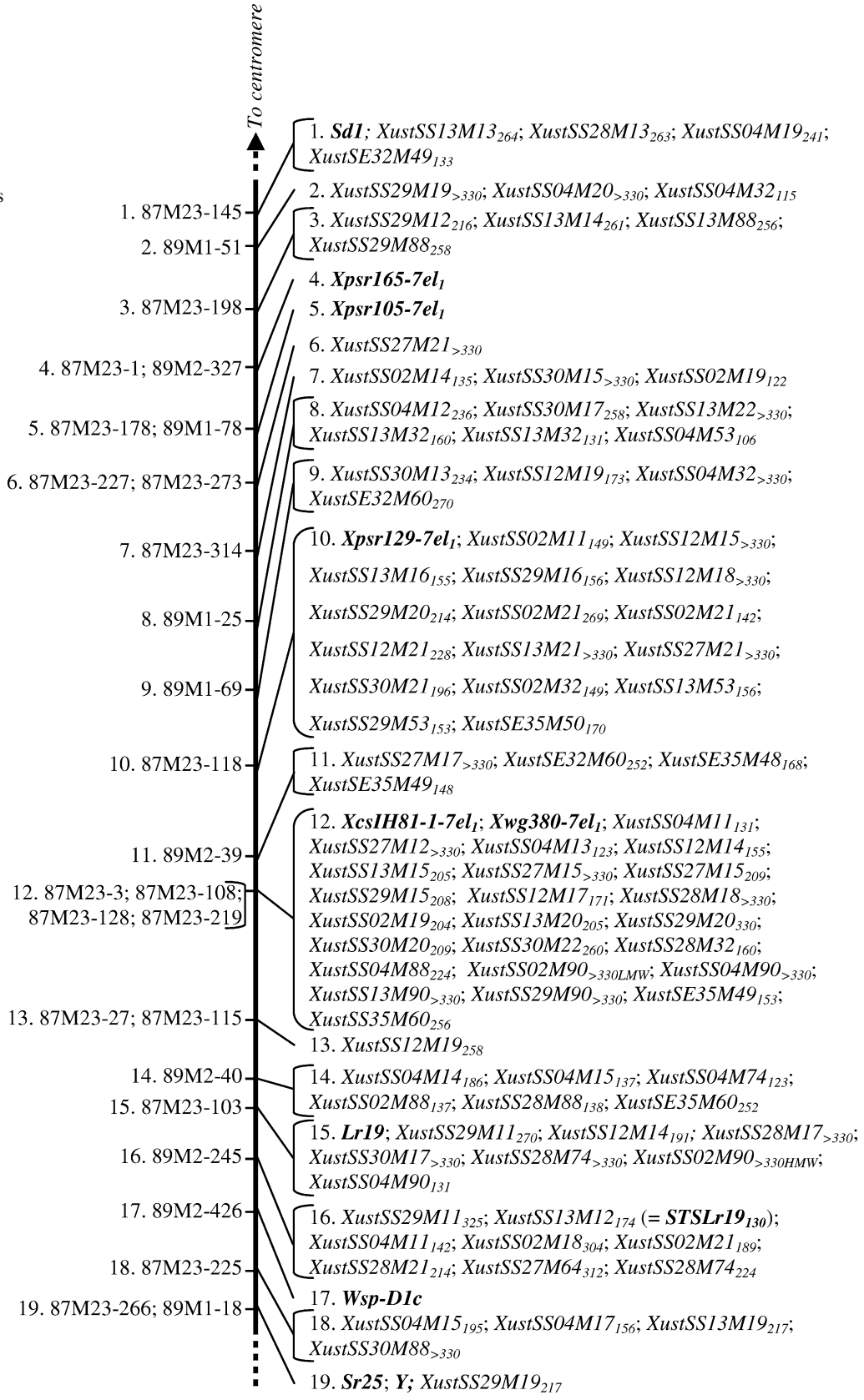

Fig. 1 Extended physical map of the *Lr19* translocation showing the order and relative distances between 19 marker bins as deduced from the positions and frequencies of irradiation breakpoints. The order of the deletion mutants (*left half of figure*) and loci (*right half of figure*) within bins is unknown. Markers other than AFLP markers are indicated in *bold* type and bin numbers are included next to the deletion mutants and loci. The deletion mutants carrying the largest deletions are at the *top* of the figure and the mutants with the smallest deletions are at the *bottom* of the figure

the recombinant lines and a subset of deletion mutants, allowed for the construction of a physical map of the tertiary recombinant (Fig. 2). The $STSLr19_{130}$ marker maps distally of *Lr19* on the tertiary recombinant and should be useful in further attempts to identify distally shortened recombinants following induction of homo-

eologous pairing. *Lr19* occurs in the same marker bin as AFLP markers *XustSS29M11*$_{270}$, *XustSS12M14*$_{191}$, *XustSS28M17*$_{>330}$, *XustSS30M17*$_{>330}$, *XustSS02-M90*$_{>330HMW}$ and *XustSS04M90*$_{131}$. However, the various mutants and recombinants could not resolve their relative locations and it was not possible to identify a potentially useful proximal marker. Several other markers are associated with the area spanned by the tertiary translocation yet their relative positions cannot be determined either (Fig. 2). Four microsatellite markers and the *Ep-B1* locus are wheat-specific and their relative positions will only be revealed once these areas have been substituted by wheat 7B chromatin in translocation recombinants. Probe TtksuE018 did not recognize a *Thinopyrum*-specific fragment, yet produced two 7B specific bands, one of which is located within the area of remaining *Thinopyrum* chromatin. Similarly, the *Sd2* locus is still associated with the tertiary translocation and its position could not be mapped using the deletion mutants as these still expressed *Sd1*, which is located nearer to the centromere and masked the effect of *Sd2*.

Discussion

Attempts to shorten the *Lr19* translocation through induction of homoeologous pairing rely on the use of *Thinopyrum* chromosome beacons that can be used to recover and/or characterize the recombinants sought. When a marker had been used successfully in one round of allosyndetic pairing induction, it is no longer useful and new markers linked closer to the target gene need to be found for subsequent use. In the absence of meiotic pairing between translocated and homoeologous wheat chromatin, physical mapping provides an alternative means of associating marker loci with specific chromosome regions. RFLP and microsatellite markers have

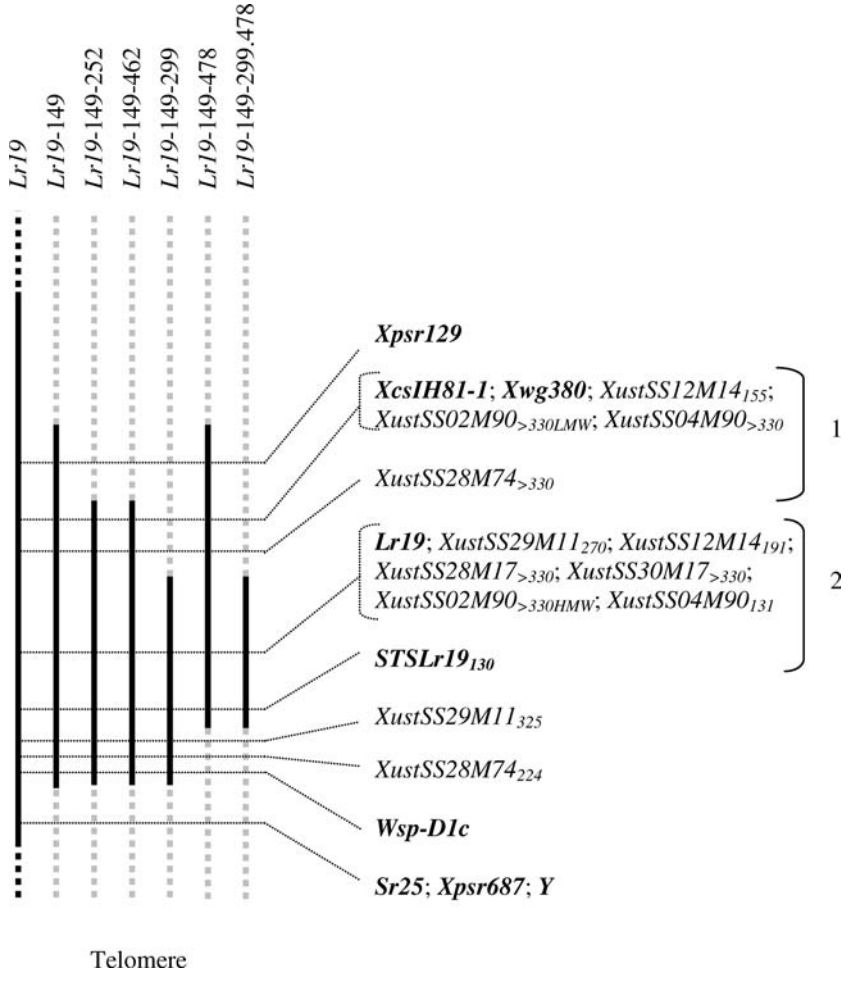

Fig. 2 A physical map that was produced to confirm the presence of a shortened *Lr19* translocation in the tertiary recombinant *Lr19*-149-299.478. Present results were integrated with the map produced by Marais et al. (2001) The map portrays the linear order of clusters of markers only, and the relative distances between clusters and order of the loci within clusters are not known. Markers other than AFLP markers are indicated in *bold* type. 1. A wheat RFLP fragment (< 2027 bp) detected by TtksuE018 maps to this region. 2. A wheat RFLP (TtksuE018) fragment (> 9146 bp); four wheat microsatellite loci (Xgwm146, Xgwm611, Xgwm577 and Xwmc276), the segregation distortion gene, Sd2, and the endopeptidase locus, *Ep-B1*, mapped to this region

proven valuable in mapping of the wheat genome yet their development is time-consuming and expensive, making them less useful for the present application. AFLP marker technology, on the other hand, provides a cost-effective, reproducible and rapid screening method that can be used to search for *Thinopyrum*-specific markers.

In organisms with complex genomes the complexity and scorability of the AFLP fingerprint are important. Addition of more selective nucleotides on the 3′ end of the *Sse*8387I or *Mse*I primers used for selective PCR amplification will decrease the complexity of the fingerprint as the selectivity of the primers increases. *Sse*8387I +1, *Sse*8387I +2 and *Sse*8387I +3 selective primers, as well as *Eco*RI +3 selective primers, in combination with *Mse*I +2 and *Mse*I +3 primers were used here for selective amplification. Most polymorphisms were found using *Sse*8387I +1 and *Sse*8387I +2 primers. Combinations of *Sse*8387I and *Mse*I were better suited for the analysis of the translocation than *Eco*RI and *Mse*I, as 47.2% of the primer combinations resulted in useful polymorphisms versus 18.8% for the latter. The low frequencies (0.60 and 0.28) of useful polymorphisms detected per combination are to be expected if only a comparatively small region of the genome is targeted (Qu et al. 1998; Bai et al. 1999). Although the AFLP technique is capable of amplifying large numbers of fragments (Vos et al. 1995), few of these will actually be linked to the gene or area of interest. The need to use a large number of AFLP primer combinations to identify linked markers is also evident from other studies. For example, Schwarz et al. (1999) used 256 *Eco*RI/*Mse*I (16 primers of each) primer combinations to screen barley NILs with resistance to powdery mildew and identified 6 polymorphic fragments associated with a large *Mla1*-carrying donor fragment in NIL P01 and 4 fragments for the small *Mla12*-carrying donor fragment in NIL P10. Only three of these fragments were found to be useful after subsequent testing in a segregating F_2 population. AFLPs were also used by Bai et al. (1999) to identify markers associated with a major QTL controlling scab resistance in wheat: 11 markers from 20 of the 300 *Eco*RI/*Mse*I primer combinations (15 *Eco*RI and 20 *Mse*I selective primers) resulted in significant association.

Using 176 primer combinations, the physical map has been extended with an additional 95 AFLP markers and allowed the ordering of 19 of the 27 deletion mutants. Seven clusters, consisting of 14 mutants, remain in which lines could still not be distinguished from one another. To allow for the possibility that the methylation status of these regions might have influenced the cutting preference of the methylation-sensitive rare cutting enzyme *Sse*8387I, the methylation-insensitive enzyme combination *Eco*RI/*Mse*I was also tested. Barrett and Kidwell (1998) found that the methylation-sensitive combination *Pst*I/*Mse*I detected significantly lower levels of diversity in wheat compared to the combination *Eco*RI/*Mse*I, and ascribed it to the possibility that hypomethylated regions might contain less DNA variation than hypermethylated regions. Screening the set of mutants with *Eco*RI/*Mse*I primer combinations did not break up the clusters any further and it is therefore unlikely that methylation is a major cause of clustering. A more plausible explanation is that some of the mutation breakpoints may simply be close to one another, reducing the chance of co-occurrence of markers. Attempts to order the mutants that are still clustered may therefore require the screening of many more primer combinations.

It was possible to derive a shorter *Lr19* translocation through homologous pairing of the proximally shortest and distally shortest secondary recombinants and confirm it making use of the AFLP map. Only limited further ordering of molecular loci that occur within the *Lr19* bin was possible by integrating data of the various *Lr19* recombinants with the AFLP map. While it has been shown that the proximal part of the translocation consists of 7BL chromatin, it was not possible to confirm that the telomeric end is composed of 7BL chromatin, and in fact, using genomic in-situ hybridization, Dr. A.J. Lukaszewski (personal communication; University of California-Riverside, USA) was not able to show the presence of wheat chromatin at the telomere. This could simply mean that the piece of telomeric wheat chromatin is very small and beyond the resolution of the technique. It could also suggest that the terminal end was lost through deletion. Although direct molecular marker evidence for the restoration of the wheat 7BL telomere is still lacking, the fact that two successive homoeologous exchanges involving the telomere occurred suggests that it must have a wheat telomere, most likely a 7BL telomere. Also, the *Thinopyrum* chromatin on recombinant *Lr19*-149-299 (which has a telomere shortened through one crossover) recombined readily (17.5% recombination) with that on recombinant *Lr19*-149-478 (whose telomeric end has been shortened twice). This suggests that pairing initiation from the telomere might have been possible, despite the presence of two bordering, non-homologous *Thinopyrum*/*Triticum* chromatin regions. The primary *Lr19*-149 recombinant has no apparent deleterious associated effects (Marais et al. 2001) and marker $STSLr19_{130}$ can detect all of the recombinants in marker-assisted selection. However, in breeding it is undesirable to have large, inaccessible linkage blocks as it restricts formation of new gene combinations. Tertiary recombinant *Lr19*-149-299.478 occupies a physically smaller region on 7BL, yet, still represents a sizeable segment of inert (recombination) chromatin. It also remains associated with *Sd2*, a gene that causes self-elimination during segregation in heterozygotes. Our ultimate aim is to employ *Lr19* in recurrent mass selection aimed at gene pyramiding. It is therefore necessary to remove *Sd2* from the translocation, as it will continually decrease the proportion of *Lr19* in the population, thus defeating the purpose. Further shortening of the translocation requires use of marker loci close to and preferably on either side of *Lr19*. While $STSLr19_{130}$ is such a useful distal marker,

the relative positions of the markers that cluster with *Lr19* could not be resolved and it may be necessary to 'blindly' use some of these or to screen directly for *Sd2* when attempting to recover and characterize new allosyndetic recombinants.

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References

Bai G, Kolb FL, Shaner G, Domier LL (1999) Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89:343–348

Barrett BA, Kidwell KK (1998) AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci 38:1261–1271

Beidler JL, Hilliard PR, Hill RL (1982) Ultrasensitive staining of nucleic acids with silver. Anal Biochem 126:374–380

Donini P, Elias ML, Bougourd SM, Koebner RMD (1997) AFLP fingerprinting reveals pattern differences between template DNA extracted from different plant organs. Genome 40:521–526

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Dvořák J, Knott DR (1977) Homoeologous chromatin exchange in radiation-induced gene transfer. Can J Genet Cytol 19:125–131

Groenewald JZ, Marais AS, Marais GF (2003) Amplified fragment length polymorphism-derived microsatellite sequence linked to the *Pch1* and *Ep-D1* loci in common wheat. Plant Breed 122:83–85

Gupta PK, Balyan HS, Edwards KJ, Isaac P, Korzun V, Röder M, Gautier M-F, Joudrier P, Schlatter AR, Dubcovsky J, De la Pena RC, Khairallah M, Penner G, Hayden MJ, Sharp P, Keller B, Wang RCC, Hardouin JP, Jack P, Leroy P (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105:413–422

Huerta-Espino J, Singh RP (1994) First report of virulence to wheat with leaf rust resistance gene *Lr19* in Mexico. Plant Dis 78:640

Knott DR (1980) Mutation of a gene for yellow pigment linked to *Lr19* in wheat. Can J Genet Cytol 22:651–654

Marais GF (1992a) Gamma irradiation induced deletions in an alien chromosome segment of the wheat 'Indis' and their use in gene mapping. Genome 35:225–229

Marais GF (1992b) The modification of a common wheat-*Thinopyrum distichum* translocated chromosome with a locus homoeoallelic to *Lr19*. Theor Appl Genet 85:73–78

Marais GF, Marais AS (1990) The assignment of a *Thinopyrum distichum* (Thunb.) Löve derived translocation to the long arm of wheat chromosome 7D using endopeptidase polymorphisms. Theor Appl Genet 79:182–186

Marais GF, Marais AS, Groenewald JZ (2001) Evaluation and reduction of *Lr19*-149, a recombined form of the *Lr19* translocation of wheat. Euphytica 121:289–295

McMillin DE, Johnson JW, Roberts JJ (1993) Linkage between endopeptidase *Ep-D1d* and a gene conferring leaf rust resistance (*Lr19*) in wheat. Crop Sci 33:1201–1203

Prins R, Marais GF, Janse BJH, Pretorius ZA, Marais AS (1996) A physical map of the *Thinopyrum*-derived *Lr19* translocation. Genome 39:1013–1019

Prins R, Groenewald JZ, Marais GF, Snape JW, Koebner RMD (2001) AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. Theor Appl Genet 103:618–624

Qu LJ, Foote TN, Roberts MA, Money TA, Aragon-Alcaide L, Snape JW, Moore G (1998) A simple PCR-based method for scoring the *ph1b* deletion in wheat. Theor Appl Genet 96:371–375

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Schwarz G, Michalek W, Mohler V Wenzel G, Jahoor A (1999) Chromosome landing at the *Mla* locus in barley (*Hordeum vulgare* L.) by means of high-resolution mapping with AFLP markers. Theor Appl Genet 98:521–530

Sharma D, Knott DR (1966) The transfer of leaf rust resistance from *Agropyron* to *Triticum* by irradiation. Can J Genet Cytol 8:137–143

Sibikeev SN, Krupnov VA, Voronina SA, Elesin VA (1996) First report of leaf rust pathotypes virulent to highly effective *Lr*-genes transferred from *Agropyron* species to bread wheat. Plant Breed 115:276–278

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Winzeler M, Winzeler H, Keller B (1995) Endopeptidase polymorphism and linkage of the *Ep-D1c* null allele with the *Lr19* leaf-rust-resistance gene in hexaploid wheat. Plant Breed 114:24–28