APPLICATION OF DNA RFLP PROCEDURES IN INTERSPECIFIC GENE TRANSFER: THE *Lr19* TRANSLOCATION OF WHEAT

R. PRINS
Agricultural Research Council (Small Grain Institute),
Department of Genetics,
University of Stellenbosch,
Stellenbosch, South Africa

G.F. MARAIS, A.S. MARAIS
Department of Genetics
University of Stellenbosch
Stellenbosch, South Africa

Z.A. PRETORIUS
Department of Plant Pathology,
University of the Orange Free State,
Bloemfontein, South Africa

B.J.H. JANSE
Department of Microbiology,
University of Stellenbosch,
Stellenbosch, South Africa

Abstract

Twenty-nine lines with deletions in the *Lr19* ('Indis') translocated chromosome segment were used to physically map *Thinopyrum* Restriction Fragment Length Polymorphism (RFLP) loci as well as the *Sr25* and *Sd1* loci. The relative distances between marker loci on the translocation were then calculated. The information was then used as an aid to characterize several recombined forms of the translocation. The data confirmed the reported homoeology between the *Lr19* segment and chromosome arm 7DL of wheat. Also, it seems that the *Lr19* translocation in 'Indis' is very similar to the *Lr19* segment in the T4 source and that the former may not derive from *Thinopyrum distichum*. Near-isogenic lines of the recombined segments were derived and used to study their expression of leaf rust resistance. It became evident that only one potentially useful recombinant was obtained in an earlier attempt to induce allosyndetic pairing between the *Lr19* translocation and 7DL of wheat.

1. INTRODUCTION

The *Lr19* translocation of wheat carries a leaf rust resistance gene which provides excellent leaf rust resistance world-wide. Unfortunately, in many countries *Lr19* cannot be used for breeding bread wheats as the translocated segment also carries a gene(s) that codes for yellow endosperm pigmentation [1-3]. Other known loci on the segment include: *Ep-D1* (endopeptidase), *Wsp-D1* (water-soluble protein), *Sr25* (stem rust resistance) and *Sd1* (segregation distortion) [4-6].

There are two sources of the *Lr19* translocation, the *Thinopyrum ponticum* derived *Lr19* or T4 [7] translocation and the 'Indis' translocation that was selected in the Dept. of Genetics, Stellenbosch in the backcross progeny of the 'Inia 66'(wheat)/*Thinopyrum distichum* cross in the early eighties. The origin of the 'Indis' translocation has, however, remained suspect as *Thinopyrum distichum*, as well as its amphiploid with common wheat are highly susceptible to local pathotypes of leaf and stem rust. The *Thinopyrum* segment (that has replaced a large portion of chromosome 7DL of wheat) does not pair with homoeologous wheat segments during meiosis, complicating attempts to recombine its genes or to study linkage relationships.

Following irradiation, Marais [5] derived 29 deletion mutants, each homozygous for a different deletion of the translocation. In an attempt to break the linkage between the leaf rust resistance and yellow endosperm loci, Marais [8] made use of pairing inhibitor *(ph1b, ph2b)* mutants in wheat ('CS') to induce allosyndetic pairing and crossovers between the *Lr19* segment and homoeologous areas of the wheat genome.

Four suspected recombinants giving white endosperm, three giving partially white endosperm, and one producing yellow endosperm but sometimes showing self-elimination, were recovered. The resistance in the four white selections was found to be associated with chromosomes other than 7D [9]. Knowledge of the relative positions of marker genes was required to characterize and select the most promising recombined forms. The deletion lines were used to determine the linear sequence of three marker genes on the translocated 7DL arm as: centromere-*Lr19-Wsp-D1-Y* [5]. They were used to characterize the suspected recombinants [8]. However, more markers were needed.

The aims of the present study were to: determine through deletion mapping the relative positions of further marker loci (especially DNA markers) on the 'Indis' translocation; use this knowledge to better characterize the suspected recombinants; study the expression of the leaf rust resistance of the original and apparently recombined forms; and determine whether the 'Indis' translocation could have been derived from *Thinopyrum distichum*.

2. MATERIALS AND METHODS

The deletion mutant lines were used to physically map three *Thinopyrum* RFLP loci, as well as the *Sr25* and *Sd1* loci. The eight suspected recombinants were also tested for the presence of the various marker loci. The original translocation and apparently recombined *Lr19* segments were incorporated through backcrossing into four common wheat backgrounds. These near-isogenic lines were compared for their resistance to five leaf rust pathotypes.

3. RESULTS AND DISCUSSION

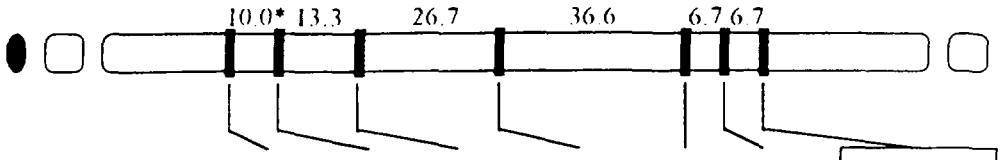
The deletion map results obtained by Marais [5] were integrated with the present data and are presented in Table I [10]. The mutants were ordered to reflect the sequence of marker genes studied. It can be assumed that irradiation induced random breaks on the translocation that resulted in these deletions. The frequencies with which the different mutants were obtained will, therefore, reflect the physical distances (arbitrary units) between loci. While the mutations were mostly caused by terminal deletions, at least two intercalary deletions may have occurred.

Three mutants had partially white endosperm which could suggest the presence of more than one *Y* locus or modified expression of a single locus. Similar phenotypes were recovered amongst the apparently recombined forms [8]. However, in view of the limited data, we chose to treat *Y* as being a single locus. Two mutants expressed stem rust resistance similar to that produced by *Sr25*, yet each has lost *Thinopyrum* chromatin on both sides of *Sr25*. It was assumed that the resistance does not derive from *Sr25* in these mutants.

Physical mapping of *Sd1* was problematic. Due to the complexity of the gametocidal interaction it was not possible to unequivocally distinguish genotypes showing complete, impaired or no gametocidal activity. The *Sd1* gene in translocation heterozygotes causes male and female gametes not carrying it to abort, and the severity of the effect depends on wheat responder genes in the genetic background [6]. Our results confirm the finding of Zhang &

Dvorák [11] that *Sd1* is situated between *Lr19* and the centromere. The absence of the *Xpsr105* and *Xpsr165* loci in three mutants, which have apparently lost all the marker loci, but still cause preferential transmission, normal transmission or self-elimination suggests that *Sd1* is situated proximal to these loci. The segregation distortion effects of 21 of the mutants were clearly different from that observed in crosses involving 'Indis' and 'Inia 66' and self-elimination occurred regularly. This would suggest that at least one further gametocidal gene may be involved. The gene probably has a distal location and was lost in the mutants described. It appears to enhance or to supplement the effect of *Sd1*.

TABLE I. DELETION MAP DATA



Mutant:	*Sd1*	*Xpsr 165*	*Xpsr 105*	*Xpsr 129*	*Lr19*	*Wsp-D1*	*Sr25*	*Y*
87M23-145	*a*	-	-	-	-	-	-	-
87M23-198	*a*	-	-	-	-	-	-	-
89M1-51	*b*	-	-	-	-	-	-	-
87M23-1	*b*	+	-	-	-	-	-	-
89M2-327	*a*	+	-	-	-	-	-	-
87M23-178	*a*	+	+	-	-	-	-	-
87M23-227	*c*	+	+	-	-	-	-	-
87M23-273	*a*	+	+	-	-	-	-	-
89M1-25	*a*	+	+	-	-	-	-	-
89M1-69	*d*	+	+	-	-	-	-	-
89M1-78	*a*	+	+	-	-	-	-	-
87M23-314	*a*	+	+	-	-	-	-	+*f,g*
87M23-3	*a*	+	+	+	-	-	-	-
87M23-27	*d*	+	+	+	-	-	-	-
87M23-108	*d*	+	+	+	-	-	-	-
87M23-115	*a*	+	+	+	-	-	-	-
87M23-118	*a*	+	+	+	-	-	-	-
87M23-128	*d*	+	+	+	-	-	-	-
89M2-416	*b*	+	-*e*	+	-	-	-	+*f,g*
87M23-219	*d*	+	+	+	-	-	+*f*	-
89M2-40	*c*	+	+	+	-	-	-	-
87M23-175	*c*	-*e*	+	+	-	-	-	-
89M2-39	*c*	+	+	+	-	-	-	-
87M23-103	*c*	+	+	+	+	-	+*f*	-
89M2-245	*d*	+	+	+	+	-	-	-
87M23-225	*d*	+	+	+	+	+	-	-
89M2-426	*d*	+	+	+	+	+	-	-
89M1-18	*b*	+	+	+	+	+	+	+*f,g*
87M23-266	*a*	+	+	+	+	+	+	+

*Percentage break points.

[a] Preferential/ normal transmission and self-elimination.

[b] Only self-elimination observed.

[c] Apparently normal segregation ratios, but low seed set.

[d] Only preferential transmission observed.

[a-d] Based on segregation of the mutated translocation in different full sib families.

[e] Intercalary deletion.

[f] Dubious classifications, see text (not considered in the calculation of physical distances).

[g] Intermediate phenotypes suggesting the possibility of more than one locus.

A total of 30 probable break points were recognized and used to estimate the physical distances between genes as indicated in this physical map. Most of the current maps for this segment simply show the markers as occurring within a linkage block. This is the first map depicting the linear sequence and estimated distances for the markers studied on this segment ($7el_1$). This also contradicts the results obtained by Kim et al. [12] that *Xpsr129* is situated distal to *Lr19*. The data confirmed the reported homoeology between the *Lr19* segment and chromosome arm 7DL of wheat.

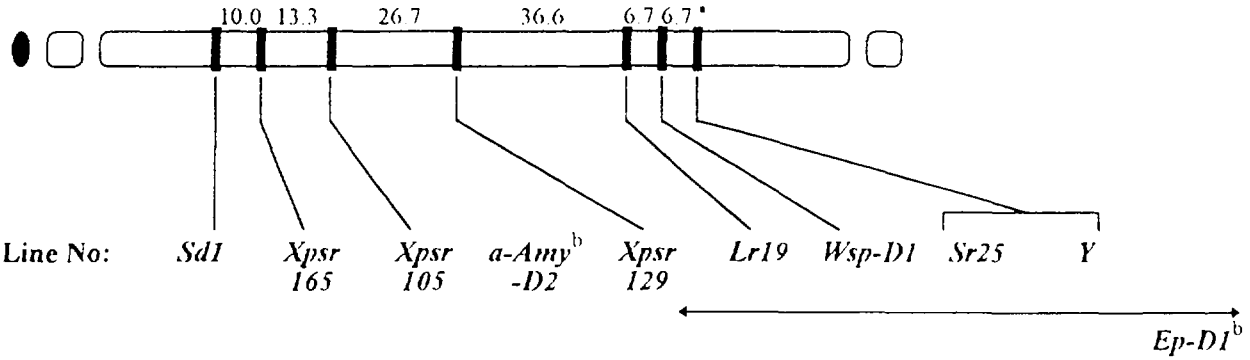
The recombinants were also characterized and rough physical maps of the apparently recombined *Lr19* segments were constructed as illustrated in Table II [13]. The selections were found to fall into two groups: those that retained large *Thinopyrum* segments, produce yellow or partially white endosperm and remained on chromosome 7D; and those that occur on different chromosomes and are not associated with yellow flour pigments. With respect to the first group, more distally located markers should be studied to determine if the distal parts of the three partially white selections have indeed been exchanged with wheat chromatin. The four promising white endosperm recombinants were initially selected on the basis of leaf rust resistance (one pathotype, UVPrt8) and absence of yellow endosperm pigmentation. Three of these selections (87M70-63, 88M22-157, 88M22-184) do not exhibit any of the *Thinopyrum* marker phenotypes except for having strong leaf rust resistance (unknown gene, *Lr?*) against one of five predominant pathotypes (UVPrt8). The latter selections have an altered resistance that could have resulted from the unintentional selection of a 'contaminating' resistance gene in the progeny following the induction of allosyndetic pairing. As will be discussed, the pedigree of 'Indis' is suspect and could have contributed such a gene.

The white endosperm recombinant 88M22-149 retained the *Thinopyrum* alleles of the *Xpsr129* and *Wsp-D1* loci, each of which can serve as a marker of its presence. It has also lost the *Xpsr129* locus on 7BL and regained the 7DL locus which would suggest that it was relocated to chromsome arm 7BL. Both the wheat (*Wsp-B1*) and *Thinopyrum*-derived (*Wsp-D1*) loci are expressed in this recombinant. This would imply that an unequal crossover event occurred which created a duplicated region. This recombinant has probably lost *Sd1* and has a very consistent tendency to self-eliminate. It is not clear from the results whether the strong self-elimination stems from the disruption of a complex of *Sd* genes or whether it is due to (or enhanced by) a disruptive chromosome duplication that occurred during recombination. It is the only true white endosperm recombinant that has retained the *Lr19* resistance and which provides complete resistance to the five pathotypes tested.

It seems likely that the 'Indis' translocation is simply a derivative of the T4 translocation. A comparison of the RFLPs obtained with a ditelosomic $7el_1$-*Thinopyrum ponticum* addition line (W743), 'Indis' and the T4 translocation, confirmed that the 'Indis' and T4 translocations and chromosome $7el_1$ of *T. ponticum* produce identical bands. Fragments corresponding to the polymorphisms in the translocation could not be detected in DNA extracts of two accessions of *Thinopyrum distichum* or its amphiploid with wheat.

The physical map of the *Lr19* segment enabled us to characterize the eight suspected recombinants. This led to the identification of only one true recombinant from the four originally suspected white recombinants. Should the *Lr19* resistance in this recombinant prove to be stable and not to be associated with any negative agronomic effects, it will be the only useful white endosperm recombinant that has been obtained following the use of the *ph* mutants. This illustrates that much effort is needed in tailoring the alien translocations for use in wheat breeding.

TABLE II. POLYMORPHISMS FOR MARKER LOCI IN SELECTIONS SUSPECTED TO HAVE RECOMBINED *LR19* TRANSLOCATED SEGMENTS (T: the *THINOPYRUM* allele; W: the common wheat allele: the alternative form which is a null condition or an allele that could not be positively identified, ?: unknown)



Translocation on chromosome arm 7DL: yellow and partially white endosperm										
87M70-348	?	T	T	-	T	T	T	T	T	-
88M22-42	?	W	W	W	T	T	T	T	T[c]	-
88M22-98	?	T	T	-	T	T	T	T	T[c]	-
88M22-103	?	T	T	-	T	T	T	T	T[c]	-
Unmodified	T	T	T	-	T	T	T	T	T	-
Resistance not associated with chromosome 7D: white endosperm										
87M70-63	-	W	W	W	W	*Lr?*[f]	W	-	-	W
88M22-157	-	W	W	W	W	*Lr?*[f]	W	-	-	W
88M22-184	-	W	W	W	W	*Lr?*[f]	W	-	-	W
88M22-149	-	W	W	W	T[d,e]	T	T[d]	-	-	W

[a] Physical deletion map constructed by Prins et al. 1996a

[b] Mapped within this region by Chao et al. 1989

[c] Partially white recombinants (see text)

[d] Retained the *Thinopyrum* locus and regained the wheat 7DL locus

[e] Lost the wheat 7BL locus

[f] Unknown resistance gene (*Lr?*)

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