Molecular characterization and further shortening of recombinant forms of the *Lr19* translocation

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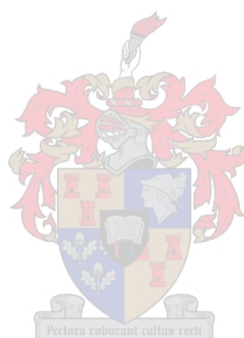
DECLARATION:

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

Signature: .... Date: ....



ABSTRACT

The *Lr19* translocation is associated with deleterious agronomic effects and as a result modified forms of the translocation have been derived by different researchers in an attempt to remove the genes responsible. The recombined forms include a primary recombinant, 'CS'-*Lr19*-149, and four secondary recombinants derived from it, i.e. 'CS'-*Lr19*-149-299, -252, -462 and –478. 'CS'-*Lr19*-149-299 is the proximally shortest recombinant, whereas 'CS'-*Lr19*-149-478 is the distally shortest recombinant. One of the aims of this study was to identify putative, tertiary double recombinants in the test cross 00M96 in which homologous recombination was induced between the latter two translocations in order to derive a still shorter translocation in which both translocation breakpoints occur. Testcross progeny were screened for double recombinants making use of a CAPS marker located proximally of *Lr19*, and a water soluble protein (WSP) marker located distally of *Lr19*. The CAPS marker is specific for the *Thinopyrum* allele, *Xpsr129-7el*$_1$, and is absent in 'CS'-*Lr19*-149-299, whilst the *Thinopyrum*-derived *Wsp-D1c* marker locus is absent in 'CS'-*Lr19*-149-478. Seventeen putative double recombinants were recovered.

An attempt was made to further characterize the primary and secondary recombinants and selected tertiary double recombinants with new and previously mapped molecular markers. The markers comprised six RFLP, 12 AFLP, seven microsatellite, an endopeptidase (*Ep-D1a*), and three STS loci. Markers which have not been mapped previously, were physically mapped using seven deletion mutants of the *Lr19* translocation (Marais, 1992). The results were integrated with existing markers on a deletion map constructed by Groenewald (2001) as well as results obtained during characterization of the various *Lr19* recombinants. Four proximal AFLP markers (27a, 137c, 138a, 126a) and two distal AFLP markers (126c and 7a) did not produce *Thinopyrum*-specific products in *Lr19*-149-299 and 'CS'-*Lr19*-149-478, respectively. None of the six *Thinopyrum*-specific AFLP fragments were amplified in the tertiary double recombinants either which confirmed their status. Four RFLP loci (*XcIH81-1-7el*$_1$, *Xwg380-7el*$_1$, *Xpsr129-7el*$_1$ and *XksuE18-7Bb*) mapped proximally to the 'CS'-*Lr19*-149-299 breakpoint. Another RFLP marker, *Xpsr687*, was mapped distally of the *Wsp-D1c* locus and was therefore not useful in characterizing the recombinants. A total of 12 molecular markers mapped in the area of *Thinopyrum* chromatin that remains in the tertiary double recombinants. These markers include six AFLP markers (7b, 27b, 54a, 56a, 137a, 138c), four microsatellite markers (*Xgwm146*, *Xgwm611*, *Xgwm577* and *Xwmc276*), one RFLP locus (*XksuE18-7Ba*), one STS marker (STSLr19$_{130}$) and the *Ep-B1* locus. The latter markers can be used in future attempts to further reduce the translocation.

OPSOMMING

Die *Lr19*-translokasie het gekoppelde nadelige agronomiese effekte en gevolglik het verskeie navorsers gemodifiseerde vorms van die translokasie geproduseer in ŉ poging om die betrokke gene te verwyder. Die gerekombineerde vorms sluit ŉ primêre rekombinant, 'CS'-*Lr19*-149, in asook vier sekondêre rekombinante, 'CS'-*Lr19*-149-299, -252, -462 en –478. 'CS'-*Lr19*-149-299 is die proksimaal korste rekombinant en 'CS'-*Lr19*-149-478 is die distaal kortste rekombinant. ŉ Oogmerk van hierdie studie was om tersiêre dubbel-rekombinante te identifiseer in die toetskruising 00M96. Die doel met hierdie kruising was om homoloë rekombinasie tussen die laasgenoemde sekondêre translokasies te bewerkstellig en ŉ korter translokasie te herwin wat beide translokasie breekpunte bevat. Die toetskruis nageslag is met ŉ CAPS-merker wat proksimaal van *Lr19* geleë is, en ŉ water-oplosbare proteïen (WSP) merker wat distaal van *Lr19* geleë is, getoets vir dubbel-rekombinante. Die CAPS-merker is spesifiek vir die *Thinopyrum*-alleel *Xpsr129-7el*$_1$ en is afwesig in 'CS'-*Lr19*-149-299, terwyl die *Thinopyrum*-verhaalde *Wsp-D1c* merker-lokus afwesig is in 'CS'-*Lr19*-149-478. Sewentien vermoedelike dubbel-rekombinante is herwin.

ŉ Poging is aangewend om die primêre en sekondêre rekombinante en die geselekteerde tersiêre dubbel-rekombinante te karakteriseer met nuwe en voorheen-gekarteerde merkers. Die merkers het ses RFLP, 12 AFLP, sewe mikrosatelliete, ŉ endopeptidase lokus (*Ep-D1a*), en drie STS-lokusse ingesluit. Merkers wat nie voorheen gekarteer is nie, is fisies gekarteer met behulp van sewe *Lr19* delesie-mutante (Marais, 1992). Laasgenoemde resultate asook resultate wat verkry is met die karakterisering van die onderskeie *Lr19*-rekombinante is geïntegreer met bestaande merkers op ŉ delesie-kaart saamgestel deur Groenewald (2001). Vier proksimale AFLP-merkers (27a, 137c, 138a, 126a) en twee distale AFLP-merkers (126c en 7a) het nie *Thinopyrum*-spesifieke produkte in 'CS'-*Lr19*-149-299, 'CS'-*Lr19*-149-478 of die tersiêre dubbel-rekombinante geamplifiseer nie. Die resultaat het bevestig dat hulle wel tersiêre rekombinante is. Vier RFLP-lokusse (*XcIH81-1-7el*$_1$, *Xwg380-7el*$_1$, *Xpsr129-7el*$_1$ en *XksuE18-7Bb*) is proksimaal van die 'CS'-*Lr19*-149-299 breekpunt gekarteer. ŉ Ander RFLP-merker, *Xpsr687*, is distaal van die *Wsp-D1c* lokus geleë en was dus nie van nut in die karakterisering van die rekombinante nie. Twaalf molekulêre merkers karteer in die *Thinopyrum* chromatien wat oorgebly het in die tersiêre dubbel-rekombinante. Hierdie merkers sluit ses AFLP merkers (7b, 27b, 54a, 56a, 137a, 138c), vier mikrosatelliet merkers (*Xgwm146*, *Xgwm611*, *Xgwm577* and *Xwmc276*), een RFLP-lokus (*XksuE18-7Ba*), een STS lokus (STSLr19$_{130}$) en die *Ep-B1* lokus in. Laasgenoemde merkers kan nuttig gebruik word in toekomstige pogings om die translokasie verder te verkort.

For my father, mother and sister

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1. Literature Review

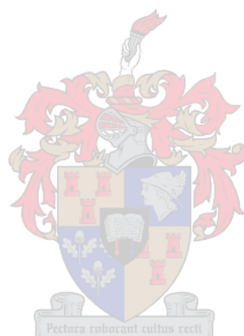
1.1 Introduction

Wheat is one of the most widely grown cereals in the world. It is planted from the borders of the arctic to near the equator in areas ranging in altitude from sea level to 4572 m above. It is therefore adaptable to a range of environmental conditions and as an annual grass it predominates in areas with a Mediterranean-type climate of hot dry summers and cooler wet winters. Wheat grows best on heavy loam and clay soils, although it makes a satisfactory crop on lighter land (Lupton, 1987; Kent and Evers, 1994).

Wheat, compared to other cereals such as rice, maize and barley, is the world's most important crop species with regard to the total land area occupied and annual production. Wheat is grown on an area of over 200 million hectares worldwide (Kent and Evers, 1994). Although there has been little increase in the area sown with wheat from 1981 to 2000, global wheat world production increased from an estimated 210 million tonnes per annum to 600 million tonnes per annum which represents an almost three-fold increase in the world average yield of wheat (Kent and Evers, 1994; Marathée and Gomez-MacPherson, 2001). China is currently the largest wheat-producing nation in the world with 112 million tonnes being produced annually (Singh, 2003). Wheat production in South Africa has greatly increased from the early 1950s at which time only 500 thousand tonnes were produced on average. Since 1990, South African wheat farmers are producing an average of two million tonnes of grain per year on approximately 1.2 million hectares. Since 1930 the yield potential in this country improved by 87 % (van Niekerk, 2001).

Almost 65 % of the 272 million tonnes of wheat that are currently being produced by developing countries, such as those in the Near East/North Africa and East Asia, are produced under irrigation with an average yield of three tonnes per hectare as compared to only 1.8 tonnes per hectare under rainfed conditions. The area under irrigation is expected to increase over the following years and at the same time the irrigated yields should also increase (Marathée and Gomez-MacPherson, 2001). Approximately 20 % of the total wheat production in South Africa is under irrigation. There are currently six major irrigation regions in South Africa, and irrigation farming is expanding into new regions (Barnard, 2003).

Of the total wheat production worldwide, an average of 66.5 % is used for human consumption, 20.2 % for animal feed, 6.7 % for industrial use and 6.6 % for seed (Kent and Evers, 1994; Marathée and Gomez-MacPherson; 2001). Over the period 1977/78 to 1990/91, on average, 19 % of the entire world wheat crop was exported from the producing countries to other countries (Kent and Evers, 1994). Wheat trade averaged 60 million tonnes during 1995-1999. North American exports represented 72 % of the wheat exported to developing countries and it will remain the main exporter for the following years (Braun *et al*., 1998). The average local consumption in South Africa is 2.4 million tonnes per annum (1998-1999) and the country is therefore a net importer of wheat along with other Southern African Development Community (SADC) countries (van Niekerk, 2001).



1.1.1 The genome of bread wheat (*Triticum aestivum* L. em. Thell)

Wheat belongs to the tribe TRITICEAE Dumort (=HORDEAE Benth) of the grass family GRAMINEAE or POACEAE which is comprised of more than 10 000 species, and includes other cultivated cereals such as barley, maize, oats and rice and related wild grass species (Evans and Peacock, 1981; Knott, 1989a). Bread or common wheat (*Triticum aestivum* L. em. Thell) is an allohexaploid species (2n = 6x = 42) which has three genomes designated A, B and D. The A and D genomes are more closely related than A with B or D with B (Naranjo *et al*., 1987). *T. aestivum* originated from hybridization events involving three different diploid progenitors classified in the genera *Triticum* and *Aegilops* (Morris and Sears, 1973; Knott, 1989a; Feldman *et al*., 1997) (Figure 1.1).

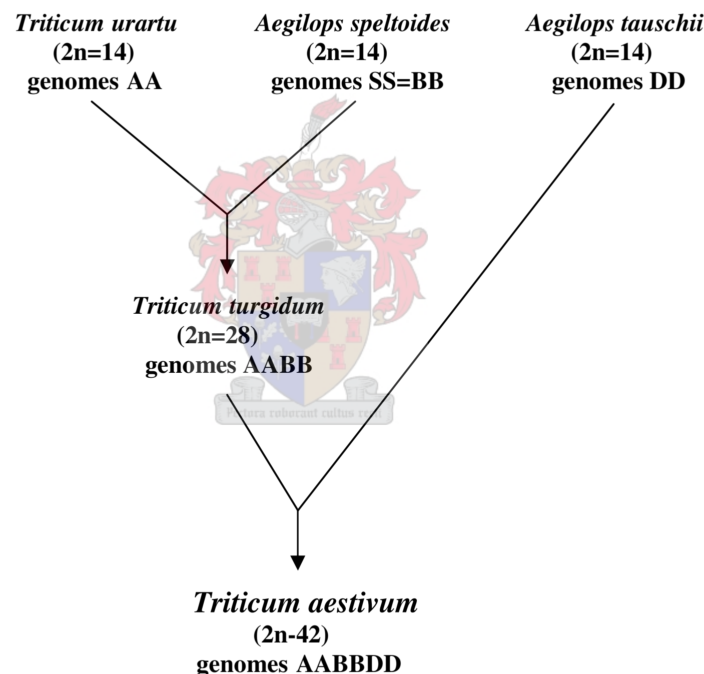


Figure 1.1 (Feldman *et al*., 1997) A diagram explaining the origin of hexaploid wheat, *Triticum aestivum*. Hexaploid wheat originated through hibridization, followed by chromosome doubling, between the tetraploid wheat *T. turgidum* and the diploid species *Ae. tauschii*. *T. turgidum* originated from hibridization, followed by chromosome doubling, between the diploid species *T. urartu* and a form closely related to *Ae. speltoides.*

The 21 pairs of homologous chromosomes fall into seven homoeologous groups, each containing one pair of chromosomes from the A, B and D genomes, respectively (Riley and Chapman, 1958; Morris and Sears, 1973, Feldman *et al.*, 1997). On the basis of specificity and distribution in the three constituent genomes of hexaploid wheat, the DNA sequences of this species can be classified into the following four categories (Liu *et al.*, 1997): (i) non-specific sequences, which map to several or to all chromosomes of the A, B and D genomes; some of these sequences are ubiquitous, present in all eukaryotes while others have a more limited distribution, being confined to the grass family or to the tribe TRITICEAE; (ii) group- or homoeologue-specific sequences, which map to a single pair of homologous chromosomes in each of the three genomes A, B and D; (iii) genome-specific sequences, which map to several or to all chromosomes of only one of the three genomes; and (iv) chromosome-specific sequences, which are those confined to one pair of homologous chromosomes. For group- or homoeologue-specific sequences, the homologous pairs of chromosomes comprise a homoeologous group, i.e. they derived from a common ancestral chromosome.

To prevent intergenomic pairing and ensure proper chromosome segregation and, hence, full fertility and disomic inheritance in the polyploid species, exclusive bivalent pairing of homologous chromosomes is essential (Feldman, 1993; Liu *et al.*, 1997). It was proposed that the diploid-like meiotic behaviour of polyploid wheat has been brought about by two independent systems that complement each other. The first system is based on non-random elimination of DNA sequences soon after formation of the polyploids, resulting in increased differentiation among homoeologous chromosomes. This differentiation provides the physical basis for the diploid-like meiotic behaviour of the polyploids (Feldman *et al.*, 1997; Liu *et al.*, 1998). The second system, involving exclusive bivalent pairing in polyploid wheat, is a genic one, brought about by the genes *Ph1* and *Ph2* (pairing homoeologous), that suppress pairing of homoeologues while allowing homologues to pair regularly (Sears, 1976; Evans and Peacock, 1981; Aragón-Alcaide *et al.*, 1997). *Ph1* and *Ph2* are located on chromosomes 5BL and 3DS, respectively, and *Ph1* is considered as the primary determinant of homoeologous pairing (Riley and Chapman, 1958; Sears, 1976; Naranjo *et al.*, 1987; Gill *et al.*, 1993b). Several studies have indicated that the *Ph1* locus also affects recombination between homologous and homoeologous segments. It was therefore suggested that the *Ph1* locus comprises two genes, the first affecting centromeric structure and hence chromosome alignments, and a second able to distinguish mismatches of sequences in heteroduplexes formed between homologous and homoeologous chromosomes (Dubcovsky *et al.*, 1995; Liu *et al.*, 1996). Genes are also found that promote homoeologous pairing between the constituent ancestral genomes (Sears, 1976; Lupton, 1987).

In the absence of activity of the *Ph1* gene, the homoeologous chromosomes may pair and recombination can occur between homoeologous chromosomes of wheat and related species. Thereby it is possible to transfer chromosome segments, carrying desirable genes such as those conferring leaf rust resistance, to the wheat genome. One method to induce homoeologous pairing in hybrids, is to use aneuploids such as monosomic 5B or nullisomic 5B lines in which chromosome 5B, containing *Ph1*, is deleted. Another method is to mutate the *Ph1* gene itself to create *ph1* mutant lines. This was done by Wall *et al*. (1971) by using a chemical mutagent, ethyl methane sulfonate; and by Sears (1977a) using radiation (X-rays). Homoeologous pairing can also be induced by introducing a gene, dominant or epistatic to *Ph*, from certain diploid species e.g. *Ae. speltoides* or *Ae*. *mutica. Ae. speltoides*, identified as the potential donor of the B genome of bread wheat (Figure 1.1), carries a gene(s) that suppresses the effect of *Ph1* and *Ph2* in wheat/*Ae. speltoides* F_1 hybrids (Morris and Sears, 1973; Sears, 1976; Evans and Peacock, 1981; Naranjo *et al*., 1987).

The genome of *Triticum aestivum* is one of the largest of the plant genomes, containing 16.5 to 19.5 picograms (pg) of DNA (1C DNA) per haploid nucleus. These amounts are equivalent to approximately 1.6×10^{10} to 1.9×10^{10} base pairs (bp) which is at least five fold that of the human genome (Bennett and Smith, 1976). The haploid complements of the three diploid genomes (A, B and D), occur within the 1C nucleus at a ratio of 1.0: 2: 0.8 (Furata *et al*., 1988). The DNA amount per cell is approximately 36.2 pg (Bonjean and Angus, 2001). Compared to other plant species, it is more than 90 times larger than the *Arabidopsis thaliana* genome (0.02×10^{10} bp), 40 times that of rice (*Oryza sativa*; 0.04×10^{10} bp), six times that of maize (*Zea mays*; 0.27×10^{10} bp) and about three times that of barley (*Hordeum vulgare*; 0.5 x 1010 bp) (Gupta *et al*., 1999). The average wheat chromosome is 10 µm in length with a size of 810 Mb (Gupta *et al*., 1999; Moore *et al*., 1995). The haploid wheat chromosome complement is 235 µM in length (Gill *et al*., 1991). The ratio for total chromatin length per nucleus in diploid, tetraploid and hexaploid species is 1: 1.5: 2 of which the differences can be attributed to chromatin elimination in polyploid wheats in the course of evolution. The diploid group has the most equal-armed chromosomes and the smallest range between the longest and shortest chromosomes, whilst the hexaploid varieties of *T. aestivum* shows the most asymmetry both in arm ratios and range in length (Morris and Sears, 1973). The average wheat chromosome is 25 times larger than the average rice chromosome (Moore *et al*., 1995). Thus, three wheat chromosomes are equal to the haploid maize genome and one-half of an average wheat chromosome equals a haploid rice genome (Gill and Gill, 1994; Gupta *et al*., 1999). The wheat chloroplast and mitochondrial genomes are approximately 135 kb and 430 kb, respectively, and both have been characterized (reviewed by May and Appels, 1987).

Due to the polyploid nature and large genome size of wheat, structural and numerical chromosome aberrations can be tolerated much better than in diploids. The lack of a chromosome within a group of homoeologous chromosomes can be compensated for by other pairs (Morris and Sears, 1973). This feature has allowed for the isolation and identification of a range of aneuploid lines of wheat in which the chromosome number is increased or decreased. Aneuploid genetic stocks such as monosomics, in which only one chromosome of a homologous pair is present (Sears, 1954); nulli-tetrasomics, in which a pair of chromosomes is deleted and replaced by an extra chromosome pair from the homoeologous group (Sears, 1966); and ditelosomics, in which long or short arms of a homologous chromosome pair are absent (Sears and Sears, 1978), have been developed by exploiting the compensating ability of homoeologous chromosomes. Nulli-tetrasomic and ditelosomic lines have been obtained for all 21 chromosomes of wheat in the variety 'Chinese Spring'.

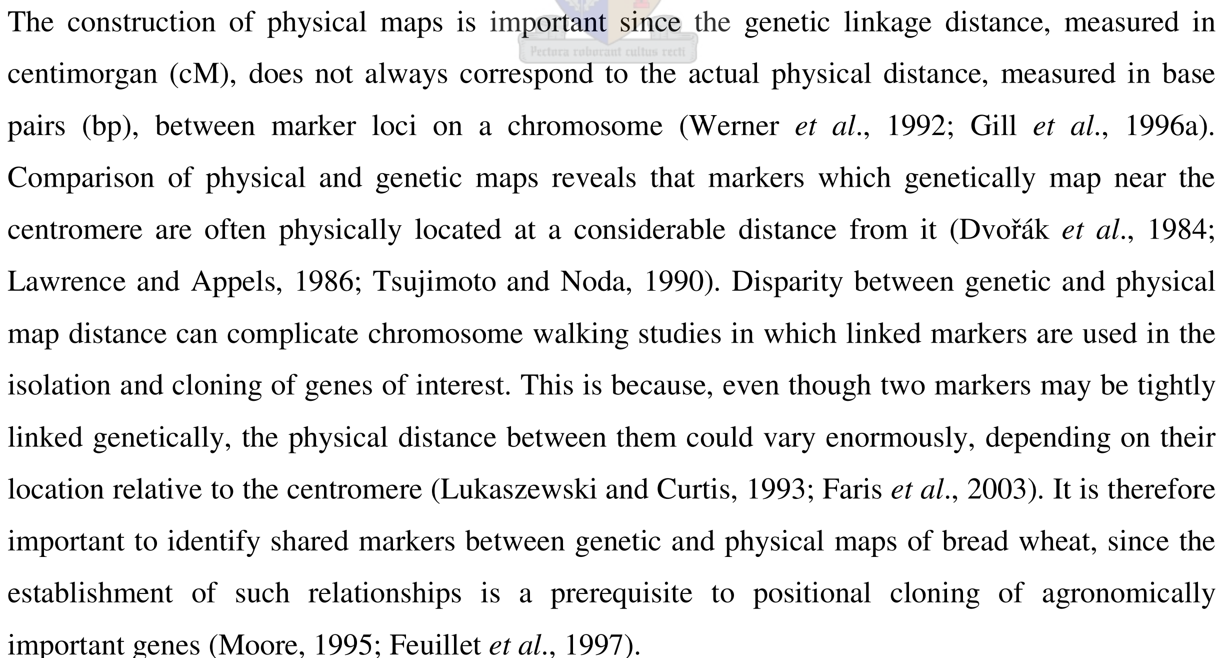
The large genome size of wheat is associated with a high amount of repetitive DNA. The wheat genome consists of more than 80 % middle and highly repeated DNA sequences and the three genomes share approximately 30 % sequence homology (Flavell *et al*., 1977; Moore *et al*., 1993). In the nuclear DNA of wheat, (AT)n sequences are the most abundant (Wang *et al*., 1994). Of the short tandem repeats (STRs) found in wheat, 48 % contains G-C basepairs. More than 30 % of the cytosine residues in the nuclear DNA of wheat are highly methylated compared to 1-7 % in animals (Shapiro, 1976; Wang *et al*., 1994). Eighty-two % of CpG dinucleotides are methylated in wheat DNA (Gruenbaum *et al*., 1981). One class of repetitive DNA, those present in tandem arrays of thousands of copies, is rDNA. The rRNA genes have been found at eight loci of 45S rDNA (1BS, 6BS, 5DS, 1AS, 7DL, 1BL, 3DS, 5DS) (Dubcovsky and Dvořák, 1995) and three of 5S rDNA (1B, 1D, 5B) (Scoles *et al*., 1988; Mukai *et al*., 1990). Many of the repetitive sequences belong to families of retrotransposons. A family of five retroelements WIS-2, WIRE-1, BIS-1, WIS-1 and Hi-10 has been identified of which BIS-1 represents a major component, accounting for more than five % of the wheat genome. These sequences are absent at centromeres, telomeres and nucleolar organising regions of wheat chromosomes (Flavell *et al*., 1987; Moore *et al*., 1991a; 1991b; 1993).

According to current studies, the wheat plant phenotype is governed by the effects of approximately 30 000 genes (Moore, 1995) with a density of approximately one gene every 5-50 kb (Keller and Feuillet, 2000). The genes represent less than three % of the wheat genome (Gill *et al*., 1996a). More than 85 % of the wheat genes are present in clusters, interspersed by blocks of nontranscribing, repetitive sequences visualized as regions of low gene density (Gill *et al*., 1996a; 1996b). There are four to six clusters on each chromosome that span only 5-10 % of the

chromosome. Distal chromosome regions possess more genes compared to proximal regions. About 30 % of the chromosomal region around the centromere is devoid of genes. More gene clusters occur towards the telomeric regions (Gill *et al*., 1996a; 1996b).

A strong correlation is observed between the distribution of genes and recombination in that recombination predominately occurs in gene rich regions (Gill *et al*., 1996a, 1996b; Gill and Gill, 1998). Therefore, recombination is not distributed evenly along the chromosome arms. Instead, it appears to be concentrated in distal chromosome segments and is infrequent or absent in the proximal segments (Dvořák and Appels, 1986; Curtis and Lukaszewski, 1991). There is a significant difference between the distribution of recombination in physically short and physically long arms. In physically short arms, recombination is almost exclusively concentrated in distal segments, whilst in physically long arms interstitial recombination is observed. Such a pattern of recombination, skewed toward terminal segments of chromosomes, is probably the result of telomeric pairing initiation and strong positive chiasma interference (Curtis *et al*., 1991; Lukaszewski and Curtis, 1993).

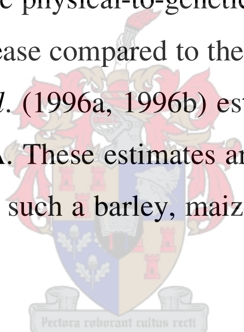
1.2 Physical mapping in wheat



The construction of physical maps is important since the genetic linkage distance, measured in centimorgan (cM), does not always correspond to the actual physical distance, measured in base pairs (bp), between marker loci on a chromosome (Werner *et al*., 1992; Gill *et al*., 1996a). Comparison of physical and genetic maps reveals that markers which genetically map near the centromere are often physically located at a considerable distance from it (Dvořák *et al*., 1984; Lawrence and Appels, 1986; Tsujimoto and Noda, 1990). Disparity between genetic and physical map distance can complicate chromosome walking studies in which linked markers are used in the isolation and cloning of genes of interest. This is because, even though two markers may be tightly linked genetically, the physical distance between them could vary enormously, depending on their location relative to the centromere (Lukaszewski and Curtis, 1993; Faris *et al*., 2003). It is therefore important to identify shared markers between genetic and physical maps of bread wheat, since the establishment of such relationships is a prerequisite to positional cloning of agronomically important genes (Moore, 1995; Feuillet *et al*., 1997).

The unit of recombination, used for measuring genetic distance, might represent anything between 10 or 100,000 kb of DNA in a specific region of the wheat genome (Werner *et al*., 1992).

According to Lukaszewski and Curtis (1993), the number of DNA base pairs that correspond to one cM may vary in wheat from approximately 1.53 Mb in the distal chromosome regions to 234 Mb in regions adjacent to the centromere. This can be ascribed to the non-random distribution of recombination along the chromosome length (Dvořák and Chen, 1984; Werner *et al*., 1992; Kota *et al*., 1993; Gill *et al*., 1996a, 1996b). Recombination hot spots more frequently occur close to the telomeres than near the centromeres. This is presumably because recombination occurs close to or even within genes (Gill and Gill, 1998; Sourdille *et al*., 2004), and as gene-rich regions or gene clusters are more numerous near the telomeres, more recombination occurs in these regions (Gill *et al*., 1996a, 1996b). Also, the gene-rich regions are expected to be highly decondensed which makes them more accessible to recombination factors compared to proximal heterochromatic regions containing highly repetitive sequences (Faris *et al*., 2000). Each chromosome arm possesses two to six gene-rich recombination hot spots (Gill *et al*., 1996a, 1996b; Weng *et al*., 2000; Sandhu *et al*., 2001). One such hot spot on chromosome 5B was evaluated in detail by saturating it with molecular markers and assessing recombination within a physical segment that accounted for four % of the arm (Faris *et al*., 2000). Estimates of the physical-to-genetic distance ratio within the hot spot were less than 200 kb per cM, a 22-fold increase compared to the average genome-wide estimate of 3000 kb per cM (Yan *et al*., 2003). Gill *et al*. (1996a, 1996b) estimated that one cM in the gene cluster regions translates to 55-120 kb of DNA. These estimates are comparable to similar estimates from other crop plants with smaller genomes such a barley, maize, oat, rice and tomato (Yu *et al*., 1996; Gill and Gill, 1998; Keller *et al*., 2001).


Pectora roborant cultus recti

There is a significant difference between the distribution of recombination in physically short and physically long chromosome arms, in that recombination is almost exclusively concentrated in distal segments in physically short arms whilst in physically long arms interstitial and proximal recombination, although very low, are observed (Lukaszewski and Curtis, 1993). Differences in the recombination frequencies can be observed in the proximal and distal chromosomal regions of wheat which can have a significant effect on the genetic map distance and therefore alter the physical-to-genetic distance ratio (Lukaszewski and Curtis, 1993; Faris *et al*., 2000; Sourdille *et al*., 2004). In studies of common wheat x *T. dicoccoides* and durum wheat x *T. dicoccoides* populations Faris *et al*. (2000) found that recombination was suppressed in a gene-rich region on chromosome 5B. Double crossover events can also be observed for certain markers or putative genes which suggests that, in wheat microrecombination, hot spots may exist within the recombination hot spots, observed at the level of whole chromosome linkage maps (Faris *et al*., 2003). In yeast, intragenic recombination frequencies were shown to correlate with specific short DNA sequences required for recombination hot spot activities (Smith, 1994). The activity of such sequences depends on binding-

specific transcription factors and/or to chromatin structure that cause hypersensitivity to nucleases (Fox *et al*., 1997; Timmermans *et al*., 1997). Occurrence of abnormal hot spots or lack of recombination may result from dysfunction of transcription factors or from modification of the chromatin structure in these regions. Higher resolution mapping and eventual partial sequencing of these regions will provide further insight to these events (Faris *et al*., 2003; Sourdille *et al*., 2004).

Physical maps can be constructed by various methods which can be categorised as either cytogenetically based or molecularly based (Delanay *et al*., 1995a). Cytogenetically based methods include chromosome banding, *in situ* hybridization and the use of aneuploid genetic stocks (Delaney *et al*., 1995a). Molecularly based methods include the cloning of DNA fragments into contigs, cosmids, yeast artificial chromosome (YAC) libraries (Schlessinger, 1990; Stuber *et al*., 1999) and bacterial artificial chromosome (BAC) libraries (Shizuya *et al*., 1992; Moullet *et al*., 1999; Lijavetzky *et al*., 1999), restriction mapping using rare cutting restriction endonucleases (Cheung *et al*., 1991), specialised DNA separation procedures such as pulse-field gel electrophoresis (PFGE) (Cantor *et al*., 1998; Moullet *et al*., 1999; Yan *et al*., 2003) and ultimately DNA sequence analysis (Wilson *et al*., 1990; Stuber *et al*., 1999).

Identification of particular wheat chromosomes is practically impossible with traditional chromosome preparations. Wheat metaphase chromosomes are approximately 5.6 µm long (Sears, 1954) and are therefore very suitable for cytological analysis, especially by chromosome banding methods. Two staining techniques, C- and N-banding, are used in wheat and reveal alternating dark (heterochromatin) and light (euchromatin) staining regions on chromosomes. The dark C-bands reveal all classes of heterochromatin, whereas N-banding only reveals heterochromatin containing polypyrimidine DNA sequences (Gill, 1987; Gill *et al*., 1991a). Application of chromosome banding techniques, especially Giemsa C-banding, reveal chromosome polymorphism within homoeologous groups and it is therefore possible to identify each wheat chromosome by its specific banding pattern with the standard banded karyotype based on Chinese Spring, which is the model cultivar for cytogenetic analysis in common wheat (Gill and Kimber, 1974; Gill, 1987; Naranjo, 1990; Gill, 1993). The C-banding method is also useful for efficient screening of chromosome aberrations such as wheat-alien translocations and especially deletions, also those induced by mutagenic treatment (Friebe *et al*., 1991; Friebe *et al*., 1994; Hohmann *et al*., 1994; Yamamori, 1994). Polymorphism for chromosomal banding patterns can be used in cytological studies of recombination in much the same way as genetic markers have been used in conventional linkage studies. The advantage of these banding patterns is that genetic and physical maps can be integrated immediately and, in species where allelic variation is minimal and gene mutations scarce, the total

number of available markers are increased significantly. In wheat, chromosomes of the B genome provide the best opportunity for a detailed study of the physical distribution of recombination because they are more heavily banded than the A and D genome chromosomes (Lukaszewski and Curtis, 1993).

Especially useful for chromosome identification and the detection of structural variation is the method of *in situ* hybridization (ISH) with specific DNA sequences (reviewed by Gill, 1995). The isolated DNA sequences are used as hybridization probes on chromosomes mounted on microscope slides (*in situ*), allowing for a detailed molecular cytogenetic analysis of the wheat genome. The probes can be labelled with radioisotopes (Appels *et al*., 1980; Hutchinson and Lonsdale, 1982) or biotin (Rayburn and Gill, 1985; Zhang and Dvořák, 1990). Different versions of the ISH protocol include FISH (fluorescence ISH – Leitch *et al*., 1991; Logojan and Molnar-Lang, 2000) and GISH (genomic ISH – Pinkel *et al*., 1986; King *et al*., 1993; Hohmann *et al*., 1996). FISH uses region-specific probes such as centromeric or chromosome specific probes to detect specific sequences on a chromosome (Logojan and Molnar-Lang, 2000). GISH uses labelled total DNA from one parent or species as probe on the unlabelled chromosomes of the target parent or species, allowing for the identification of alien chromatin present in the unlabelled chromosomes (Hohmann *et al*., 1996; Chen *et al*., 1998; Aghaee-Sarbarzeh *et al*., 2001). The repetitive sequence pSc119.2 isolated from rye was used as a probe for ISH to wheat chromosomes and allowed the identification of all chromosomes of the B genome and several pairs of chromosomes in the A and D genomes (Mukai *et al*., 1993). Distribution of simple-sequence repeats (SSRs) and their *in situ* hybridization pattern can also be used for the detection of alien chromatin and chromosome rearrangement (Cuadrado and Schwarzacher, 1998). Sequential C-banding and ISH can be used for both chromosome identification and detection of targeted sites (Cuadrado *et al*., 1996; Aghaee-Sarbarzeh *et al*., 2001). However, *in situ* hybridization techniques are more effective or successful in detecting and characterising small alien chromatin transfers in which C-bands are absent, especially when they replace unbanded chromosome regions in the wheat complement (Hohmann *et al*., 1996).

1.2.1 Deletion mapping

Aneuploid genetic stocks of wheat, in which whole chromosomes, chromosome-arms or chromosome-arm segments are missing, greatly facilitates the physical mapping of phenotypic and molecular markers in wheat. Monosomic and telosomic lines (Sears, 1954) have been used to identify chromosomes carrying particular genes and to map them relative to the centromere. Nulli-tetrasomic (Sears, 1966) and ditelosomic (Sears and Sears, 1978) lines are widely employed to assign molecular markers such as restriction fragment length polymorphisms (RFLPs, Anderson *et al*., 1992; Devey and Hart, 1993) and microsatellites (Plascke *et al*., 1995; Röder *et al*., 1995) to specific chromosomes and chromosome arms. A higher level of resolution can be obtained by using deletion mutant lines (Werner *et al*., 1992; Gill, 1993; Endo and Gill, 1996; Sourdille *et al*., 2004). These lines have deletions of chromosome segments, usually from the telomere, and can be used to assign markers to particular regions of a chromosome. Once the arm location is known, the deletion lines can be screened to identify the deletions where the marker is missing. As a result of the genetic triplication in hexaploid wheat, multiple fragments detected in southern blots by probe hybridization are most efficiently mapped by deletion stocks (Gill and Gill, 1998). There is no need for polymorphism as DNA fragments are scored for presence or absence. Deletion breakpoints may serve as additional chromosome markers for constructing physical maps of wheat (Werner *et al*., 1992; Gill *et al*., 1996a). Deletion lines showing different morphological, physiological and biochemical traits are powerful tools for identification of chromosome regions for molecular tagging and may provide the starting points for gene cloning (Endo and Gill, 1996; Sutka *et al*., 1999; Faris *et al*., 2002).

Deletion mutants can occur spontaneously (Payne *et al*., 1984; Snape *et al*., 1985; Kota and Dvořák, 1986) or can be induced by gamma irradiation (Pienaar and Van Niekerk, 1973; Marais, 1992a), fast neutron bombardment (Okubara *et al*., 1994; Faris *et al*., 2003) or gametocidal genes (Endo, 1988, 1990; Endo and Gill, 1996). Marais (1992a) produced deletion mutants in a translocated chromosome segment derived from *Thinopyrum ponticum* by making use of gamma irradiation. The translocated segment, containing the leaf rust resistance gene *Lr19*, was characterized with this set of deletion mutants using isozymes and a water soluble protein (WSP) marker (Marais, 1992a), RFLPs (Prins and Marais, 1998), AFLPs (Groenewald, 2001) and a sequence tagged site (STS) marker (Prins *et al*., 2001). Deletion mutants were produced by Faris *et al.* (2003) using fast neutrons to evaluate the M_2 for spike morphology and to construct a physical contig spanning the major domestication locus, *Q*, in wheat.

Most wheat deletion lines were produced with the use of gametocidal chromosomes or genes that cause breakage in wheat chromosomes (Endo, 1988, 1990; Endo and Gill, 1996). An alien chromosome from *Aegilops cylindrica*, *A. triuncialis* or *A. speltoides* can induce chromosome mutations in the wheat genome, including deletions (Tsujimoto and Noda, 1988; Marais and Pretorius, 1996). More than 436 deletion stocks of this type are available and the breakpoints, with some exceptions, are more or less randomly distributed along the length of the 21 chromosomes of wheat (cv Chinese Spring; Endo, 1988; Werner *et al*., 1992; Gill *et al*., 1993a; Endo and Gill, 1996; Gill and Gill, 1998). They provide, on average, 60 breakpoints for a consensus homoeologous group physical map (Gill and Gill, 1998). The deletion lines are essential in the construction of cytologically based physical maps (CBPMs) in bread wheat (Werner *et al*., 1992; Gill *et al*., 1996a, 1996b). Mapping can be done in either hemizygous or homozygous chromosome conditions and most genetic markers can be used without the necessity of polymorphism (Werner *et al*., 1992).

A CBPM consists of an ideogram of a chromosome depicting C-banding patterns, deletion breakpoints, and molecular markers allocated to each deletion interval (Werner *et al*., 1992). A CBPM can be aligned with a genetic linkage map for the same chromosome to compare marker distribution and variations in cM/bp along the chromosome (Delaney *et al*., 1995a). Individual physical maps of homoeologous chromosomes can be combined to generate a consensus physical map (Gill *et al*., 1993a). The consensus physical map can also be compared with genetic linkage maps of wheat and its wild or cultivated related species (Gill *et al*., 1996a). Apart from the possibility of map-based cloning, wheat CBPMs have provided important insights into cereal chromosome structure and distribution of recombination and markers along the chromosomes (reviewed by Gill and Gill, 1994; Delaney *et al*., 1995a, 1995b; Gill *et al*., 1996a, 1996b).

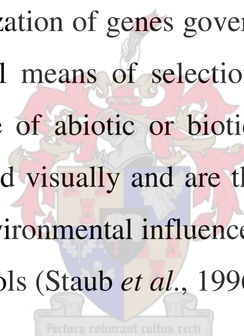
The deletion lines, obtained by the gametocidal gene chromosome breakage system, were used to physically map RFLP probes onto sub-arm chromosomal regions of homoeologous group 1 (Kota *et al*., 1993; Gill *et al*., 1996a; Tsujimoto *et al*., 1999; Ma *et al*., 2001), 2 (Delaney *et al*., 1995a), 3 (Delaney *et al*., 1995b; Ma *et al*., 2001), 4 (Mickelson-Young *et al*., 1995), 5 (Gill *et al*., 1996b), 6 (Gill *et al*., 1993a; Weng *et al*., 2000) and 7 (Werner *et al*., 1992; Hohmann *et al*., 1995a, 1995b). Röder *et al*. (1998b) physically mapped a set of microsatellites on the homoeologous group two chromosomes. Sourdille *et al*. (2004) characterized a set of 84 deletion lines covering the 21 chromosomes of wheat using 725 microsatellites, mapped to chromosome deletion bins. The mapped microsatellite loci could be useful not only for deletion stock verifications, but also for allocating associated quantitive trait loci (QTL) to deletion bins where numerous expressed sequence tags occur (ESTs) that could be potential candidate genes. Zhang *et al*. (2000) saturated

the satellite region on the short arm of chromosome 1B with amplified fragment length polymorphism (AFLP) markers.

The deletion mapping strategy has also allowed the delineation of chromosomal regions for important genes like *Ph1* on 5BL (Gill *et al*., 1993b), the vernalization gene (*Vrn1*) on 5AL (Sarma *et al*., 1998; Sutka *et al*., 1999), the frost resistance gene (*Fr1*), also on 5Al (Sutka *et al*., 1999), *Ha* on 5DS (Sarma *et al*., 2000) and *Q* on 5AL (Kojima *et al*., 2000; Faris *et al*., 2002). A subset of deletion lines was also used by Qi *et al*. (2004) to construct a chromosome bin map of the 21 chromosomes of bread wheat using 7104 EST loci (Southern hybridization).

1.3 Genetic mapping

With conventional plant breeding methods, plants are selected directly based on their phenotype without the identification or characterization of genes governing the plants' phenotypic expressions (Staub *et al*., 1996). These traditional means of selection may be hampered by environmental conditions. For example, the presence of abiotic or biotic stress symptoms resulting from frost damage or disease resistance are scored visually and are therefore not so reliable (Koebner *et al*., 2001; Langridge *et al*., 2001). Such environmental influences can be eliminated or reduced by using genetic markers as indirect selection tools (Staub *et al*., 1996).



Markers used in plant breeding programs are broadly classified into four categories: morphological, linked disease resistance genes, biochemical, and DNA-based or molecular markers (Staub *et al*., 1996; Eagles *et al*., 2001). Morphological markers produce phenotypes which can be readily identified, but which are not usually of direct economic importance. Their value is due to close linkage with economically important traits (Eagles *et al*., 2001). One such morphological marker that has been used for breeding for resistance to rust is leaf-tip necrosis, conditioned by the *Ltn* gene. This gene is closely linked with *Lr34*, a gene conferring resistance to leaf rust, and *Yr18*, a gene conferring resistance to stripe rust. Leaf-tip necrosis, along with leaf rust development pattern, is used to predict the presence of *Lr34/Yr18* in advanced breeding lines (Singh, 1992). Although codominant morphological markers have been useful as predictors of genetic response to selection, they can be influenced by environmental and genetic factors or modifying genes and its usefulness may therefore be limited as a genetic marker (Staub *et al*., 1996). In the case of closely linked disease resistance genes such as *Lr19*/*Sr25* (Marais *et al*., 1988) or *Sr31*/*Lr26*/*Yr9*/*Pm8* (Lukaszewski, 2000), resistance to more than one disease are selected indirectly while selection is

performed for another (McIntosch, 1998). Biochemical markers produce an enzyme or storage protein, which can be identified by biochemical assays. As with morphological markers, their value can be due to close linkage with a trait of economic importance (Koebner *et al*., 1987). However, in some cases these proteins are the determinants of the economic trait, for example, glutenin proteins and dough properties (Gras *et al*., 2001). Because recombination does not occur between these markers and the trait of interest, they have in some cases been called 'perfect' or 'diagnostic' markers (Eagles *et al*., 2001). *In situ* hybridization, morphological and isozyme markers are also convenient tools for identifying addition lines or direct chromosome introgressions. However, these tools have limited use in tracking small segments, such as that of barley (*Hordeum chilense*) chromatin in a wheat background because they either lack resolution or mark only a small fraction of the alien genome (Hernández *et al*., 1999).

The genome of wheat, like other living organisms, contains a vast amount of DNA sequence variation, and molecular markers, which are based on DNA polymorphism, are therefore much more abundant than biochemical markers which are based on protein polymorphisms (Langridge *et al*., 2001; Burr, 2001). Furthermore, molecular markers are not influenced by environmental factors, can be scored at all stages of plant growth and are not limited by tissue specificity (Demeke *et al*., 1992; Gupta *et al*., 1999).

One of the most important applications of molecular markers is the construction of detailed genetic maps. In bread wheat, molecular markers that have been used for mapping can be broadly classified into three groups: (1) those having triplicate homoeoloci, one locus each on three chromosomes of a homoeologous group, (2) those having multiple loci, but not on homoeologous chromosomes, and (3) those which are chromosome specific, each with a single locus (Gupta *et al*., 1999; Gupta *et al*., 2002).

Genetic maps are used for several purposes such as allowing the localisation of genes of interest; facilitating trait tagging, marker-assisted breeding and map-based cloning, and providing the framework for understanding the biological basis of complex traits (Kam-Morgan *et al*., 1989; Chalmers *et al*., 2001; Koebner *et al*., 2001). In marker-assisted selection (MAS), DNA-based markers are used to identify molecular or DNA sequence differences among genes determining traits of interest, or of DNA segments linked to genes determining the traits of interest. The target trait or gene could be a major locus or a quantitative trait locus (QTL) (William *et al*., 1997; Seyfarth *et al*., 1999; Feuillet *et al*., 2003). In wheat, molecular markers have been identified for around 40 traits of economic importance which cover many aspects of wheat selection (Gupta *et al*.,

1999). Knowing the location of these genes or traits and specific alleles offers the possibility to apply MAS or backcross assisted selection (BCAS) to wheat. For breeders, the two strategies permit plant selection at the seedling stage and in early generations. Unwanted alleles can therefore be eliminated or greatly reduced early in selection, focusing the selection in the field on reduced numbers of mature plants (Staub *et al*., 1996; Stuber *et al*., 1999; Koebner *et al*., 2001). BCAS allows the selection of plants carrying a favourable recessive allele in each generation, limiting the need for a progeny test which is the norm in traditional backcrossing (Stuber *et al*., 1999). MAS can be very effective in selecting and breeding resistant varieties by combining or pyramiding several resistance genes to a single pathogen in one variety and creating the possibility of selecting for resistant genotypes in the absence of the pathogen (McIntosch, 1998; Langridge *et al*., 2001).

The cytogenetic ladder mapping (CLM) strategy has shown that a majority of wheat genes are present as clusters and that small chromosome regions encompassing these gene clusters are highly recombinogenic. These chromosome regions are suitable for molecular analysis comparable to those possible in other crops with small genomes such as rice and sorghum (Gill and Gill, 1994; Gill *et al*., 1996a). Studies on the distribution of the sites of recombination along the cereal chromosomes using physical markers all show that recombination is predominantly in the distal regions (Dvořák and Chen, 1984; Lawrence and Appels, 1986; Delaney *et al*., 1995a; Gill *et al*., 1996). As the result of reduced recombination towards the centromere, markers physically located in these regions are clustered on recombination-based or genetic maps when compared to those located in the distal chromosome regions (Lukaszewski and Curtis, 1993; Delaney *et al*., 1995a).

Wheat is more difficult to map than other crop species as a result of its very large genome, polyploidy and complex organisation consisting of unique or low-copy sequences surrounded by regions of highly repetitive DNA (Moullet *et al*., 1999; Langridge *et al*., 2001). Problems such as locus duplication, or triplication as in hexaploid bread wheat, lack of allelic variation, and epistatic effects are associated with polyploidy (Kan-Morgan *et al*., 1989). There is generally a low level of polymorphism in wheat relative to other cereal species and this means that a larger number of markers usually needs to be screened than is the case for rice, maize, or barley (Chao *et al*., 1989; Langridge *et al*., 2001). Further, the level of polymorphism is not consistent across the genomes or crosses. The D genome is more highly conserved between varieties and is, consequently, substantially more difficult to map. If random markers are being used in a mapping strategy, then the maps of the D genome tend to have the poorest coverage (Chao *et al*., 1989; Gill *et al*., 1991b; Chalmers *et al*., 2001; Sourdille *et al*., 2004). To overcome this lack of polymorphism, ancestors or donors of the D genome eg. *Triticum taushii* (Gill *et al*., 1991b; Boyko *et al*., 1999), or of the A

genome e.g. *Triticum monococcum* (Pal *et al*., 1998), along with the cytogenetic stocks of the variety, Chinese Spring (Huang *et al*., 2000) are employed.

Syntenic maps in which the genetic maps of different grass species are aligned and compared, can also be used to deduce chromosome organization in species, such as wheat, with large genomes (Devos and Gale, 1997). Comparative genomic analysis at the genetic map level has shown extensive conservation of the gene order between the different grass genomes in many chromosomal regions (Keller *et al*., 2001). It has been shown in these types of studies that markers that have been mapped on rice chromosomes, are maintained in the same order as on barley and wheat chromosomes (Moore and Gustafson, 1997). It is therefore possible to map the available molecular markers of one species such as rice, in another related species, such as wheat, to regions in the genome which have little or no markers (Gale and Devos, 1998).

Wheat molecular markers can be broadly classified in three groups (Gupta *et al*., 1999):

(i) Hybridization-based DNA markers such as restriction fragment length polymorphism (RFLPs) and oligonucleotide fingerprinting;

(ii) PCR-based DNA markers such as random amplified polymorphic DNAs (RAPDs), sequence characterized (SCARs), amplified fragment length polymorphism (AFLPs), sequence-tagged sites (STS), cleaved amplified polymorphic sequences (CAPS), simple sequence repeats (SSRs) or microsatellites, inter-simple sequence repeat amplification (ISA) and amplicon length polymorphism (ALPs)

(iii) DNA chip, sequence-based DNA markers such as single nucleotide polymorphisms (SNPs), Expressed Sequenced Tags (ESTs) and matrix assisted laser desorption ionization, time of flight (MALDI-TOF) mass spectrometry.

The molecular and biochemical markers which were used in this study, will be discussed further in the following sections.

1.3.1 Isozymes

Isozymes (isoenzymes) are structurally different molecular forms of an enzyme system with, qualitatively, the same catalytic function. They are accepted as species-specific variants of an enzyme system according to the classification of the 'Enzyme Commision' (Müller-Starck, 1998). Isozymes originate through amino acid alterations which cause changes in net charge or the spatial structure or conformation of the enzyme molecules and also, therefore, in their electrophoretic mobility. In enzyme electrophoresis, the position to which any particular enzyme has migrated is identified by immersing the gel in a solution containing a substrate for the enzyme along with a dye that precipitates where the enzyme-catalyzed reaction take place. In this way the position of an enzyme in the gel is marked by the appearance of a dark band (Hart and Langston, 1977; Staub *et al*., 1996).

Enzyme electrophoresis indirectly identifies changes in the nucleotide sequence of the gene encoding the protein molecules. These protein products can be separated and isozymes can therefore mark allelic variation at a single structural gene locus, such that different alleles are represented by isozymes with different electrophoretic mobility. Bands visualised from specific enzymes that represent the protein products, provide genetic information as codominant markers and homozygous and heterozygous genotypes can therefore be distinguished (Müller-Starck, 1998). Isozymes which are encoded by different alleles of the same gene locus are designated as 'allozymes' or 'alloenzymes' (Müller-Starck, 1998).

Different electrophoretic methods are available such as horizontal gel-electrophoresis in which polyacrylamide or hydrolysed starch can be used as a gel medium. If a high-resolution separation is required, the conventional methods can be replaced by isoelectric focusing, polyacrylamide gradient gel electrophoresis or two-dimensional electrophoresis (Koebner *et al*., 1987; Müller-Starck, 1998). Since allelic variation within wheat is essential in order to produce a genetic map, isozyme studies, especially those that are used to uncover intraspecific polymorphism, have shown that isoelectric focusing is a more powerful and flexible technique (Ainsworth *et al*., 1985; Koebner *et al*., 1987). Electrophoretic variation is common between homoeallelic products, both between the genomes within hexaploid wheat and between wheat and its wild relatives. Many reports have been restricted to the study of the aneuploids of 'Chinese Spring' that allow for the determination of chromosome arm location only (Tang and Hart, 1975; Hart and Langston, 1977; Koebner *et al*., 1987).

Various isozymes have been studied in wheat such as lipoxygenase (Hart and Langston, 1977); aminopeptidase (Tang and Hart, 1975; Hart and Langston, 1977); glutamate oxaloacetate transaminase (Tang and Hart, 1975; Gill *et al*., 1991b); grain esterase (Ainsworth *et al*., 1984; Gill *et al*., 1991b); α-amylase (Chao *et al*., 1989; Weining and Langridge, 1991); endopeptidase (Tang and Hart, 1975; Hart and Langston, 1977; Koebner *et al*., 1987; Marais and Marais, 1990); acid phosphatase and alcohol dehydrogenase (Tang and Hart, 1975; Hart and Langston, 1977; Gill *et al*., 1991b); β-amylase, glucosephosphate isomerase, malate dehydrogenase and shikimate dehydrogenase (Gill *et al*., 1991b). Different electrophoretic and staining procedures are used for these isozymes (Hart and Langston, 1977; Gill *et al*., 1991b). Endopeptidase variation was studied in this investigation and will be described further.

In bread wheat endopeptidases have been described as BANA (N-α-benzoyl-DL-arginine-β-naphthylamide) hydrolysing enzymes of which a homoeoallelic series of structural genes have been located on the long arms of the homoeologous group 7 chromosomes (Hart and Langston, 1977). McMillin and Tuleen (1977) reported three structural genes on chromosome arm 7AL and two on 7BL of the wheat 'PI 357307'. Tang and Hart (1975) observed three endopeptidase bands on a zymogram of 'Chinese Spring' of which an intermediate band has been shown to be composed of two isozymes. The extracts were obtained from leaves of 7 to 21 day-old green seedlings. The zymogram phenotype was determined using acrylamide gel disk electrophoresis and starch gel electrophoresis. Four endopeptidase bands were described by Hart and Langston (1977) in the zymogram of 'Chinese Spring' following starch gel electrophoresis of extracts of etiolated 7-day-old seedlings. The genes coding for the isozymes in order of increasing mobility were assigned to chromosomes 7DL, 7AL, 7BL and 7BL, respectively. The controlling loci were named *Ep-A1*, *Ep-B1*, and *Ep-D1*. The genes of the endopeptidase-1 (*Ep-1*) homoeoallelic series were found to map to the long arms of the group 7 chromosomes (Koebner *et al*., 1987) following isoelectric focusing of aqueous extracts of mature embryo tissue of 'Chinese Spring'. The study also revealed three alleles at the *Ep-A1* locus, five at the *Ep-B1* locus and three at the *Ep-D1* locus of which one allele at each locus was null. The only variation found at the *Ep-D1* locus was contributed by the *Aegilops-ventricosa*-derived translocation in '*Rendezvous*' and a null allele proposed to exist in '*Synthetic*'. Marais *et al*. (1998) identified an additional endopeptidase allele, *Ep-D1e*. Close linkage was found between *Ep-B1* and the leaf rust resistance gene *Lr14a* following endopeptidase analysis of single chromosome recombinant lines derived from the cross of monosomic 7B of 'Chinese Spring' and 'Hope' (Koebner *et al*., 1987). The *Lr19* translocation located on chromosome 7DL of 'Indis' does not express an *EpD1* product (Marais and Marais, 1990).

1.3.2 Water soluble proteins (WSPs) in wheat

WSPs are water soluble proteins found in mature wheat grains and have proven to be highly variable and thus potentially useful as markers in wheat-alien introgression studies and in intervarietal selection programs (Gale *et al*., 1989; Liu *et al*., 1989). The function of the genes encoding these proteins are unknown and the protein name and gene symbol, *Wsp-1*, have therefore been temporarily assigned (Liu *et al*., 1989). Nine major water soluble wheat endosperm proteins of ‘Chinese Spring’ have been resolved with isoelectric focusing of which three have been shown to be controlled by genes on the long arms of chromosomes 7A, 7B and 7D. Temporary symbols *Wsp-A1*, *Wsp-B1* and *Wsp-D1* were given to the genes on the respective chromosomes. Another three proteins were shown in the same study to be controlled by genes on chromosome arms 2DS, 4DS and 7DS (Liu *et al*., 1989).

Liu *et al*. (1989) found considerable intervarietal variation among a sample of 44 hexaploid wheat varieties. Five alleles at *Wsp1-A*, three at *Wsp-B1* and two at *Wsp-D1* were identified. Intrachromosomal mapping showed linkage of *Wsp-B1* with *Ep-B1* and the RFLP locus, *Xpsr121*, both of which are located in the distal region of 7BL as was previously determined by Chao *et al*. (1989) and Liu *et al*. (1989). Alien homoeoloci were identified in species related to wheat such as in *Agropyron elongatum* in which the WSP-1 homoeolocus was designated *Wsp-E1* (Liu *et al*., 1989). A homoeolocus, designated *Wsp-D1c*, was mapped distal to *Lr19* on the *Thinopyrum ponticum* derived *Lr19* translocation by Marais (1992a).

1.3.3 Restriction Fragment Length Polymorphisms (RFLPs)

In restriction fragment length polymorphism (RFLP) analysis labelled probes are hybridized to filters containing genomic DNA which had been digested with restriction enzymes. The resultant fragments are separated by gel electrophoresis and transferred onto nitrocellulose or nylon filters using the Southern blotting procedure (Southern, 1975; Botstein *et al*., 1980). The enzymes commonly used for RFLP analysis recognize 4-6 base-pair sequences (Brettschneider, 1998). The hybridization probes can be labelled with radioactive isotopes, such as ^{32}P (Dvořák *et al*., 1988), enzymes such as horseradish peroxidase or hapten molecules such as digoxigenin (Brettschneider, 1998). A polymorphism in a restriction pattern occurs when mutation of a single base-pair results in the loss, or, creation, of a new restriction site, or when, by insertion or deletion, the size of a

restriction fragment is altered. These alterations are detected on an autoradiograph when these fragments bind the hybridization probe (Sharp *et al*., 1989; Grant and Shoemaker, 1997).

RFLP analysis is time-consuming, labour-intensive, expensive and low levels of polymorphisms are usually detected in wheat. This low level of polymorphism can be ascribed to the polyploid nature, high proportion of repetitive DNA and large genome size of wheat (Gupta *et al*., 1999; Gao *et al*., 2004). Nevertheless, the RFLP technique has been used for a variety of purposes in wheat, including varietal identification, genome mapping, characterization of wheat-rye recombinants and identification of homoeoelogous chromosome arms (Devey and Hart, 1993; Gupta *et al*., 1999). The problems of using RFLPs are greatest in the genetic mapping of qualitative and quantitative traits of agronomic importance, and marker-assisted selection, which require informative markers in an intraspecific context (Gupta *et al*., 1999; Hayden *et al*., 2001). However, numerous disease resistance genes such as leaf rust resistance genes, have been mapped with RFLP markers (Autrique *et al*., 1995; Schachermayr *et al*., 1995; Nelson *et al*., 1997; Seyfarth *et al*., 1999; Huang and Gill, 2001).

The first (human) genetic map using RFLPs was constructed by Botstein *et al*. (1980). Thereafter, these markers were used to map plant genomes (Weber and Helentjaris, 1989) and the first RFLP maps of wheat chromosomes were produced by Chao *et al*. (1989). There are more than 1700 DNA clones listed that have been used for linkage and/or deletion and/or aneuploid mapping of one or more *Triticum* species (McIntosh *et al*., 1998). Wheat reference genetic maps with 300 to more than a 1000 loci have been constructed for the seven homoeologous groups. Such maps were mainly based on RFLP markers and mapping populations derived from crosses involving wild species, for example synthetic (a tetraploid wheat x *Aegilops tauchii* cross) and a cultivated wheat (Van Deynze *et al*., 1995; Nelson *et al*., 1995), interspecific *T. spelta* x *T. aestivum* crosses (Liu and Tsunewaki, 1991; Schachermayr, 1995; Messmer *et al*., 1999) and inter-varietal crosses (Cadalen *et al*., 1997; William *et al*., 1997). The value of using wide crosses instead of varietal variation were illustrated by the studies of Chao *et al*. (1989) in which 18 group 7 cDNA clones and 13 restriction enzymes were used. Among all pair-wise combinations of six of the 10 varieties used, an average of only 8.7 % polymorphism was revealed. However, a test for 7D polymorphisms between Hobbit 'S' and 'VPM1', which contains a 7D that is mostly derived from *Aegilops ventricosa*, revealed 23.3 % polymorphism.

The majority of the DNA clones used to map RFLP loci in *Triticum* species are either anonymous cDNA clones or anonymous genomic DNA (gDNA) clones isolated from *T.aestivum*, *T. tauschii*,

oat, barley, and other related plant species (Gill *et al*., 1991; Anderson *et al*., 1992). Genomic DNA clones were found to be almost twice as efficient as cDNA clones and generally chromosome-specific clones and clones that hybridize to sequences in non-homoeologous chromosomes, were the most polymorphic (Devos *et al*., 1992). Most cDNA clones hybridize to fragments located in each of the three members of one or sometimes two homoeologous chromosome-arm groups of hexaploid wheat. Very few clones hybridize to fragments from only one or two chromosomes in a group or to chromosomes in more than two groups (Sharp *et al*., 1989; Chao *et al*., 1989a, 1989b; Devos *et al*., 1992). Low-copy-number anonymous gDNA clones hybridize less frequently than cDNA clones to sequences located in each of the three members of a homoeologous group (Gupta *et al*., 1999). Liu and Tsunewaki (1991) found that only 32 % of 72 *Pst*I gDNA clones tested detected loci in all three groups and that 49 % of the clones detected loci in the chromosomes of two or more groups. Ten of 15 homoeologous group-3 gDNA clones studied by Devos *et al*. (1992) hybridized to one or more fragments located in non-homoeoelogous chromosomes or to one or more fragments located in one chromosome only. Anderson *et al*. (1992) determined the chromosome arm locations of over 800 DNA fragments using 210 barley and oat cDNA clones and wheat gDNA clones. Seventy-seven % of the clones hybridized to fragments in one chromosome arm group only and almost all of the clones hybridized to at least one fragment in each of the three arms. It is therefore useful to test many different clone-enzyme combinations to reveal adequate polymorphisms. RFLP analysis is used preferably in comparative genetic mapping in plants, because it is amenable to the use of heterologous DNA probes to detect DNA polymorphisms at corresponding loci in different taxa (Ahn *et al*., 1993; Gupta *et al*., 1999).

1.3.4 Amplified Fragment Length Polymorphisms (AFLPs)

The AFLP technique is a sequence-arbitrary, amplification or polymerase chain reaction (PCR)-based method (Vos *et al*., 1995). The technique is based on the selective amplification of a subset of restriction fragments from a complex mixture of DNA fragments obtained after digestion of genomic DNA with restriction endonucleases. Polymorphisms or differences in the length of the amplified fragments are then detected by polyacrylamide gel electrophoresis (Vos *et al*., 1995; Reiter, 2001). Specific double-stranded DNA adapters of known sequence are ligated to the digested genomic DNA after which the DNA is amplified using primers with sequences complementary to those of the adapters. AFLP products can be detected using ^{33}P-labelled primers, fluorescently labelled primers, or silver staining (Langridge *et al*., 2001; Reiter, 2001).

The number of observed polymorphisms is dependent upon both primer and restriction endonuclease selection. For the analysis of cereals with larger genomes, including wheat, selective-PCR-amplification requires the presence of additional selective nucleotides at the 3' ends of the primers (Vos *et al*., 1995). The addition of selective nucleotides effectively reduces the number of bands (Vos *et al*., 1995). The use of AFLP in wheat has been reported using both *Pst*I and *Eco*RI primers. In wheat more polymorphisms are detected by the *Pst*I/*Mse*I combination than with the *Eco*RI/*Mse*I combination, and analysis reveals that the distribution of markers generated by the two types of enzyme combinations is different (Langridge *et al*., 2001). Generally, AFLP markers are distributed throughout the genome, although clustering of markers in centromeric regions has also been reported (Chalmers *et al*., 2001). However, there is also evidence that AFLP markers are located outside or between regions that are heavily populated with RFLPs and this frequently leads to increases in map lengths due to the addition of AFLP markers to terminal and interspersed chromosome regions (Campbell *et al*., 2001). In addition, some primer combinations generate more polymorphisms than others and this can be used to optimise the number of bands visualised (Campbell *et al*., 2001; Koebner *et al*., 2001). Most plant genomes are AT-rich, and use of AT-poor primers can reduce the band complexity in plants with large genomes such as wheat (Langridge *et al*., 2001).

AFLPs are dominant markers and can be used for genetic diversity studies (Koebner *et al*., 2001), DNA fingerprinting (Powell *et al*., 1996) and QTL mapping (Campbell *et al*., 2001). One of the most important applications of AFLP is in genetic mapping, especially intraspecific mapping Hayden *et al*., 2001). Reported AFLP polymorphism observed in mapping populations of bread wheat amounted to 12.8 % (Chalmers *et al*., 2001). AFLP loci appear to be evenly distributed across the A and B genomes but there is a significant reduction in the level of polymorphism detected in the D genome, as is also observed in RFLP and microsatellite mapping studies (Röder *et al*., 1998a; Langridge *et al*., 2001).

1.3.5 Microsatellite markers

Microsatellites are simple sequence repeats (SSRs) of 1-6 nucleotides. They appear to be ubiquitous in higher organisms, although the frequency and type of SSRs vary between species (Gupta *et al*., 1999; Hayden *et al*., 2001). The frequency of SSRs in the wheat genome was estimated by hybridization of synthetic AC and AG oligomers to genomic libraries by Röder *et al*. (1998a) and Ma *et al*. (1996). The calculated frequencies were: once every 292 kb or 704 kb for AC repeats and

once every 440 kb or 212 kb for AG repeats. Trinucleotide repeats were approximately 10 times less common than the two dinucleotide repeats tested and tetranucleotide repeats were rare.

SSRs are abundant, dispersed throughout the genome, and show higher levels of polymorphism than other genetic markers. SSRs are also not as labour intensive as RFLPs due to the lower amount of DNA required and the ability to automate assays. SSR markers can easily be exchanged as information between researchers, because each locus is defined by the primer sequences rather than the physical exchange required for clones used as RFLP probes. SSRs assays are more robust than RAPDs and more transferable between populations than AFLPs. The co-dominant nature of most SSR markers is also an advantage for genetic mapping. In contrast to RFLP markers, most wheat SSR markers are genome specific and amplify only one specific locus containing an SSR in the A, B, or D genome of bread wheat. However, the development costs are extremely high (Röder *et al*., 1998; Stephenson *et al*., 1998; Gupta *et al*., 1999; Koebner *et al*., 2001; Langridge *et al*., 2001; Somers *et al*., 2004).

Various microsatellite maps have been constructed and sometimes the microsatellite loci were integrated with other molecular markers (Röder *et al*., 1998; Harker *et al*., 2001; Gupta *et al*., 2002; Somers *et al*., 2004; Gao *et al*., 2004; Sourdille *et al*., 2004). Somers *et al*. (2004) mapped 1.235 microsatellite loci, covering 2569 cM, giving an average interval distance of 2.2 cM (Somers *et al*., 2004). This consensus map is comprised of four maps and is one of the highest-density public microsatellite maps of wheat. In the map constructed by Gao *et al*. (2004), 478 microsatellite markers were derived from expressed sequence tags (EST-SSRs) of which 101 were mapped.

1.4 Leaf rust of wheat

Various factors cause yield loss in wheat, of which the three wheat rusts are probably the most significant (Kolmer, 1996). Wheat stem rust is caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn, wheat leaf rust by *Puccinia recondita* Roberg ex Desmasz f. sp. *tritici* Eriks. & E. Henn, and wheat stripe or yellow rust by *Puccinia striiformis* Westend (Wiese, 1977; McIntosch, 1998).

Wheat leaf rust, also known as brown rust, is the most common and widely distributed of the three wheat rusts and is often regarded as the most important foliar pathogen of wheat with the potential to cause extensive losses in grain yield of wheat and triticale (Kolmer, 1996; Sayre *et al*., 1998;). The effect of foliar rusts on plant development and wheat quality depends on the onset of disease, yield potential and the level of cultivar resistance (Boshoff *et al*., 2002). Damage to wheat depends on its growth stage at the time of infection. Epidemics that occur before or during flowering are most serious, especially when the flag leaf becomes severely infected (Kolmer, 1996; Sayre *et al*., 1998). Under epidemic conditions wheat leaf rust causes yield losses of between 7 and 65 % depending on cultivar susceptibility. Leaf rust causes losses by reducing the number of kernels per head, by reducing the size of kernels, by lowering test weights, and by lowering the protein content of the grain (Sayre *et al*., 1998; Huang and Gill, 2001; Boshoff *et al*., 2002; Prabhu *et al*., 2004).

Leaf rust pustules are small, up to 1/16 mm long, oval fruiting bodies (uredia) of the rust fungus. Reddish-orange urediospores develop within the uredia and rupture the epidermis of the leaf surface as the spores mature (Figure 1) (Wiese, 1977; Knott, 1989a; Hoch and Staples, 1987; Murray *et al*., 1998).

Figure 1. Wheat leaf with rusty red pustules of leaf rust

Figure 2. Black leaf rust pustules on mature plant leaf.

Pustules can be either scattered or clustered on the leaves and leaf sheaths of infected plants and contains thousands of clonally produced dikaryotic urediniospores. These spores can be wind-blown for thousands of kilometres from initial infections sites, causing epidemics on a continental scale (Roelfs, 1989). When moisture is adequate, urediospores germinate and infect leaves within six to eight hours after settling on the plant surface. Once established, a new generation of urediospores may be produced every seven to 14 days if environmental conditions are favourable. Frequent heavy dew, light rain, or high humidity and temperatures of 15 to 22 ^{o}C are ideal for rust development (Knott, 1989a; Murray *et al*. 1998; Boshoff *et al*., 2002). Usually, infections occur first on the upper leaves due to spores that have been deposited out of the air during spore showers. Under severe epidemics, pustules may develop on the awns and glumes of the heads or occasionally on the stem below the head. During favourable weather conditions for rust development, pustules increase in number until 30 to 50 % of the leaf surface is covered. As the plant matures, black, submerged pustules develop on the leaves and leaf sheaths (Figure 2). These pustules (telia) contain the winter spores (teliospores). Teliospores do not infect wheat, and telia may not develop when plants become infected near maturity (Hochs and Stapels, 1987; Knott, 1989a; Murray *et al*., 1998).

1.4.1 Genetic control of leaf rust

The rusts are controlled primarily by either genetic resistance or the use of chemicals, and to a lesser extent by cultural methods (Knott, 1989a). The development of resistant cultivars is the most effective method of biological control of the rusts in that it is the most economical and environment-friendly strategy, compared to chemicals or fungicides (Knott, 1989a; Singh *et al*., 1998; Akem *et al*., 2000). To date more than 50 leaf rust-resistance genes (*Lr*) have been identified, of which 19 *Lr* genes have been tagged by molecular or cytological markers (McIntosch *et al*., 1995; Seyfarth *et al*., 1999; Seah *et al*., 2000; Spielmeyer *et al*., 2000; Aghaee-Sarbarzeh *et al*., 2001; Prabhu *et al*., 2004). Most of them are expressed at the seedling stage (one or two leaves) and usually remain effective throughout the life of the plant (seedling resistance), and some of the expressed resistance is effective at the adult plant growth stages only (adult plant resistance; APR) (Seyfarth *et al*., 1999). Genetic studies have indicated that seedling resistances is usually controlled by single genes, many of which have been rendered ineffective by changes in rust pathogens following their deployment (Messmer *et al*., 2000; Singh *et al*., 2001b). Single gene APRs to rust diseases have also been identified in cereals, these include *Lr12* (Dyck *et al*., 1966) and *Lr35* (Sawhney *et al*., 1998), and in some cases these have also been rendered ineffective by the development of matching virulence in the respective pathogens (Singh *et al*., 2001b). Despite this, genetic studies of cereals have shown that APRs are often important components of durable rust resistance (Seyfarth *et al*., 1999; Kaur *et al*., 2000; Singh *et al*., 2001b).

Pectora roborant cultus recti

There are two main breeding strategies to improve leaf rust resistance: pyramiding of the major resistance genes conferring complete resistance and/or the accumulation of minor resistance genes conferring quantitative resistance (DeLacy *et al*., 2000; Messmer *et al*., 2000). To obtain more durable resistance, quantitative resistance, so-called partial or slow rusting resistance, is preferred, in which the infection is not completely stopped but the spread of disease is delayed (Kaur *et al*., 2000; Messmer *et al*., 2000). In general, slow rusting wheat has longer latent periods, fewer uredinia, and smaller uredinia size at 10-14 days after inoculation with wheat leaf rust (Kolmer, 1996). Several genetic studies have been performed in order to determine the inheritance of slow rusting in wheat (reviewed by Geiger and Heun, 1989). In most of these studies transgressive segregation for leaf rust resistance was found as well as partial dominance for susceptibility. The inheritance of slow rusting resistance could be attributed to only one to three genes with predominantly additive gene action and, in some crosses, also with significant epistatic effects (Geiger and Heun, 1989). A genetic analysis of latent period of *Puccinia recondita* in wheat performed by Shaner *et al*. (1997) provided evidence that four genes with unequal and epistatic

effects controlled the character, whereas Vander-Gaag and Jacobs (1997) found at least five genes to be involved in the prolongation of the latent period. The leaf rust resistance conferred by the adult plant resistance gene *Lr34,* i.e., longer latent period, fewer uredinia and smaller uredinia size, matches the description of slow rusting (Kolmer, 1996; Seyfarth *et al*., 1999). In contrast, the resistance gene *Lr13* induces a hypersensitive reaction upon infection with an avirulent leaf rust race (McIntosch *et al*., 1995). Since many wheat lines characterized by slow rusting were derived from sources containing *Lr34*, it is likely that *Lr34* was segregating together with other genes in these studies (Kolmer, 1996). *Lr13* is probably one of the most widely distributed resistance genes worldwide (McIntosch *et al*., 1995) but it shows enhanced effectiveness only in combination with other resistance genes (Kolmer, 1996; Sawhney *et al*., 1998). The combination of the adult plant resistance genes *Lr13* and *Lr34* appears to be the basis of most of the durable leaf rust resistance (Roelfs, 1988). This example shows that a combination of adult plant resistance genes is an important factor for successful resistance breeding. Singh *et al*. (1998) studied the inheritance of adult plant resistance of the spring wheat variety 'Pavon 76' and found another gene involved in slow rusting resistance, designated as *Lr46*, which is located on chromosome 1B.

Wheat rust fungi are highly specific obligate parasites that interact with wheat in a gene-for-gene relationship, as was hypothyzed by Flor (1971). This high degree of specificity has made durable rust resistance in wheat difficult to achieve because the virulence of wheat rust fungi against wheat resistance genes is highly diverse, resulting in the existence of many different pathogenic races (Liu and Kolmer, 1998; Sawhney *et al*., 1998). The gene pool of cultivated wheat for resistance to pests and pathogens is unfortunately inadequate to respond to the evolution of different pathogen populations. Replacement of highly variable land races by higher yielding, pure-line varieties in many parts of the world has further reduced the wheat gene pool (McIntosch, 1998; Akem *et al*., 2000). The narrow genetic base for rust resistance and the evolution of new rust pathotypes still necessitate a continuous search for new sources of resistance (McIntosch, 1998; Aghaee-Sarbarzeh *et al*., 2001). It is therefore important to import alternative genes from other sources such as wheat diploid species, *T. monococcum*, *Aegilops speltoides* and *Aegilops tauschii* (Friebe *et al*., 1996; Dubcovsky *et al*., 1998; Helguera *et al*., 2000). The introduction of alien genetic variation into wheat is also a valuable and proven technique for wheat improvement (Sharma and Knott, 1966; Friebe *et al*., 1996; McIntosch, 1998; Aghaee-Sarbarzeh *et al*., 2001). The specific interactions between resistance genes in wheat and avirulence genes in the rusts serve as extremely useful markers for characterizing rust populations. Near-isogenic (Prabhu *et al*., 2004) or single-gene lines (Inagaki *et al*., 1998) of wheat, which differ only by the presence of a single rust resistance gene, are used to identify races of rust fungi. Two genes for leaf rust resistance in wheat, *Lr10* (Feuillet *et*

al., 2003) *and Lr21* (Huang *et al*., 2003), have been isolated, cloned and sequenced. Both genes have sequences that encode nucleotide-binding site (NBS) and leucine-rich repeat (LRR) regions, which are characteristic of disease resistance genes in plants (Huang and Gill, 2001).

1.4.2 The Leaf Rust Resistance Gene *Lr19*

Sharma and Knott (1966) originally produced the *Lr19* (=T4) translocation when they transformed a leaf rust resistance gene from the $7el_1$ chromosome of *Thinopyrum ponticum* (*Agropyron elongatum*) to the long arm of chromosome 7D of common wheat. *Lr19* is an effective source of leaf rust resistance worldwide, despite the first report by Huerta-Espino and Singh (1994) of virulence in *Puccinia triticina* to *Lr19*. Unfortunately, the translocation also carries a gene(s) for yellow endosperm that renders the resistance useless in countries where the trait is regarded as undesirable (Prins *et al*., 1996). The 'Indis' translocation line, which was selected in the B_2F_3 following backcrossing of an 'Inia 66' / *Thinopyrum distichum* amphiploid to 'Inia 66', was found to carry a translocation that showed identical polymorphisms to the T4 translocation at various loci. Both translocations expressed *Lr19*, *Sr25* (stem-rust resistance), *Sd1* (segregation distortion), *Wsp-D1c* (water soluble protein), *Y* (yellow endosperm pigmentation) and null alleles for *Ep-D1* and *α-Amy-D2* (Prins *et al*., 1997). Prins *et al*. (1996) compared polymorphisms at three RFLP loci in 'Indis' and W743 (a $7el_1$ ditelosomic addition line having the *Th. ponticum* chromosome arm that carries *Lr19*). They concluded that the translocation in 'Indis' did not derive from *Th. distichum* and that it was the *Lr19* segment from *Th. ponticum.*

Kim *et al*. (1993) located the breakpoint of the *Lr19* translocation in the middle of the long arm of chromosome 7D and concluded that the distal half of 7DL was replaced by *Thinopyrum* chromatin. The *Thinopyrum* segment on 7DL does not pair with homoeologous wheat segments during meiosis, complicating attempts to recombine its genes or to study linkage relationships (Knott, 1980; Marais and Marais, 1990). Knott (1980) obtained two white endosperm mutants ('Agatha-28' and 'Agatha-235') of the *Lr19* translocation after treatment with ethyl-methanesulphonate (EMS), but the agronomic performance of these lines was impaired (Knott, 1986, 1989b). Following genomic *in situ* hybridization and C-banding of the two derived mutants and 'Agatha', Friebe *et al*. (1994, 1996) suggested that the T4 translocation breakpoint is closer to the centromere. Following gamma-irradiation, Marais (1992a) derived 29 deletion mutants, each homozygous for a different deletion of the *Lr19* translocation segment in 'Indis'. Through deletion mapping, the relative positions of a number of marker loci on the *Thinopyrum* segment were determined as: centromere,

Sd1, *Xpsr165*, *Xpsr105*, *Xpsr129*, *XcsIH81-1*, *Xwg380*, *Xmwg2062*, *Lr19*, *Wsp-D1*, *Sr25/Y* (Marais 1992a; Prins *et al*., 1996, 1997; Prins and Marais, 1998). Bournival *et al*. (1994) deduced the gene order on the *Lr19* translocation as being *Lr19-Sr25-Y*. The set of mutant lines were further analysed by Groenewald (2001) using 144 *Sse*8387I/*Mse*I and 32 *Eco*RI/*Mse*I AFLP primer combinations, extending the physical map with 95 novel AFLP markers (86 *Sse*8387I/*Mse*I and 9 *Eco*RI/*Mse*I markers), seven of which mapped close to *Lr19*. One of the AFLP markers closest to *Lr19*, $XustSS12M14_{155}$, was converted into a sequence-tagged-site (STS) marker (STS$Lr19_{130}$) (Prins *et al*., 2001). This assay generated a 130-bp PCR fragment that could be mapped distally to *Lr19*. Although STS$Lr19_{130}$ is not closely associated with *Lr19*, this does not pose problems for its use in marker-assisted selection, since the alien segment in both the original *Lr19* translocation and recombined forms does not recombine with its wheat homoeologue in the presence of the homoeologous pairing gene, *Ph1* (Prins *et al*., 2001).

Sears (1972a, 1972b) identified 12 ('Transfer') lines carrying translocated *Thinopyrum* segments from chromosome $7el_1$ after homoeologous recombination was induced between wheat chromosome 7D and cromosome $7el_1$ of *Th. ponticum*. Ten of the 'Transfer' lines proved to be true recombinants between chromosome $7el_1$ and chromosome 7DL of wheat (Sears, 1977b; Eizenga, 1987). By studying the extent of metaphase I pairing of each recombinant chromosome with selected chromosome stocks, Sears (1973, 1977b) could infer the approximate position of the homoeologous crossover in each. Zhang & Dvořák (1990) used a *Thinopyrum*-specific repetitive sequence, pLeUCD2 to characterize the 'Transfer' lines and to derive the approximate positions of the exchanges. By integrating their results with those of Sears (1973, 1977b) they were able to map the *Sd1* gene proximal to *Lr19*. Prins and Marais (1998) characterized six of the 'Transfer' lines, using RFLP markers, some of which have previously been physically mapped to the *Lr19* translocation (Prins *et al*., 1996, 1997).

In an attempt to break the linkage between *Lr19* and *Y*, Marais (1992b) made use of the ph_1b and ph_2b mutants of 'Chinese Spring' to induce allosyndetic pairing and crossovers between the 'Indis' *Lr19* segment and homoeologous areas of the wheat genome. The 'Chinese Spring' ph_1b mutant contains a deletion of the *Ph1* gene (pairing homoeologous) and promotes homoeologous chromosome pairing at meiotic metaphase I (MI) (Sears, 1977b). Wheat geneticists have suggested the use of this mutant for transfer of desirable gene(s) from the wild relatives to cultivated wheat (Morris and Sears, 1973; Sears, 1976). Marais (1992b) reported the recovery of resistant suspected recombinants following an attempt to induce homoeologous pairing between the *Lr19* translocation and 7DL of wheat. The putative recombinants included four with white endosperm, three with

partially white endosperm and one recombinant that produced yellow endosperm but sometimes showed self-elimination. Of these, only one was proved to be a true recombinant, 88M22-149 (henceforth referred to as *Lr19*-149) (Prins *et al*., 1997).

The original *Lr19* translocation is often transmitted preferentially in heterozygotes due to the presence of the segregation distortion locus, *Sd1* (Kibirige-Sebunya and Knott, 1983; Marais, 1990; Zhang and Dvořák, 1990). The *Lr19*-149 recombinant has lost *Sd1*, and regularly shows self-elimination in heterozygotes (Prins *et al*., 1997) due to the presence of a further segregation factor, *Sd2*, which was retained in this recombinant (Prins and Marais, 1999). In crosses of *Lr19*-149 with local wheats, only 1% of the F_2 progeny were resistant homozygotes, complicating the recovery of homozygotes during selection (Prins *et al*., 1997). However, in certain genetic backgrounds, *Lr19*-149 may show strong prefential transmission. The degree of prefential transmission or self-elimination induced by the translocation appears to be determined by responder polygenes in the wheat genetic background (Prins and Marais, 1999). Marais *et al*. (2000) showed that in *Lr19*-149 heterozygotes the translocation generally showed reduced pollen transmission whereas its transmission through egg cells was mostly normal. A yield trial with near isogenic lines of both the original and shortened translocations suggested that *Lr19* may cause a small reduction in kernel size and an increase in loaf volume, effects which are not associated with *Lr19*-149 (Marais *et al*., 2000).

The translocated segment in *Lr19*-149 has lost the yellow pigment gene and was apparently relocated from chromosome arm 7DL to 7BL in a double crossover event. Comparison of the group 7 consensus map constructed by Werner *et al*. (1992) and the *Lr19* deletion map derived by Prins *et al*. (1997) suggests that the *Lr19*-149 translocation probably overlaps with coding regions at the chromosome 7BL distal end. Physical maps of the *Lr19* and *Lr19*-149 translocations have been constructed (Prins *et al*. 1996, 1997; Prins and Marais, 1998). Six of the *Thinopyrum* marker genes, *Xpsr129-7el*$_1$, *XcsIH81-1*, *Xwg380*, *Xmwg2062*, *Lr19* and *Wsp-D1c*, utilised in mapping *Lr19* were retained on *Lr19*-149. An attempt was made by Marais *et al*. (2000) to further shorten *Lr19*-149 through allosyndetic recombination in the absence of *Ph*$_1$*b*. A plant (cross: 96M1 = 'Chinese Spring' monosomic 5B/'CS'-*Lr19*-149//'CS' *ph*$_1$*b* mutant) was derived which was heterozygous for the *Lr19*-149 translocation on chromosome arm 7BL and also lacked the *Ph*$_1$*b* locus. The absence of *Ph*$_1$*b* in 96M1 was confirmed making use of the probe KSU008 (Gill *et al*., 1991). In an attempt to recover allosyndetic recombinants for the translocated region, 96M1 clones were pollinated with 'CS'. Four recombinants (*Lr19*-149-252, -299, -462, and -478) were produced which were characterized with RFLP markers *XcsIH81-1-7el*$_1$ and *Xpsr129-7el*$_1$ previously mapped on *Lr19*-

149 (Prins *et al*., 1997; Prins and Marais, 1998) and nine of the AFLP marker loci previously mapped to the *Lr19* segment (Groenewald, 2001). Absence of the *Xpsr129-7el*$_1$ allele in the resistant progeny was used as a reporter of homoeologous recombination of *Thinopyrum* chromatin proximal to *Lr19*. To facilitate its detection, the PSR129 RFLP marker was converted to a PCR-based CAPS marker (Marais *et al*., 2000). In three of the secondary recombinants (252, 299 and 462) recovered, the *Xpsr129-7el*$_1$ locus was lost which indicated that *Thinopyrum* chromatin proximally to *Lr19* was exchanged for wheat chromatin. In recombinant 478 the *Wsp-D1c* locus was lost which is situated distally from *Lr19*. Recombinant 299 appeared to be the shortest translocation produced, and it could not be determined which of recombinants 252 or 462 were the shortest. Based on the physical map distance estimates it appeared that the *Lr19* translocation in 299 may have been reduced to approximately one third or less of its original size (Figure 3). To obtain a further decrease in the size of the translocation, an attempt was made to induce normal crossover during meiosis between the *Thinopyrum* regions of recombinants 299 and 478.

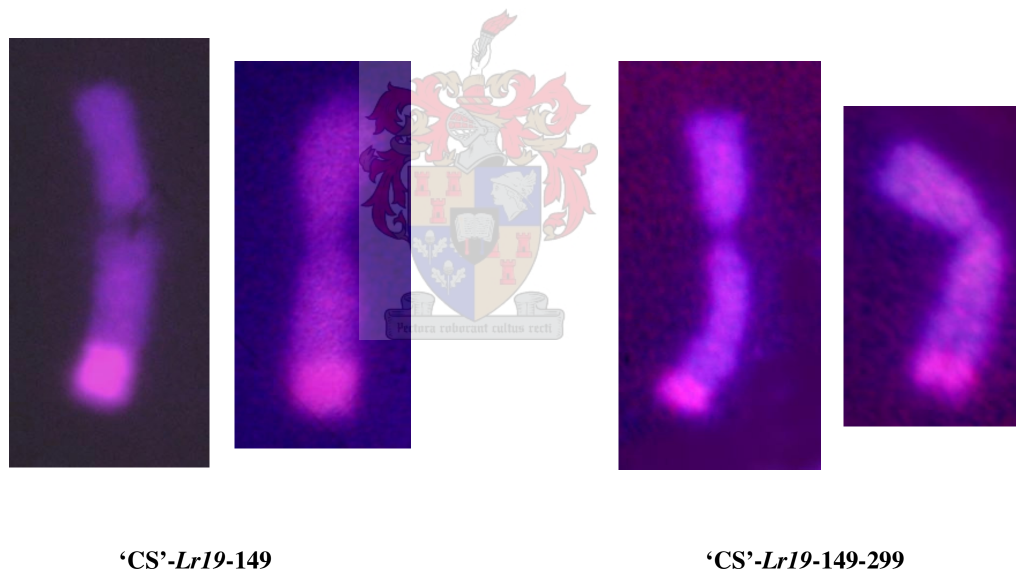


Figure 3 Photo images of recombinants 'CS'-*Lr19*-149 and 'CS'-*Lr19*-149-299 using genomic *in situ* hybridization (A. Lukaszewski; University of California-Riverside; Personal communication)

In this study, one of the aims was to identify and select putative double recombinants from the cross between recombinants 299 and 478 which have retained *Lr19* yet have lost both the *Thinopyrum* loci, *Xpsr129-7el*$_1$ and *Wsp-Dlc*, present in 478 and 299, respectively. The markers, *Xpsr129-7el*$_1$ and *Wsp-Dlc*, which are specific for each translocation, were used to characterize all the progeny. A

second aim was to confirm tertiary (double) recombinants thus obtained and to further characterize the primary, secondary and tertiary recombinants making use of existing and new molecular markers. The molecular markers included RFLP's, AFLP's, STS's and microsatellites. The markers which have not been previously mapped, were first physically mapped to the *Lr19* translocation by making use of a set of *Lr19* deletion lines derived by Marais (1992b).

Two translocation lines, produced by Prof. A. Lukaszewski (Dept. of Botany & Plant Genetics, University of California-Riverside) were also studied. Based on his GISH results, the terminal breakpoints were closer to *Lr19* (proximal end) than the breakpoint in recombinant 299. Provided one or both occur on 7B, it might have been possible to use these in crosses with recombinant 478 and shorten the translocation even more drastically. It was hoped that the marker study would provide information on their chromosome location and confirm that they are in fact shorter on the proximal end than recombinant 299.

2. Materials and Methods

2.1 Characterization of *Lr19* recombinants and mutants

2.1.1 Plant material

The different sources of *Lr19* that were used in the study are listed in Table 2.1. These included the original T4 translocation on 7DL produced by Sharma & Knott (1966) (accessions 'Indis' and 'CS'-*Lr19*); an allosyndetic recombinant ('CS'-*Lr19*-149) of the T4 translocation produced by Marais (1990) which occurs on 7BL; four allosyndetic recombinants of the 'CS'-*Lr19*-149 translocation (Marais *et al*., 2000) that occur on 7BL and two $7el_1$ translocations to group 7 chromosomes of wheat that were produced by A.J. Lukaszewski, Dept. of Botany & Plant Genetics, University of California-Riverside.

A set of seven homozygous, terminal deletion lines of the 'Indis' *Lr19* translocated segment (Marais, 1992) were used to physically map a number of polymorphic molecular markers on the translocation. The deletion mutants, ranked from the largest to the smallest deletions, were 89M2-40, 87M23-103, 89M2-245, 89M2-426, 87M23-225, 89M1-18 and 87M23-266. All of the mutants, except 89M2-40, carry *Lr19*. 'Inia 66', W84-17 and 'CS' were used as common wheat controls, while the 'CS' group 7 nulli-tetrasomics were employed to confirm the chromosomal location of molecular markers. Selected resistant F_1 seeds of the testcross, 00M96 = ('CS'-*Lr19*-149-299 / 'CS'-*Lr19*-149-478 // W84-17), were characterized in an attempt to recover double recombinants.

2.1.2 DNA extraction

Total genomic DNA was extracted from approximately 2-week-old seedlings grown in a greenhouse, following the procedure of Doyle and Doyle (1990) with minor modifications. Fresh leaf tissue (1 g) was ground in 10 ml preheated CTAB extraction buffer (2% w/v CTAB (cetyltrimethylammonium bromide), 1.4 M NaCl, 0.2% beta-mercaptoethanol, 20 mM EDTA, 100mM Tris-HCL pH 8.0), followed by incubation in a waterbath at 60 °C for approximately one hour. This mixture was then extracted once with an equal volume of chloroform:isoamylalcohol (24:1), centrifuged for 10 minutes at 7000 rpm (room temperature), and the nucleic acids in the upper aqueous phase precipitated with 2/3 volumes isopropanol at –20 °C for one hour. After

centrifugation for five minutes at 7000 rpm, the pellet was washed in 15 ml washing buffer (76% ethanol; 10mM NH_4OAc (pH 7.7) for approximately 16 hours (4 °C) and centrifuged at 10000 rpm for 10 minutes at 10-25 °C. After the supernatant was removed, the pellet was air-dried briefly and resuspended in 1 ml of TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH 8.0). The dissolved DNA was treated with Rnase A for 60 minutes at 37 °C, diluted with two volumes of TE buffer (pH 8.0), and precipitated with 7.5 M NH_4OAc (pH 7.7) and 2.5 volumes of cold 100% ethanol for one hour at –20 °C. After the sample was centrifuged for 10 minutes at 10000 rpm (12°C), the pellet was air-dried, dissolved in dH_2O and then subjected to phenol:chloroform:isoamylalcohol (25:24:1) and chloroform:isoamylalcohol (24:1) treatments. Finally the DNA was precipitated at –20 °C for 60 minutes in the presence of 0.5 volumes 7.5 M NH_4OAc (pH 7.7) and double the volume cold 100% ethanol. After centrifugation for 40 minutes at 12000 rpm (4 °C), the genomic DNA was washed two times with 70% ethanol and centrifuged for 30 minutes at 12000 rpm (4 °C) (Sambrook *et al*., 1982). The resulting pellet was air-dried and dissolved in dH_2O. The DNA concentrations were determined by comparing the DNA samples with standard λ DNA controls in an ethidium bromide stained gel. If any phenol and/or salt residues were suspected to be present, the final washing phase was repeated with washing buffer instead of 70% ethanol.

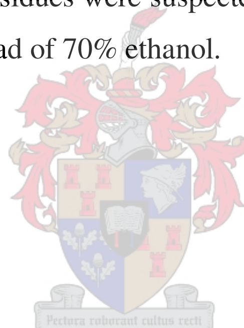


Table 2.1 Wheat lines and cultivars used as sources of *Lr19* in the study

Name / Code	Description
'Indis'	An accession which has the complete *Lr19/*(T4) translocation on 7DL (Marais *et al*., 1988)
'CS'-*Lr19*	A near-isogenic line of 'CS' that has the complete *Lr19/*(T4) translocation on 7DL (Marais, 1990)
'CS'-*Lr19*-149	An allosyndetic recombinant of *Lr19* developed by Marais (1990) which occurs on 7BL
'CS'-*Lr19*-149-252 'CS'-*Lr19*-149-462 'CS'-*Lr19*-149-299	Allosyndetic recombinants of 'CS'-*Lr19*-149 that have exchanged *Thinopyrum* chromatin proximally to the *Lr19* gene on 7BL for wheat chromatin (Marais *et al*., 2000)
'CS'-*Lr19*-149-478	Allosyndetic recombinant of 'CS'-*Lr19*-149 that has exhanged *Thinopyrum* chromatin distally of the *Lr19* locus on 7BL for wheat chromatin (Marais *et al*., 2000)
4772	*Thinopyrum ponticum*-common wheat ($7el_1$/7D) translocation produced by A.J. Lukaszewski (2003, Personal communication, Dept. of Botany & Plant Genetics, University of California-Riverside)
4773[1] 4774[1]	*Thinopyrum ponticum*-common wheat ($7el_1$/7A) translocation produced by A.J. Lukaszewski (2003, Personal communication, Dept. of Botany & Plant Genetics, University of California-Riverside)
89M2-40 87M23-103 89M2-245 89M2-426 87M23-225 89M1-18 87M23-266	A set of terminal deletion mutants for the T4 translocation developed by Marais (1992a). The breakpoint in 89M2-40 occurs proximal to *Lr19*, whereas the breakpoints of the remaining mutants occur distally of *Lr19.* The mutants are given in order of deletion size, with 89M2-40 carrying the largest deletion and 87M23-266 the smallest (Marais, 1992)

[1] These are two samples of the same line.

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2.1.3 Endopeptidase analysis

Endopeptidase analyses were performed according to Marais and Marais (1990). The embryo half of a mature kernel was incubated in 120 µl dH_2O overnight at 4 ^oC. Following maceration, the extracts were centrifuged at 10 000 rpm for 25 minutes at 2 – 5 ^oC, after which 30 µl of supernatant was loaded directly on the cathodal end of a 150 mm x 125 mm x 0.25 mm 8% w/v acrylamide:*bis*-acrylamide (37.5:1 w/w) gel with 2% w/v ampholyte (consisting of two parts Pharmalyte 4.2 – 4.9 and one part Pharmalyte 4 – 6.5) and 13% v/v glycerol. 1 M glycine was used as catholyte and 0.5 M glacial acetic acid as anolyte. Electrophoresis was performed at 4 ^oC on a Hoefer Isobox unit after prefocusing for 30 minutes at 13 W and running for three hours at the same setting. Staining of the gel was done for 30 minutes according to Tang and Hart (1975). The stained gel was washed from the glass plate with distilled water, spread onto a white paper sheet and air-dried.

2.1.4 Restriction fragment length polymorphism (RFLP) analysis

Six RFLP loci that map to chromosome arm 7DL, *viz. Xpsr129*, *XcsIH81-1*, *XmWg380*, *Xpsr687*, *XtksuE18* and *Xcdo414* were analyzed. Probe csIH81-1 was supplied by Dr. E. Lagudah (CSIRO Division of Plant Industry, Canberra ACT, Australia); the PSR129 and PSR687 probes by the Plant Science Laboratory, Norwich, UK; the WG380 and CDO0414 probes by Prof. M.E. Sorrels (Department of Plant Breeding and Biometry, 353 Emerson Hall, Ithaca, NY 14853-1902 USA) and the TtksuE018 probe by Dr. Jon Raupp (Department of Plant Pathology, the Wheat Genetics Resource Center, Throkmorton Hall, Kansas State University, Manhatten, KS 66506-5502 USA).

An attempt was made to physically map RFLP loci *Xpsr687*, *XksuE18* and *Xcdo414* with a set of seven deletion lines (Table 2.3, p.48) of the 'Indis' *Lr19* translocation segment. Markers *Xpsr129, XcsIH81-1* and *XmWG380* were previously mapped proximally to *Lr19*, with *XmWG380* being the closest to *Lr19* (Prins *et al*., 1996, 1998). The mapped markers were used to characterize 'CS'-*Lr19*-149 and the four secondary derived recombinants, 'CS'-*Lr19*-149-252, -462, -478 and –299; the putative tertiary recombinants derived in this study as well as recombinants 4772 and 4773. 'CS', 'CS'-*Lr19* and the 'CS' group 7 nulli-tetrasomics were included as control genotypes to identify the wheat group 7BL and 7DL fragments, as well as the *Thinopyrum* fragments of chromosome $7e_1$.

2.1.4.1 RFLP methodology

RFLP analysis was performed according to Groenewald (2001). Approximately 10 µg of gDNA was digested (37 ^{o}C) overnight to completion with 50 units *Hind*III or *Eco*RI restriction endonucleases (Roche). The restriction reactions were size fractionated in a 0.8% agarose gel, initially at 100 V for 10 minutes, then 30 V overnight, and increased to 40 V the following morning, using 1x TBE (100 mM Tris-HCl, 100 mM boric acid, 2 mM Na_2EDTA, pH 8.3) as running buffer. Lambda (λ) DNA (Roche) digested with *Hind*III was used as size marker and electrophoresed on the same gel. Following gel electrophoresis, the gel was depurinated for 15 minutes at room temperature in 0.2 N HCl, briefly rinsed in sterile dH_2O and submerged twice with gentle shaking in denaturing solution (0.5 M NaOH, 1.5 M NaCl) for 15 minutes each at room temperature. The gel was then submerged twice with gentle shaking (15 minutes) in neutralisation solution (0.5 M Tris-HCl, pH 7.5; 3.0 M NaCl) at room temperature. The gDNA was then blotted overnight onto a Hybond – N+; positively charged nylon membrane (Roche), using 20x SSC (3 M NaCl, 300 mM sodium citrate, pH 7.0) as transfer buffer. The DNA was UV fixed to the blot by cross-linking the DNA side of the blot for three minutes on a UV transilluminator. The blot was rinsed briefly in sterile 2x SSC, sealed between plastic sheet protectors and stored at 4 ^{o}C for future use.

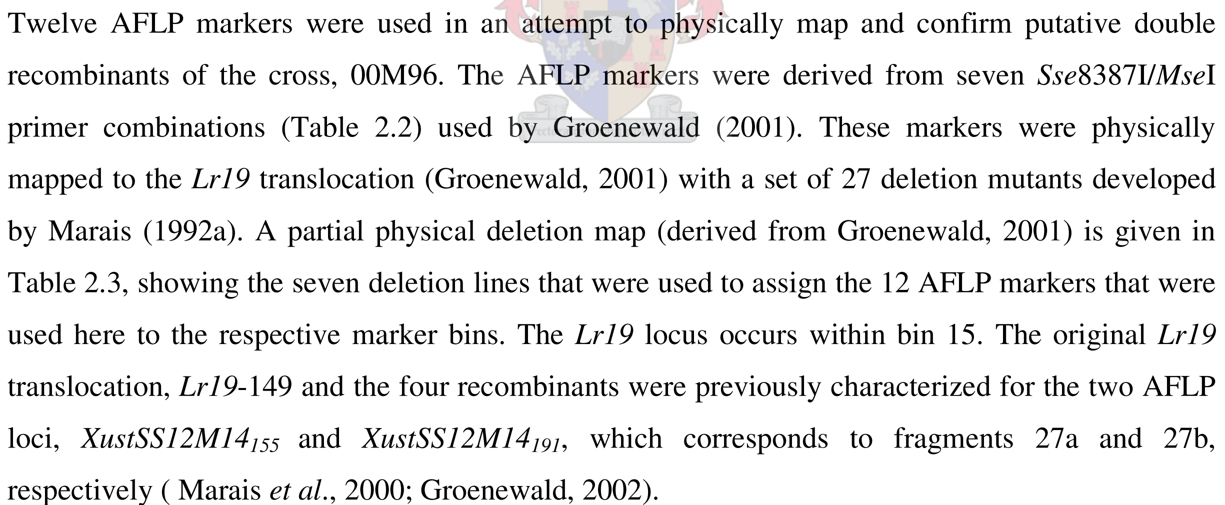
The insert of the plasmid containing the probe was PCR labelled using a dNTP stock mix consisting of 1 mM each of dATP, dGTP, dCTP, 0.75 mM dTTP and 0.3 mM digoxigenin-11-2'-deoxy-uridine-5'-triphosphate (alkali labile – Roche). PCR amplification was performed in 0.2 ml PCR tubes in a 50 µl total reaction volume consisting of 2 ng plasmid DNA, 15 pmol each of M13 forward and reverse primer, 20% v/v dNTP mix, 2.5 units *Taq* DNA polymerase (Bioline), 1x PCR buffer, 1.5 mM $MgCl_2$ and AFLP-grade water. The PCR profile was run on a Hybaid PCR Express or GeneAmp® PCR System and involved (a) seven minutes at 94 ^{o}C; (b) 36 cycles each consisting of 45 seconds at 94 ^{o}C, one minute at 60 ^{o}C, two minutes at 72 ^{o}C; (c) two minutes at 72 ^{o}C and (d) a final soak temperature of 4 ^{o}C.

Membranes were prehybridized with DIG Easy Hyb (Roche) for three hours at 42 ^{o}C and reused membranes for two to three hours at the same temperature in a Techne Hybridizer (Lasec) oven. The hybridization solution contained DIG Easy Hyb (Roche) and approximately 300 ng denatured labelled probe. Only CDO0414 was hybridized on the membranes containing the genomic DNA that was restricted with *Eco*RI. Following overnight hybridization at 42 ^{o}C, the membrane was washed twice in a solution consisting of 2x SSC and 0.1% SDS, each wash lasting five minutes at room temperature. This was followed by another two washes, this time in a solution consisting of

0.5x SSC and 0.1% SDS, each wash lasting 20 minutes at 68 °C. The time and temperature of this washing step was eventually reduced to 15 minutes and 65 °C, respectively, to create less stringent conditions to obtain stronger hybridization signals of the labelled probe. Prior to detection, the membrane was rinsed briefly in 2x SSC to avoid the formation of salt precipitants. Detection of the hybridized probe was performed using CDP Star (Roche) and the DIG Wash and Block Buffer Set (Roche) according to the manufacturer's instructions. Hybridization products were visualised after exposure to a high performance chemiluminescence X-ray film (Amersham) for one to two hours. The membrane was then rinsed in dH_2O for one minute at room temperature. The probe was stripped from the membrane by washing the membrane twice for 15 minutes at 37 °C in a solution consisting of 0.2 N NaOH and 0.1 % SDS, followed by a final rinse in 2x SSC for five minutes at room temperature. The stripped membrane was sealed between plastic sheet protectors and stored at 4°C.

2.1.5 Amplified fragment length polymorphism (AFLP) analysis

2.1.5.1 AFLP markers



Twelve AFLP markers were used in an attempt to physically map and confirm putative double recombinants of the cross, 00M96. The AFLP markers were derived from seven *Sse*8387I/*Mse*I primer combinations (Table 2.2) used by Groenewald (2001). These markers were physically mapped to the *Lr19* translocation (Groenewald, 2001) with a set of 27 deletion mutants developed by Marais (1992a). A partial physical deletion map (derived from Groenewald, 2001) is given in Table 2.3, showing the seven deletion lines that were used to assign the 12 AFLP markers that were used here to the respective marker bins. The *Lr19* locus occurs within bin 15. The original *Lr19* translocation, *Lr19*-149 and the four recombinants were previously characterized for the two AFLP loci, $XustSS12M14_{155}$ and $XustSS12M14_{191}$, which corresponds to fragments 27a and 27b, respectively (Marais *et al*., 2000; Groenewald, 2002).

Table 2.2 Twelve AFLP markers derived from seven *Sse*8387I and *Mse*I adaptor-specific oligonucleotides used as selective primers by Groenewald (2001).

AFLP markers	Marker bin	*Sse*8387I[1] primers	*Mse*I[1] primers	AFLP marker code	Polymorphic fragment size (bp)
27a:S-AC/M-AT$_{155}$	12	S12-AC	M14-AT	27 a	155
137c:S-C/M-TGT$_{>330}$	12	S02-C	M90-TGT	137 c	>330
138a:S-T/M-TGT$_{>330}$	12	S04-T	M90-TGT	138 a	>330
7b:S-AGG/M-AA$_{270}$	15	S29-AGG	M11-AA	7 b	270
27b:S-AC/MAR$_{191}$	15	S12-AC	M14-AT	27 b	191
54a:S-AGC/M-CG$_{>330}$	15	S28-AGC	M17-CG	54 a	>330
56a:S-AGT/M-CG$_{>330}$	15	S30-AGT	M17-CG	56 a	>330
126a:S-AGC/M-GGT$_{>330}$	15	S28-AGC	M74-GGT	126 a	>330
137a:S-C/M-TGT$_{>330}$	15	S02-C	M90-TGT	137 a	>330
138c:S-T/M-TGT$_{131}$	15	S04-T	M90-TGT	138 c	131
7a:S-AGG/M-AA$_{325}$	16	S29-AGG	M11-AA	7 a	325
126c:S-AGC/M-GGT$_{224}$	16	S28-AGA	M74-GGT	126 c	224

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[1]The primers are labelled in such a way that the first three characters represent the primer identifier, followed by the selective nucleotides. For example S02-C represents the *Sse* adaptor specific primer number S02 and the selective nucleotide that followed the common adaptor sequence was a ‘C’. The AFLP marker code also represents the specific primer combination used by Groenewald (2001). The AFLP marker bins are listed in their physical map order from the proximal (centromeric) end to the distal (telomeric) end on the *Lr19* translocation.

2.1.5.2 AFLP methodology

The DNA samples used for AFLPs included the control genotypes 'Indis' (*Lr19*), 'Inia-66' (non-*Lr19*) and 'Chinese Spring' (non-*Lr19*); the five 'CS'-*Lr19* primary and secondary recombinants; a number of putative tertiary 00M96 double recombinants; and University of California recombinants, 4772 and 4773. The AFLP reactions were repeated up to eight times for most of these genotypes which meant that three to four different DNA samples, that were obtained on separate occasions, were used for analysis of each recombinant and control genotype.

The technique involves three main steps: 1) the restriction digestion of genomic DNA followed by the ligation of oligonucleotides adapters; 2) amplification of the restriction fragments using selective primer pairs and 3) the detection of the amplified products (Vos *et al*., 1995). The protocol described by Donini *et al*. (1997), as modified from Vos *et al*. (1995), was used.

Total genomic DNA (500 ng) was digested with five units *Sse*83871 (AEC Amersham) and five units *Mse*I (New England Biolabs) restriction enzymes in a total reaction volume of 40 µl containing 0.1 µg/µl BSA (New England Biolabs), 1x One-Phor-All buffer (Pharmacia) and AFLP-grade water. The reaction was incubated for two to three hours at 37 ^{o}C, after which 10 µl of adapter ligation solution was added. The adapter ligation solution consisted of five pmol *Sse*8387I adaptor, 50 pmol *Mse*I adaptor, 1 mM ATP (Pharmacia), one unit T4 DNA ligase (Pharmacia), 0.1 µg/ul BSA, 1x One-Phor-All buffer (Pharmacia) and AFLP-grade water. The restriction-ligation reaction was then incubated overnight at 37 ^{o}C. A 1:1 – 1:10 dilution using 1x $TE_{0.1}$ buffer (10 mM Tris-HCl, 0.1 mM Na_2EDTA, pH 8.0) was made using 45 µl of the restriction-ligation reaction and the remaining 5 µl of each reaction was loaded on a 1.5% agarose gel [1x TBE buffer (100 mM Tris-HCl, 100 mM boric acid, 2 mM Na_2EDTA, pH 8.3] and electrophoresed for three to five hours at 75 V to confirm complete digestion of the gDNA.

Pre-selective amplification of the diluted reactions was performed in thin-walled 0.2 ml PCR tubes. The total volume of each amplification reaction consisted of 13 µl diluted restriction-ligation template, 75 ng S00 primer (5'- AGA CTG CGT ACA TGC AGG –3'), 75 ng M00 primer (5'-GAT GAG TCC TGA GTA A –3'), 50 µM of each dNTP, AFLP-grade water, as well as one unit of *Taq* polymerase (GIBCO), 1x PCR buffer and 1.5 mM $MgCl_2$. The PCR amplification consisted of elongation at 72 ^{o}C for five minutes, 30 cycles of 94 ^{o}C for 30 seconds, 56 ^{o}C for 60 seconds, 72 ^{o}C for 60 seconds, followed by a final extension for five minutes at 72 ^{o}C and a soak temperature of 4

^{o}C. amplification was performed in a GeneAmp® PCR System (Applied Biosystems). A 1:1-1:10 dilution using 1x $TE_{0.1}$ buffer (pH 8.0) was performed for 45 µl of each of the amplification reactions and the remaining 5 µl of each reaction was tested on a 1 % agarose gel (1x TBE buffer, three hours at 75 V) to confirm successful amplification.

For each selective reaction, 2.5 ng of the *Sse*8387I selective primer was always end-labelled with 0.5 µCi {γ-^{33}P}ATP (74 TBq/mmol, 2000 Ci/mmol, 370 MBq/ml, 10 mCi/ml; NEN Life Science Products, Inc.) in the presence of 0.05 units of T4 polynucleotide kinase (USB), 1x One-Phor-All buffer (Pharmacia) and AFLP-grade water. The reaction was incubated for two hours at 37 ^{o}C after which the enzyme was heat-inactivated by incubation for 10 minutes at 65 ^{o}C. The labelled primer was stored at 4 ^{o}C until required. AFLP ladder (30-330 bp) (Life Technologies) was labelled according to the manufacturer's instructions.

Selective amplification ('hot reaction') was performed in 0.2 ml PCR tubes in a 10 µl total reaction volume. This consisted of 2.5 µl diluted template DNA (diluted pre-selective amplification product), 15 ng unlabelled *Mse*I primer (5'- GAT GAG TCC TGA GTA ANN N –3', where N denotes the specific selective nucleotides), 2.5 ng labelled *Sse*8387I primer (5'- GAC TGC GTA CAT GCA GGN NN –3', where N denotes the specific selective nucleotides), 50µM of each dNTP, 0.25 units *Taq* DNA polymerase (Bioline), 1x PCR buffer, 1.5 mM $MgCl_2$ and AFLP-grade water. The PCR profile consisted of one cycle of 94 ^{o}C for 30 seconds, 65 ^{o}C for 30 seconds, 72 ^{o}C for 60 seconds, followed by 12 cycles in which the annealing temperature in each cycle was lowered by 0.7 ^{o}C. This was followed by 23 cycles of 94 ^{o}C for 30 seconds, 56 ^{o}C for 30 seconds, 72 ^{o}C for 60 seconds and a final soak temperature of 4°C. The PCR amplification was performed in a GeneAmp® PCR System(Applied Biosystems). The amplification reactions were stored at 4 ^{o}C until size-separation on a denaturing polyacrylamide gel.

Samples were size-fractionated on a 0.4 mm thick 6% w/v acrylamide:*bis*-acrylamide (19:1 w/w) denaturing sequencing gel containing 6 M urea in 1x TBE buffer (100 mM Tris-HCl, 100 mM boric acid, 2 mM Na_2EDTA, pH8.3) using a Model S2001 sequencing gel electrophoresis apparatus (Life Technologies) according to the manufacturer's instructions. Prior to loading the samples, 5 µl of AFLP loading buffer (98% formamide, 10 mM EDTA pH 8.0, 0.05% w/v bromophenol blue, 0.05% w/v xylene cyanol FF) was added to each sample after which the samples were denatured at 90 ^{o}C for three to five minutes and immediately quenched on ice. Five to 6.5 µl of the denatured samples and 2 µl of the labelled 30–330 bp AFLP ladder (Life Technologies) were loaded on the

gel. Denaturing polyacrylamide gel electrophoresis was then performed at a constant power of 80 W for approximately three to five hours (when the xylene cyanol front was approximately seven cm from the bottom of the glass plate). The gel was transferred to Whatmann 3 MM Chr chromatography paper and dried on a Savant Gel Dryer for two hours at 80 ^{o}C. Amplified products were visualised after exposure to an X-ray film (Kodak BioMax MR) for up to seven days.

Fragments were scored manually for the absence and presence of an amplification product. Fragments present as a 'doublet' were scored as a single marker. The results were repeated for verification purposes and/or where the bands were not clearly visible on the autoradiograph.

2.1.6 STS and SCAR markers

2.1.6.1 STSLr19$_{130}$

The sequenced-tagged-site (STS) marker, STSLr19$_{130}$, was developed by Prins *et al.* (2001) and was the first molecular marker to be mapped on the distal side of *Lr19*. The STS was derived from an AFLP marker, *12c:S-AG/M-AC174*, which mapped between *Lr19* and *Wsp-Dlc* (Prins *et al*., 2001; Groenewald, 2002). This dominant STS marker generated a 130 bp PCR fragment in all *Lr19*-carrying lines tested, except for one deletion mutant, while non-carrier template failed to amplify any product.

STSLR19$_{130}$ was used in this study to characterize recombinants 'CS'-*Lr19*-149-299, -478, -252 and –462. The controls used were 'Inia-66' and 'Indis'. PCR amplifications were performed in a total reaction volume of 25 µl containing 50 ng genomic DNA, 12.5 pmol of each primer, 0.2 mM of each dNTP, 0.625 units of *Taq* polymerase (Bioline), 1x PCR buffer and two mM $MgCl_2$. The PCR reaction was performed in a GeneAmp® PCR System(Applied Biosystems) and involved four minutes at 94 ^{o}C, 30 cycles of 30 seconds at 94 ^{o}C, 30 seconds at 60 ^{o}C, 30 seconds at 72 ^{o}C and a final extension for five minutes at 72 ^{o}C. The PCR products were separated on a 3% w/v agarose gel (1x TBE buffer, two hours at 60 V) and visualized under UV light after ethidium bromide staining.

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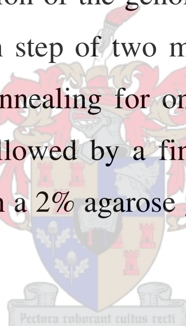
2.1.6.2 Sequence characterized amplified region (SCAR) marker, SCS73$_{719}$

The SCAR marker, SCS73$_{719}$, was converted from a randomly-amplified polymorphic DNA (RAPD) marker S73$_{728}$ by Cherukuri *et al*. (2003). This SCAR marker was reportedly linked to *Lr19* which mapped at 6.4 +/- 0.035 cM distance. The SCAR primer pair amplified a characteristic single band of 719 bp in lines carrying *Lr19* that was absent in near-isogenic lines (NILs) in which other specific *Lr* genes were present and in susceptible varietal backgrounds in which *Lr19* was absent. Marker SCS737$_{719}$ was therefore found to be linked in coupling to the *Lr19* (Cherukuri *et al*., 2003). Since SCS737$_{719}$ could be useful in the present study it was tested on the *Lr19* material and an attempt was made to map it.

The initial PCR amplification with the SCAR primers, was as described by Cherukuri *et al*. (2003). The PCR reactions were performed in a 25 µl volume which included 25 ng of genomic DNA, 50 ng of SCAR primers, 2 mM $MgCl_2$, 200 µM of each dNTP, 0.75 units *Taq* DNA polymerase (Bioline) and 1x PCR buffer. Amplification of the genomic DNA was performed in a GeneAmp® PCR System with an initial denaturation step of two minutes at 94 ^{o}C followed by 35 cycles of denaturation for one minute at 94 ^{o}C, annealing for one minute at 55 ^{o}C and extension for one minute at 72 ^{o}C. The last cycle was followed by a final extension for seven minutes at 72 ^{o}C. Amplification products were separated on a 2% agarose gel and visualized and photographed under UV light.



This PCR protocol, provided by the authors, did not result in amplification of the specified single band of 719 bp in the control genotypes. Optimisation was therefore attempted. The reaction volume was reduced to 15 µl and consisted of 25-50 ng template DNA; 12, 25, 40, or 50 pmol of each primer; 50-200 µM of each dNTP; 0.75 units *Taq* DNA polymerase (Bioline); 1x PCR buffer; 2-6 mM $MgCl_2$ with increments of 0.5 mM; and sterile distilled water (Sabex). The amplification reactions involved a) two minutes at 94 ^{o}C; b) 25, 35 or 45 cycles, each consisting of one minute at 94 ^{o}C, one minute at the annealing temperature (55°C or 60°C); c) 1.30 or 2.30 minutes of extension at 72°C, with a final extension of seven minutes at 72 ^{o}C and final soaking temperature of 4°C. The lines that were used in the preliminary tests were 'Indis' (*Lr19*),' Inia-66' (non-*Lr19*), 'CS' (non-*Lr19* carrier) and the mutant 887M23-266 (*Lr19* carrier with the smallest deletion) (Marais *et al*., 1992).

2.1.7 Microsatellite analysis of ‘CS’-*Lr19*-149 recombinants

Seven microsatellite loci that map to chromosome arm 7BL (Röder *et al*., 1998; Gupta *et al*., 2002), *viz. Xgwm68*, *Xgwm611*, *Xgwm577*, *Xgwm146*, *Xgwm344, Xcnl2, Xwmc216* and *Xwmc276* were selected for further characterization of four secondary recombinants of ‘CS’-*Lr19*-149. ‘CS’, ‘CS’-*Lr19* and ‘CS’-nullisomics for chromosomes 7A, 7B and 7D were included as controls.

PCR amplification of the microsatellite locus was performed in 0.2 ml PCR tubes in a 20 µl total reaction volume. This consisted of 100 ng template DNA, 10 pmol of each primer, 200 µM of each dNTP, 1 unit *Taq* DNA polymerase (Bioline), 2x PCR buffer, 1.5-3 mM $MgCl_2$ and sterile distilled water. Optimal $MgCl_2$ concentrations were 2 mM for all of the microsatellite markers. The annealing temperatures were 55 ^oC for *Xgwm344*, *Xgwm577*, *Xgwm611*, *Xwmc216* and *Xwmc276*; and 60 ^oC for *Xgwm68*, *Xgwm146* and *Xcnl2*. The PCR reaction was performed in a GeneAmp® PCR System(Applied Biosystems) and involved (a) four minutes at 94 ^oC; (b) 30 cycles each consisting of 30 seconds at 94 ^oC, 30 seconds at the optimal annealing temperature, 90 seconds at 65 ^oC; (c) three minutes at 65 ^oC and (d) a final soak temperature of 4 ^oC.

The PCR reactions were analysed on a 2% w/v agarose gel (1x TBE buffer, two hours at 75 V) to verify PCR product formation by loading 5 µl of each sample on the gel, using 10 µl of ‘Orange Ficoll’ as loading buffer. A 100 bp ladder (Promega) was used to verify that the correct size product was amplified for each marker. The remaining 15 µl amplification reactions were stored at 4^oC until size-separation on a denaturing polyacrylamide gel.

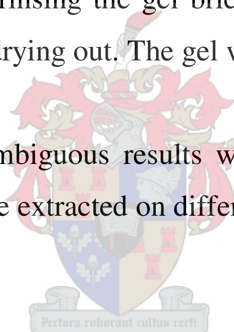
The samples were size-fractionated on a 0.8 mm thick 6% w/v acrylamide:*bis*-acrylamide (19:1 w/w) denaturing sequencing gel containing 6 M urea in 1x TBE buffer (100 mM Tris-HCl, 100 mM boric acid, 2 mM Na_2EDTA, pH 8.3) using a Model S2001 sequencing gel electrophoresis apparatus (Life Technologies) according to the manufacturer’s instructions. Prior to loading the samples, an equal volume of AFLP loading buffer (98% formamide, 10 mM EDTA pH8.0, 0.05% w/v bromophenol blue, 0.05% w/v xylene cyanol FF) was added to each sample. An equal volume (1.5 µl) of the loading buffer was also added to 1.5 µl of a 30-330 bp AFLP ladder (Life Technologies). The samples were then denatured at 90 ^oC for three to five minutes and immediately quenched on ice. The denatured samples (12 µl of each) and all of the AFLP ladder (3 µl) were loaded on the gel. Denaturing polyacrylamide gel electrophoresis was then performed at a constant power of 70 W for five hours (when the xylene cyanol front was aproximally 10 cm from the

bottom of the glass plate). Amplified products were visualized after the polyacrylamide gel was silver-stained.

2.1.7.1 'Silver-staining' procedure

After electrophoresis the two glass plates were separated and the glass plate to which the gel adhered was placed with the gel side up in a large photography plastic tray. Fresh fixing solution (10% w/v EtOH, 0.5% w/v acetic acid) was added and the gel was gently shaken at room temperature for 10 minutes after which it was rinsed three times in distilled water for one minute or until the gel did not appear oily any more. The gel was then placed in a separate photographic tray, containing 0.1% silver nitrate solution and incubated for 10 minutes, whilst shaken gently at room temperature. Following silver impregnation, the gel was briefly rinsed for five seconds in distilled water and placed in chilled developing solution (375 mM NaOH, 0.41% w/v 37% formaldehyde). The gel was shaken gently until the amplification products and ladder were visible. The development reaction was stopped by rinsing the gel briefly in distilled water. The gel was then covered with a plastic sheet to prevent drying out. The gel was photographed using a digital camera.

Markers were scored manually and ambiguous results were double-checked and/or repeated, in some cases with DNA samples that were extracted on different occasions.



2.2 Recombination between the 'CS'-*Lr19*-149-299 and 'CS'-*Lr19*-149-478 translocations

The aim of this part of the study was to select shortened translocations from the F_1: 00M96 = ('CS'-*Lr19*-149-299 / 'CS'-*Lr19*-149-478 // W84-17) in which a heterozygote for the two recombinants, -299 and –478, was derived and testcrossed with the leaf rust susceptible line, W84-17 (Figure 2.1). Any crossover within the chromatin common to the two translocations was expected to yield a shortened, double tertiary recombinant.

Two loci were used as markers for screening the testcross progenies, i.e. the proximally situated locus, *Xpsr129*, and the distally occurring locus, *Wsp-D1.* Recombinant –299 expresses the wheat allele for *Xpsr129*, but the *Thinopyrum* allele for *Wsp-D1.* Conversely, recombinant –478 has the *Thinopyrum* allele for *Xpsr129* but lacks the *Thinopyrum* allele, *Wsp-Dlc* (Marais *et al*., 2001).

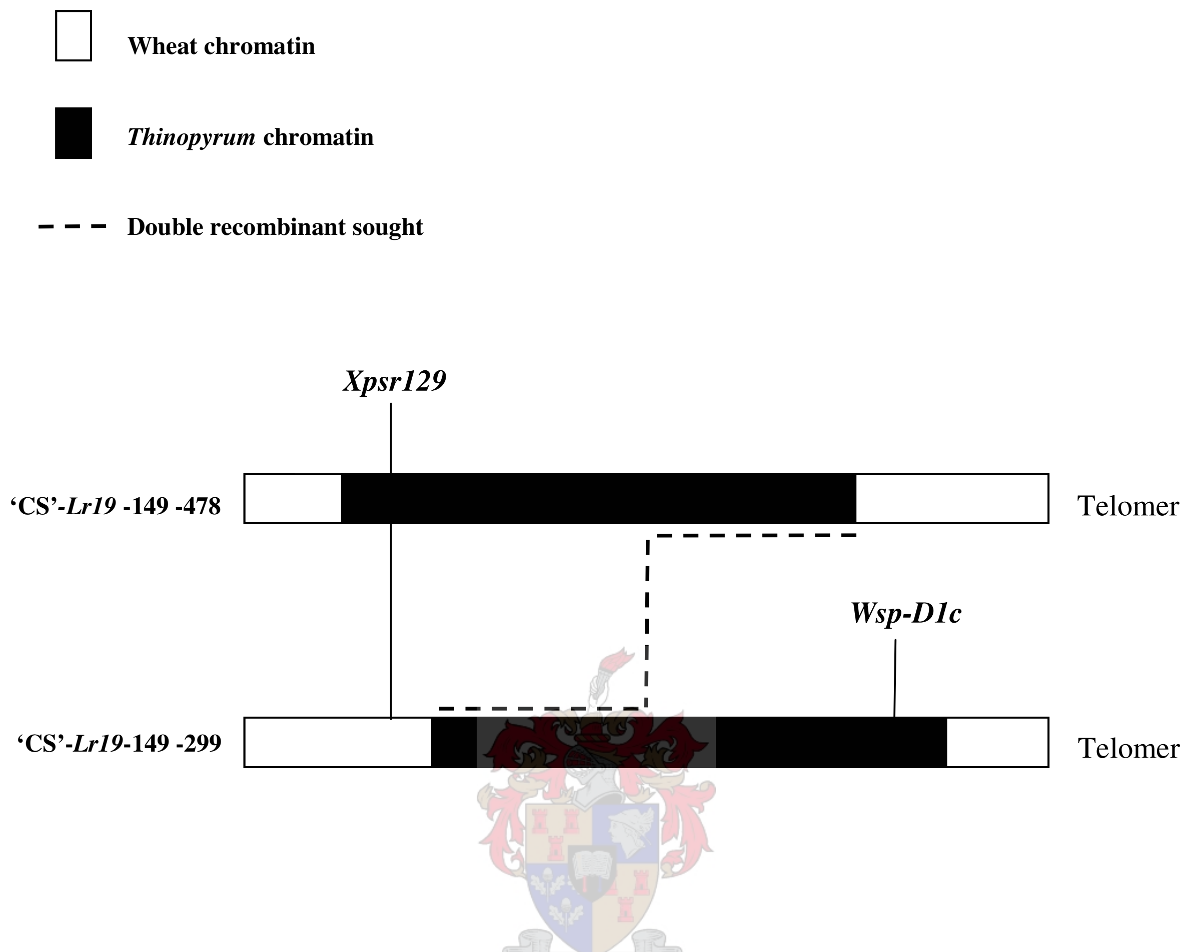


Figure 2.1 Anticipated recombination of the 'CS'-*Lr19*-149-299 and 'CS'-*Lr19*-149-478 translocations in the F_1:00M96 to produce a double tertiary recombinant.

A total of 200 testcross F_1 seeds were numbered, then halved and the embryos planted. The endosperm halves were used to extract and separate water soluble proteins (WSPs) to test for the presence of *Wsp-D1c*, whereas the seedlings were inoculated with the leaf rust pathotype UVPrt8 to test for the presence of *Lr19*. Resistant seedlings that did not express the *Wsp-D1c* allele, were used for DNA extraction (carborandum) and CAPS marker analysis (a *Xpsr129* specific SCAR). Testcross F_1 plants that were resistant yet lacked both markers, were selected. These were characterized for the physically mapped markers to authenticate them as being shortened translocations. Recombinants 4772, 4773 and 4774 were also characterized for the *Wsp-D1c* and *Xpsr129* loci.

2.2.1 *Wsp-D1c*-analysis

The water-soluble proteins (WSPs) were separated by isoelectric focusing, utilizing a modification of the procedure described by Liu *et al*. (1989). The endosperm half of each mature F_1 seed was crushed and incubated in 70 µl of distilled water overnight at room temperature. Prior to loading the samples, the extracts were centrifuged at 15 000 rpm for 25 minutes at room temperature, after which 30 µl of supernatant was loaded directly on the anodal end of a 150 mm x 125 mm x 0.25 mm 7% w/v acrylamide:*bis*-acrylamide (35.5:1 w/w) gel with 2% w/v ampholyte (consisting of three parts Pharmalyte, pH 5-8; one part Pharmalyte, pH 8-10.5; and one part Servalyte, pH 9-11) and 13% glycerol. 0.25 M HEPES was used as anolyte and 0.2 M ethylene diamine as catholyte. Electrophoresis was performed at 4 ^{o}C on a Hoefer Isobox unit after prefocusing for 30 minutes at 13 W and running for three hours at the same setting.

The gels were stained following the procedure of Radola (1980). After electrophoresis was completed, the gel was fixed in 20% TCA for 10 minutes, separated from the carrier plate using distilled water and stained for two minutes with 'Serva Violet-49' (80% w/v) which was solubilized in a solvent containing 25% methanol and 10% glacial acetic acid. The gel was destained in small aliquots of solvent until the background was clear and spread onto a white paper sheet and air-dried.

2.2.2 CAPS marker used to test for *Xpsr129-7el$_1$*

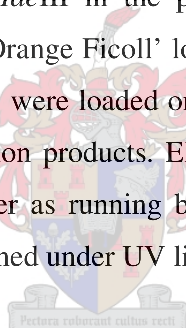
The SCAR primer pair was derived from the sequence of the RFLP probe PSR129 and used to test for the presence of the *Xpsr129-7el$_1$* allele in *Lr19* forms (Marais *et al*., 2000). The SCAR primer pair amplifies fragments of one length (1.14 kb) which are then digested with the restriction endonuclease, *Hae*III (Roche), to produce wheat specific and *Thinopyrum* specific digestion products that are separated on an agarose gel.

A 'PCR Master Mix' (Promega) solution was used for optimisation of the PCR reaction. 'PCR Master Mix' is a premixed, ready-to-use solution containing 0.05 units/µl *Taq* DNA polymerase supplied in a proprietary reaction buffer (pH 8.5), 400 µM of each dNTP and 3 mM $MgCl_2$. 'PCR Master Mix' has been optimised by the manufacturers for use in routine PCR reactions for amplifying DNA template in the range of 0.2 – 2 kb. PCR reactions were performed in 0.2 or 0.5 ml PCR tubes, depending on the type of PCR thermal cycler that was used. A total volume of 25 µl was used for each reaction, which consisted of 20-250 ng template DNA, 0.3-0.5 pmol of each

primer (10 pmol), 12.5 µl of 'PCR Master Mix' and nuclease-free water. Optimal reactions included 50 ng template DNA and 1.5 µl of each primer (10 pmol). The PCR reaction was performed in a GeneAmp® PCR System (Applied Biosystems) or a Hybaid PCR Sprint Thermal Cycler and involved (a) two to four minutes at 94-95 ^{o}C; (b) 33 or 40 cycles, each consisting of one minute at 94 or 95 ^{o}C, one minute at 55 ^{o}C, two minutes at 72 ^{o}C; (c) five minutes at 72 ^{o}C and (d) a final soak temperature of 4°C. The optimal PCR program involved an initial denaturation step at 95 ^{o}C for two minutes with subsequent denaturation steps at 95 ^{o}C for one minute, and 40 cycles.

PCR amplification of a 1.14 kb fragment was confirmed on a 1% agarose gel by loading 5 µl of the PCR reaction, using 'Orange Ficoll' as loading buffer. The gel was electrophoresed for one hour at 65 V, using 1x TBE buffer (100 mM Tris-HCL, 100 mM boric acid, 2 mM Na_2EDTA, pH 8.3) as running buffer. Products were visualized after ethidium bromide staining.

Following PCR product confirmation, the remaining 20 µl of the amplification reaction was digested overnight with two units of *Hae*III in the presence of 1x reaction buffer at 37 ^{o}C. Following overnight digestion, 5 µl of 'Orange Ficoll' loading buffer was added to each sample to stop the enzymatic reaction. The samples were loaded on a 1.5% agarose gel to separate the wheat specific and *Thinopyrum* specific digestion products. Electrophoresis was allowed to proceed for two hours at 65 V, using 1x TBE buffer as running buffer. The products were visualized after ethidium bromide staining and photographed under UV light.



Presence of the *Thinopyrum* allele for *Xpsr129* results in a *Thinopyrum* specific fragment of 0.6 kb, which indicates the presence of an unaltered copy of translocation –299. SCAR reactions were repeated for those samples that tested negative for the *Xpsr129-7el*$_1$ locus. F_2 seeds were harvested from each resistant testcross F_1 plant that tested negative for both *Wsp-D1* and *Xpsr129-7el*$_1$. Resistant F_2 seedlings from each of the selected families were tested again for the presence or absence of *Lr19* and *Wsp-D1* employing DNA extracts, performed according to Doyle & Doyle (1990).

Table 2.3 Excerpt of physical map data of the *Lr19* translocation relevant to the present study (+ indicates presence and – absence of a gene).

	Centromere◀------------------------Marker bin[1]----------------------------▶Telomere												
	1-3	**4**	**5**	**6-9**	**10**	**11**	**12**	**13-14**	**15**	**16**	**17**	**18**	**19**
Number of markers in bins	**11**	**1**	**1**	**14**	**17**	**4**	**25**	**7**	**9**	**9**	**1**	**4**	**5**
Markers relevant to present study		*Xpsr165*	*Xpsr105*		*Xpsr129*		*Xgw380*		***Lr19***	7a	*WspD1c*		**Y**
							XcsIH81-1		7b	126c			*Sr25*
							27a		27b	12c=SCAR(*STSLr19*$_{130}$)[4]			*Xpsr687*[4]
							137c		54a				
							138a		56a				
									126a				
									137a				
									138c				
									XksuE18-7Bb[4]				
Subset of mutants that delineate bins 15-19													
89M2-40	+	+	+	+	+	+	+	+	-	-	-	-	-
87M23-103	-[2]	+	+	-[2]	+	+	+	+	+	-	-	-	-
89M-245	+	+	+	+	+	+	+	+	+	+	-	-	-
89M2-426	+	+	+	+	+	+	+	+	+	+	+	-	-
87M23-225	+	+	+	+	+	+	+	+	+	+	-[3]	+	-
89M1-18	+	+	+	+	+	+	+	+	+	+	+	+	+
87M23-266	+	+	+	+	+	+	+	+	+	+	+	+	+

[1]Markers were assigned to bins making use of a set of 27 deletion lines (Groenewald, 2001).

[2]Mutant 87M23-103 has an intercalary deletion in bin 1, as well as an intercalary deletion spanning bins 7 and 8**.**

[3]This line has an intercalary deletion in bin 17.

[4]Additional markers added in the present study.

3. Results and Discussion

3.1 Recombination between the 'CS'-*Lr19*-149-299 and 'CS'-*Lr19*-149-478 translocations

Of the 200 embryo halves that were planted, four did not germinate. One of 196 seedlings was susceptible to leaf rust pathotype, UVPrt8 while another one died. Testing of 194 surviving resistant seedlings for the presence of the distally located *Thinopyrum* allele, *Wsp-D1c* (Figure 3.1), showed that 120 retained it. Of the remaining 74 that had lost the allele, 57 expressed the *Thinopyrum* allele for *Xpsr129* and 17 did not (Figure 3.2). The latter 17 plants were putative recombinants.

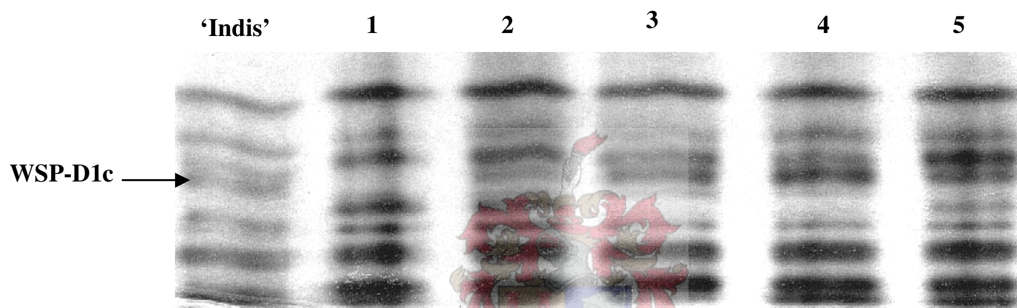


Figure 3.1 Isoelectric focusing of water soluble proteins. The WSP-D1c band is present in lanes 2, 3, 4 and 5, but absent in lane 1. 'Indis' (*Lr19* carrier) is the control genotype that has *Wsp-D1c*.

Pectora roborant cultus recti

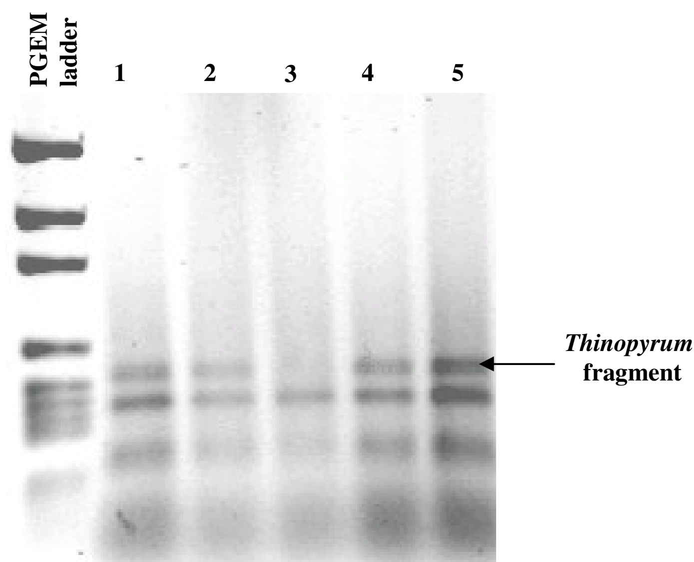


Figure 3.2 SCAR analysis to test for the presence of the *Xpsr129-7el$_1$* allele. Lane 1 is 'Indis' (positive control), lanes 2, 4 and 5 also expressed the locus, whereas 3 did not.

3.1.1 Segregation of resistance and *Wsp-D1c*

Seventeen TF_1 00M96 progeny were resistant, yet did not retain the *Thinopyrum* alleles *Wsp-D1c* and *Xpsr129-7el$_1$*. Four of these (00M96-88, -142, -173, -176) were selected for further characterization although in some instances some of the other recombinants had also been studied. Segregation of the *Lr19* resistance was confirmed in the F_2 of the selected families by inoculating 28 seedlings of each with UVPrt 8. On average, nine seedlings of each for the four families were susceptible and an average of 18 were resistant (segregation ratio 66:34). These results confirmed that the segregation distortion factor, *Sd2*, associated with recombinant *Lr19*-149 (Prins and Marais, 1998) as well as with the secondary recombinants (*Lr19*-149-252, -299, -462 and -478) (Marais *et al*; 2001) was also present in the four double recombinants. Segregation of the resistance was also studied in a number of F_2-derived F_3 families in an attempt to derive homozygous lines. Homozygous lines were obtained of selections 00M96-88, -142, and –173 but not of 00M96-176.

3.2 Endopeptidase screening of *Lr19* recombinants

The endopeptidase (*Ep-1*) polymorphisms of the original *Lr19* translocation (7DL), the primary recombinant; *Lr19*-149 (7BL) and the four secondary recombinants (*Lr19*-149-252, -299, -462, -478) were compared (Figure 3.3).

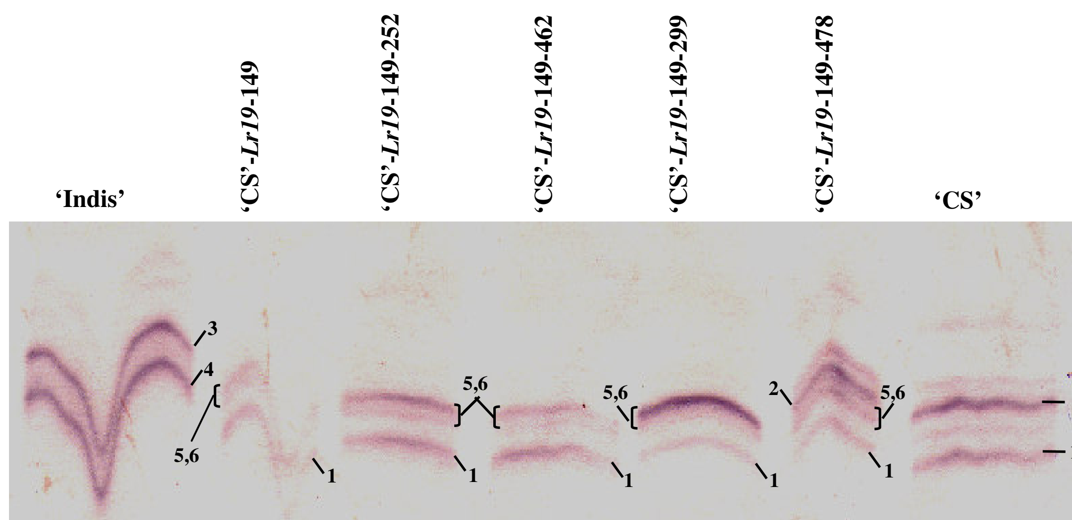


Figure 3.3. Zymogram of 'CS'-*Lr19*-149 recombinants (1=*Ep-D1a*; 2=*Ep-B1a*; 3=*Ep-A1b*; 4=*Ep-B1c*; 5+6=undesignated loci)

'Indis' and 'CS' were used as controls. Absence of an *Ep-D1a* product is linked to the presence of the complete *Lr19* translocation which is located on chromosome 7DL in 'Indis' (Marais and Marais, 1990). The *Ep-D1a* band is, however, expressed in 'CS', since it lacks *Lr19*. The zymogram (Figure 3.3) shows expression of the *Ep-D1a* band in all the recombined translocation lines since they carry *Lr19* on 7BL. The *Ep-B1a* band was absent in all the recombinants barring 478 which confirms the relocation of the *Lr19* segment from 7DL to 7BL in *Lr19*-149 and derivatives as was reported by Prins *et al*. (1996). In recombinant 'CS'-*Lr19*-149-478 the *Ep-B1a* locus was also expressed. This would suggest that either (a) the 'CS'-*Lr19*-149-478 source used segregated and that the profile represented a heterozygote or, (b) that in recombinant 478 the normal wheat *Ep-B1a* locus was restored during the allosyndetic recombination event (Marais *et al*., 2001)

To determine which, endopeptidase analysis was done on seedling leaves of each of 'CS', 'CS'-Nullisomic-7B, 'CS'-*Lr19*, 'CS'-*Lr19*-149-478 and F_2:00M76 (= 'CS'-Nullisomic-7B/'CS'-*Lr19*-478 which contained only a translocated chromosome 7B from 'CS'-*Lr19*-149-478) (Figure 3.4). All genotypes were first tested for resistance to confirm the presence or absence of *Lr19*. It was clear from the results that 'CS'-*Lr19*-149-478 does not express *Ep-B1* and that the plant in Figure 3.3 was a heterozygote.

Pectora roborant cultus recti

Figure 3.4 Endopeptidase zymograms of *Lr19* recombinants and control genotypes

Endopeptidase analysis was also done on 10 putative double recombinants (00M96-88, -101, -119, -129, -142, -154, -169, -170, -173, -176) of which six to seven seeds were used of each. The *Ep-D1a* band was present in all of the recombinants which confirmed presence of a normal chromosome 7D. Figure 3.5 shows a zymogram of seven of the putative double recombinants.

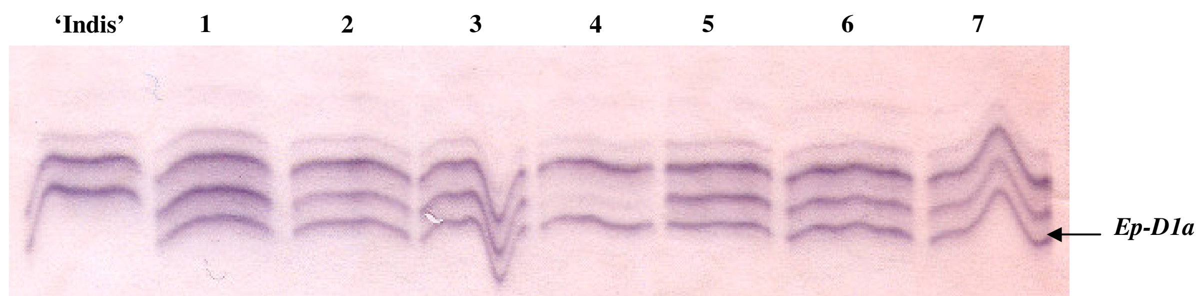
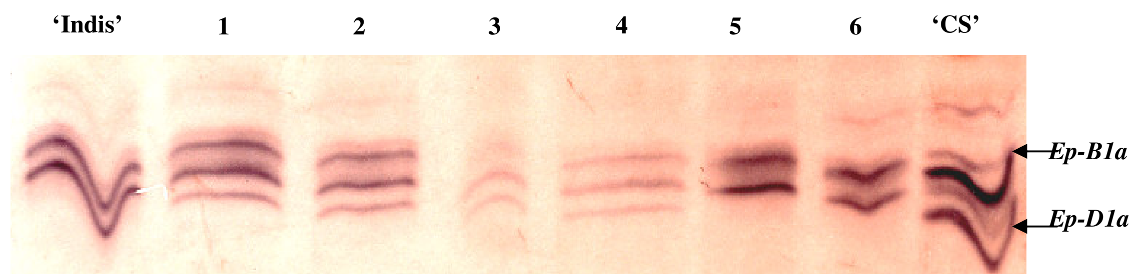


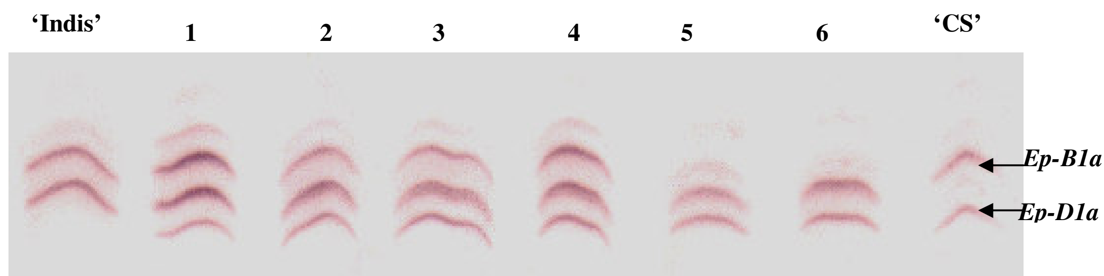
Figure 3.5 Endopeptidase zymogram of suspected double recombinants

Endopeptidase zymograms were also obtained of six segregates from each of three families segregating for the University of California recombinants 4772, 4773 and 4774. In the zymogram of recombinant 4772 (Figure 3.6a), the *Ep-Dlb* band was absent in two of the extracts. This would suggest that a section of *Thinopyrum ponticum* chromosome 7el$_1$ carrying *Lr19* was translocated to 7DL of wheat in this line. Since the seeds derived from a segregating population, some of the plants (translocation heterozygotes) did have a normal 7DL arm and thus expressed *Ep-D1a*.

(a) 4772 segregates



(b) 4773 segregates



(c) 4774 segregates

Figure 3.6 a, b and c. Endopeptidase zymograms of segregating progenies of University of California *Lr19* recombinants 4772, 4773 and 4774, respectively.

Since the 4772 translocation is associated with 7DL whereas *Lr19*-149-478 occurs on 7BL, it will not be possible to combine the two translocations in order to derive a shorter recombinant through meiotic crossover. In the zymograms of 4773 and 4774, however, an *Ep-D1a* band was always expressed while there was reduced expression or absence of a band with a more negative iso-electric point in some of the extracts (Figures 3.6 b and c). Since the genetic background of these lines was not 'CS', the identity and chromosome location of the loci involved cannot be inferred. Thus, the translocation in 4773/4774 occurred to either 7AL or 7BL.

3.3 Physical mapping of RFLP loci and analysis of recombinants

3.3.1. Characterization of recombinants with mapped RFLP probes PSR129, CSH81-1 and WG380

3.3.1.1 Physical map location of *Xpsr129*, *XcsIH81-1* and *XmWg380*

Groenewald (2001) extended a physical map of the *Lr19* translocation developed by Marais (1992a), Prins *et al.* (1996), and Prins and Marais (1998). The map summarizes data obtained with 104 markers grouped into 19 bins making use of a set of deletion lines (Table 2.3, p. 48). Only the marker data relevant to the present study are summarized here. Also included in Table 2.3 are information about the subsets of mutants that were used in this study to organize new and existing markers on the physical map.

3.3.1.2 Confirmation of recombinants with RFLPs PSR129, CSH81-1 and WG380

The RFLP probes (PSR129, CSH81-1 and WG380) were previously used to derive a physical map of the *Lr19* translocation and recombinants *Lr19*-149, *Lr19*-149-252, *Lr19*-149-462, *Lr19*-149-478 and *Lr19*-149-299 (Prins *et al*., 1996, 1997, 1998; Marais *et al*., 2000). These markers mapped proximally to *Lr19* with *Xwg380* being closest to *Lr19* (Table 2.3). The three markers were applied to all the primary, secondary and tertiary recombinants employed in the present study (Figures 3.7-3.9).

The present results confirmed those obtained by Marais *et al.* (2000) namely that the three secondary recombinants, i.e. *Lr19*-149-252, -299 and –462, have each lost the *Xpsr129-7el*$_1$ locus, whilst recombinant –299 has also lost the *XcsIH81-1-7el*$_1$, and *Xwg380* loci. *Xwg380* maps closest to *Lr19* (Prins and Marais, 1998). The 7el$_1$-alleles of all three markers were therefore replaced by corresponding 7BL-alleles in *Lr19*-149-299 as well as in the 00M96 putative double recombinants. This confirmed that the proximal break point in the tertiary recombinants is the same as in *Lr19*-149-299. These results are integrated in an updated physical map of the translocation presented as Figure 3.25 (p. 78).

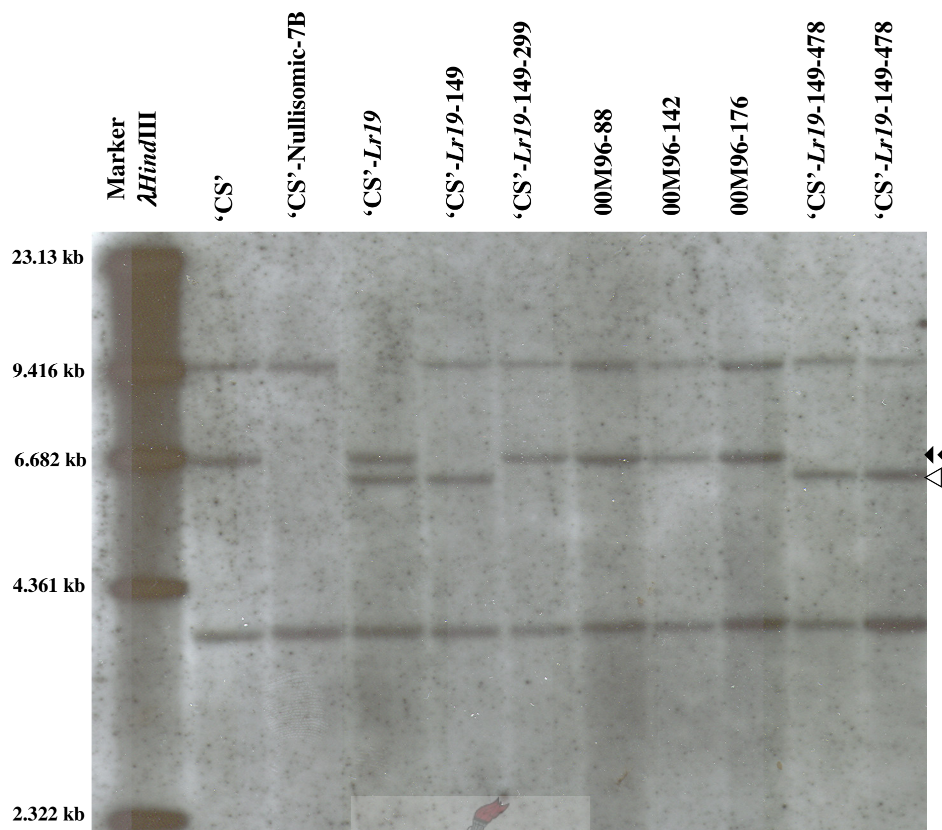


Figure 3.7a Hybridization pattern of PSR129 on the control genotypes, *Lr19* recombinants and 00M76 derivatives, which were digested with *Hind*III. ◁ *Thinopyrum* 7el$_1$ fragment, and ◀◀ wheat chromosome 7BL fragment.

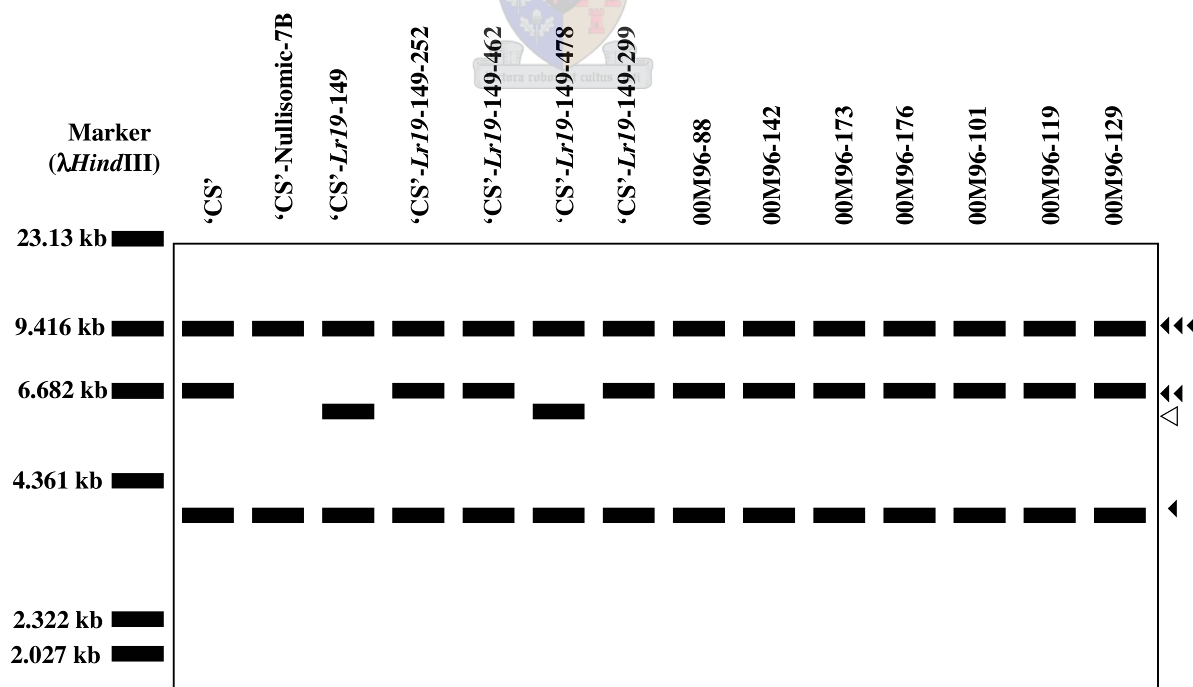


Figure 3.7b Schematic representation of RFLP data for probe **Psr129** control genotypes, *Lr19* recombinants and putative double recombinants (00M96 entries), which were digested with *Hind*III. Lambda DNA (λ *Hind*III) was used as size marker. ◁ *Thinopyrum* 7el$_1$ fragment, ◀ and ◀◀◀ wheat chromosome 7AL and 7DL fragments, respectively (Prins and Marais, 1998), and ◀◀ wheat chromosome 7BL fragment.

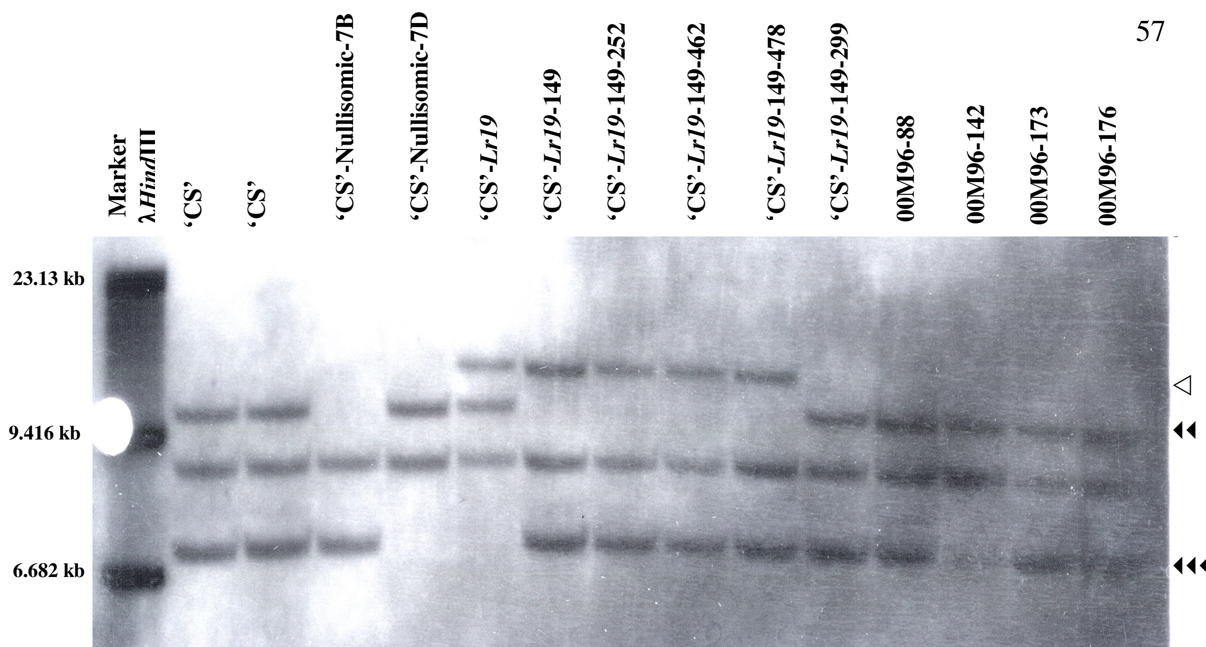


Figure 3.8 Hybridization patterns for **CSH81-1** on the control genotypes, *Lr19* recombinants and putative double recombinants (00M96 entries), which were digested with *Hind*III. ◁ *Thinopyrum* 7el$_1$ fragment, ◀◀ wheat chromosome 7BL fragment, and ◀◀◀ wheat chromosome 7DL fragment.

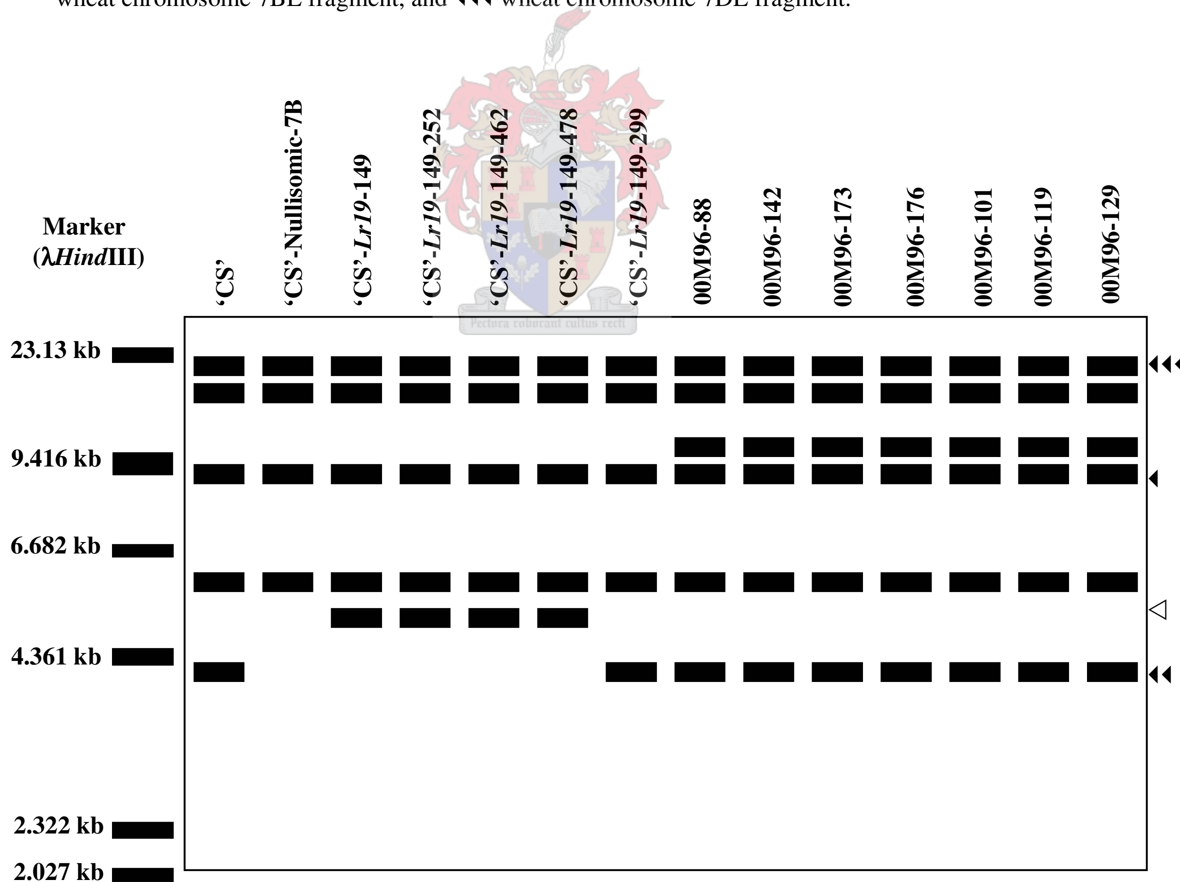


Figure 3.9 Schematic representation of RFLP data for **WG380**, control genotypes, *Lr19* recombinants and putative double recombinants (00M96 entries), which were digested with *Hind*III. Lambda DNA (λ *Hind*III) was used as size marker. ◁ *Thinopyrum* 7el$_1$ fragment, ◀ and ◀◀◀ wheat chromosome 7AL and 7DL fragments, respectively (Prins and Marais, 1998), and ◀◀ wheat chromosome 7BL fragment.

3.3.2 RFLP probe PSR687

3.3.2.1 Physical mapping of PSR687

PSR687 detected a distinct restriction fragment associated with the *Lr19* translocation (Figure 3.10). RFLPs were therefore also produced using seven previously characterized *Lr19* deletion mutants (Table 2.3). The *Thinopyrum* specific fragment was present in the two mutants (H and I) which have the smallest and most distal deletions (Figure 3.10). The results therefore indicate that PSR687 is located distally of the bin 18 markers (Table 2.3).

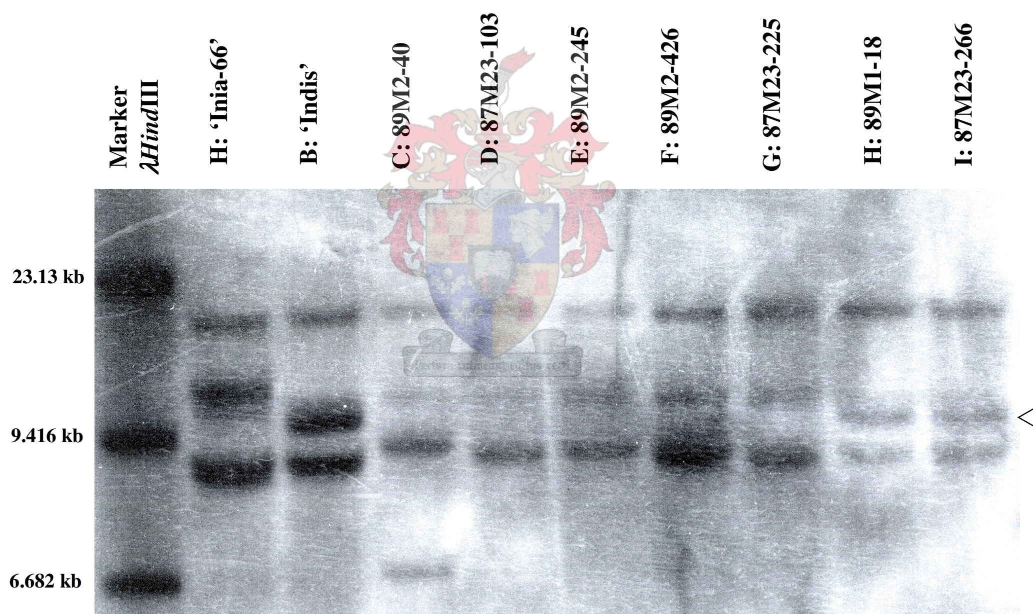


Figure 3.10 Hybridization pattern of PSR687 on control genotypes and *Lr19* deletion mutants. ◁ *Thinopyrum* $7el_1$ fragment.

3.3.2.2 Characterization of recombinants with PSR687

PSR687 was subsequently also tested on a further set of controls, *Lr19* recombinants and putative double recombinants (Figure 3.11).

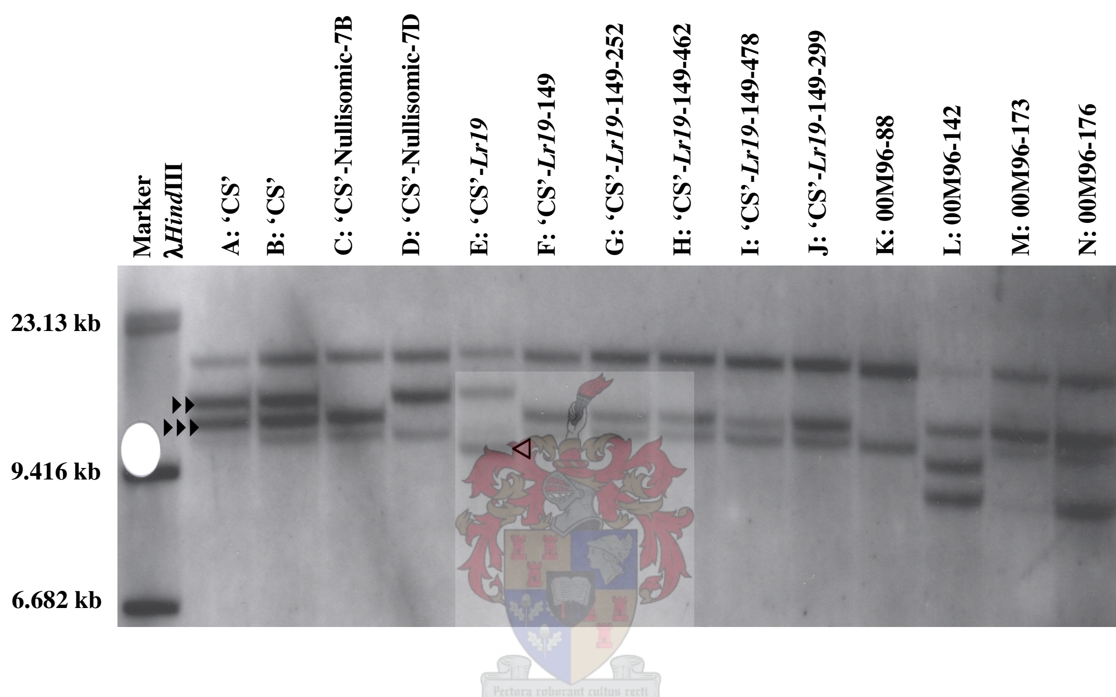


Figure 3.11 Hybridization patterns for **PSR687** on control genotypes, *Lr19* recombinants and putative double recombinants, which were digested with *Hind*III. ◁ *Thinopyrum* 7el$_1$ fragment, ◀◀ wheat chromosome 7BL fragment, and ◀◀◀ wheat chromosome 7DL fragment.

The restriction fragments hybridized by PSR687 appears to be very polymorphic. Genotypes A-J all have a 'CS' background and shows variation for the same set of alleles. Genotypes K-N on the other hand derive from a testcross with W84-17 and shows segregation of a nul allele for *Xpsr687-7D*. The derivatives also show variation for fragments other than that associated with the *Xpsr687-7D* and *Xpsr687-7B* loci; among these a fragment of similar size to *Xpsr687-7el$_1$* (lane L). However, similar to the recombinants F-J, the double recombinants do not appear to express *Xpsr687-7B*, while three of them express *Xpsr687-7D*.

As could be expected if the locus occurs in bin 19 (Table 2.3), none of the recombinants or putative double recombinants expressed the *Xpsr687-7el1* allele. The approximate location of the locus on the *Lr19* physical map is given in Figure 3.25. None of the *Lr19* derivatives (F-N) expressed a *Xpsr687-7b* product either, which would suggest one of the following: (a) the loss of the yellow pigment gene in *Lr19*-149 resulted from deletion rather than recombination, (b) the ends of the *Lr19* translocation and 7BL may differ structurally and recombination has not restored the 7BL chromatin on which *Xpsr687-7b* occurs, (c) during recombination a restriction site was altered which resulted in the loss of a *7B* band.

3.3.3 RFLP probe TtksuE018

3.3.3.1 Characterization of recombinants with TtksuE018

RFLP results obtained following analysis of the recombinants and controls with probe TtksuE018 are summarized in Figure 3.12. TtksuE018 appears to recognise two chromosome 7B loci. The larger of the two 7BL fragments appears to be absent in the recombined translocations on chromosome 7B (lanes F-J), as well as in double recombinants K and M. Double recombinants L and M are probably heterozygous for the translocation and therefore express the band. The locus must therefore be located in the region of the translocation that was not effected by any of the recombination events between the *Lr19*-149-299 and *Lr19*-149-478 break point (Figure 3.25). This locus will be referred to as *XksuE18-7Ba*.

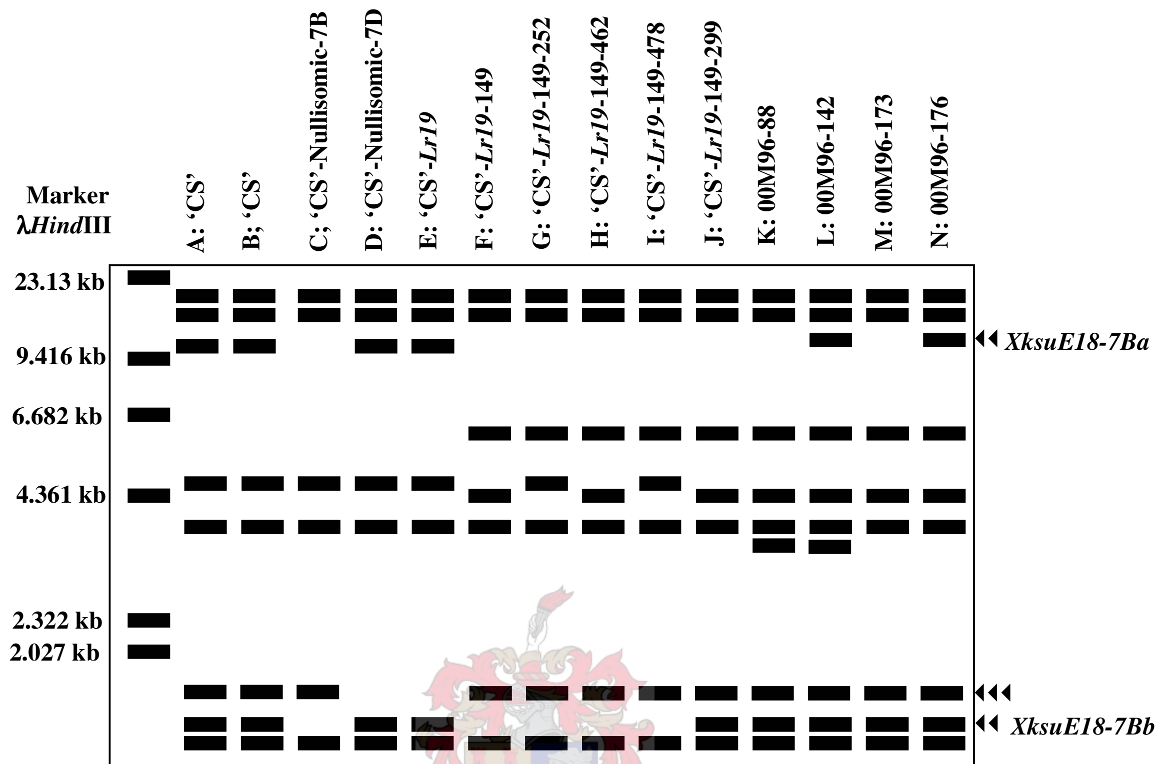


Figure 3.12 Schematic representation of RFLP data for probe **TtksuE018** on the control genotypes, *Lr19* recombinants and putative double recombinants (00M96 members), which were digested with *Hind*III. ◂◂ wheat chromosome 7BL fragment, and ◂◂◂ wheat chromosome 7DL fragment.

The smaller fragment (< 2.027 bp) is present in the controls that have a normal chromosome 7B but is absent in recombinants *Lr19*-149, -252, -462 and –478. It reappears in recombinant –299 as well as the four 00M96 double recombinants which would suggest that it is situated proximally to *Lr19* but distally from *Xpsr129-7el1* or between the *Lr19*-252/462 and *Lr19*-299 break points (Figure 3.25). The latter locus is referred to as *XksuE18-7Bb*.

3.3.3.2 Physical mapping of TtksuE018

The seven *Lr19* deletion mutants were characterised using probe TtksuE018 (Figure 3.13). None of the deletion lines showed a *Thinopyrum* specific band but all of them expressed both *XksuE18-7Ba* and *XksuE18-7Bb*. This is to be expected as the mutants occur on the chromosome 7D translocation and therefore have normal 7B chromosomes.

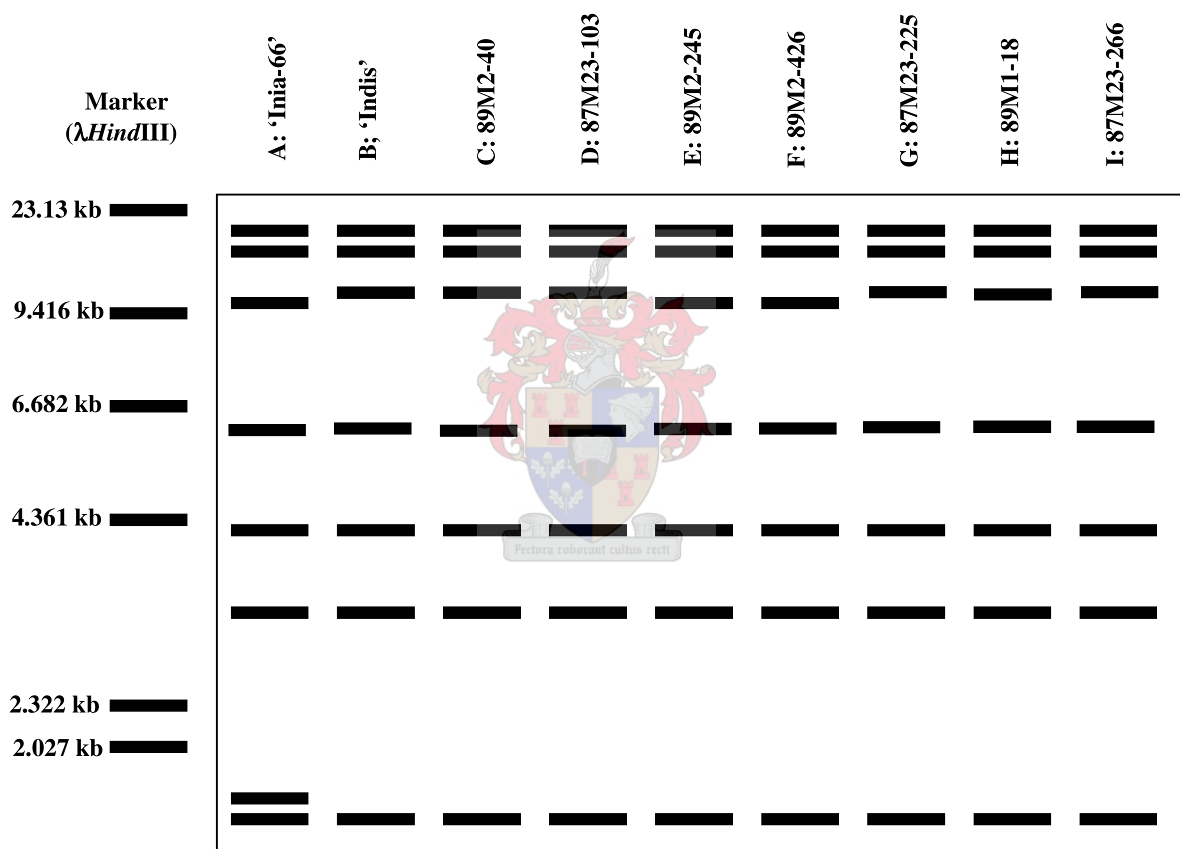


Figure 3.13 Schematic representation of RFLP data for probe **TtksuE018** on the control genotypes and seven deletion mutants of the *Lr19* translocation. ◄ *XksuE18-7D*.

3.3.4 RFLP probe CDO0414

3.3.4.1 Physical mapping of CDO0414

The hybridization pattern of CDO0414 shows a polymorphic fragment that is absent in three deletion mutants i.e. 89M2-245, 89M2-426 and 87M23-225 (Figure 3.14). However, it is present in mutants 89M2-40, 87M23-103, 89M1-18 and 87M23-266. This pattern is not reconcilable with the established physical map (Table 2.3). It is therefore unlikely to be a *Thinopyrum* locus.

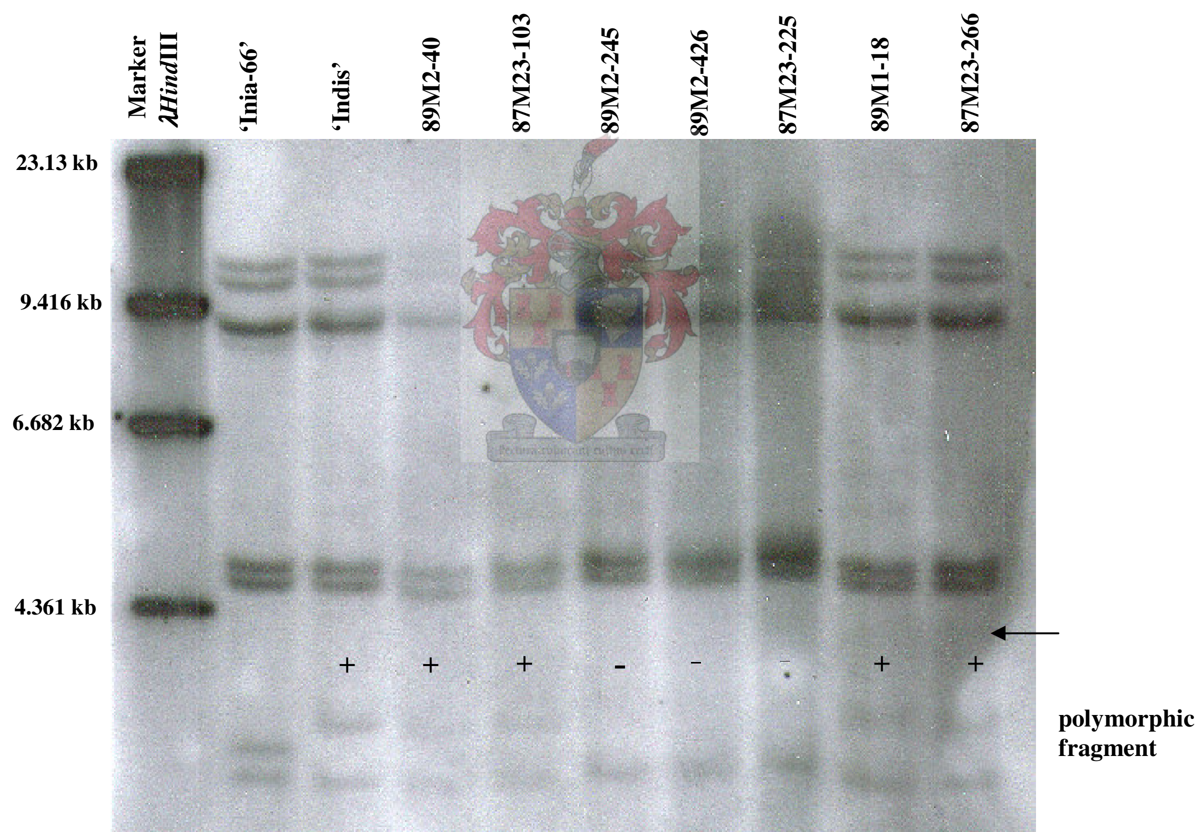


Figure 3.14 Hybridization pattern for CDO0414 with restriction (*Eco*RI) fragments produced using DNA of the deletion mutants.

3.3.4.2 Characterization of recombinants with CDO0414

Following RFLP analysis of the controls and *Lr19* recombinants with probe CDO0414, no polymorphisms, except for 'CS'-Nullisomic-7D, were seen (Figure 3.15). The polymorphic fragment, indicated in Figure 3.14 with a size smaller than 4.361 kb, could not be detected in Figure 3.15, although the same stringency conditions were used. In an attempt to obtain stronger hybridization signals, the blots were hybridized and washed employing different lower stringency conditions. A small number of bands with extremely poor hybridization signals could be visualized in only some of the genotypes of some of the blots and no polymorphism(s) could therefore be detected.

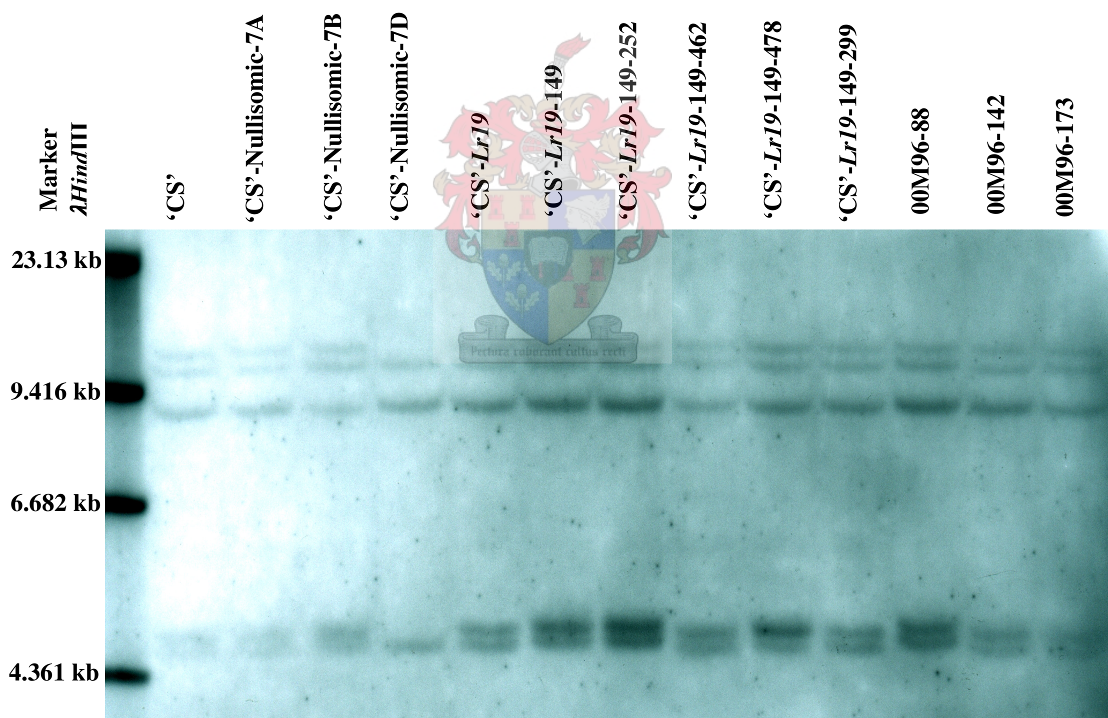


Figure 3.15 Hybridization pattern obtained with CDO0414 using DNA of controls, *Lr19* recombinants and putative double recombinants, which were digested with *Eco*RI

3.4 Amplified fragment length polymorphism (AFLP) analysis

Groenewald (2001) mapped a number of AFLP loci in the proximity of *Lr19* making use of a full set of deletion mutants. Twelve of these markers were used here in an attempt to characterize the recombinants. Absence (-) or presence (+) of the 12 polymorphic AFLP products are summarized in Table 3.1 whereas autoradiographs obtained with two of the primer sets are given in Figures 3.16 and 3.17.

The AFLP profile of marker 138c is shown in Figure 3.16. This marker was one of seven AFLP markers that mapped in the same bin as *Lr19* (Groenewald, 2001; Table 2.3). The *Thinopyrum*-specific fragments amplified with markers 27a, 137c, 138a and 126a did not occur in 'CS'-*Lr19*-149-299, which confirms that this translocation is proximally shorter than 'CS'-*Lr19*-149-252 and 'CS'-*Lr19*-149-462 (Table 3.1) as was concluded by Marais *et al.* (2000). The AFLP profile for marker 27a is shown in Figure 3.17. The distal markers 7a and 126c were not amplified in 'CS'-*Lr19*-149-478 which confirms that this is the distally shortest translocation as concluded by Marais *et al.* (2000).

The fragments produced by the four proximal markers (27a, 137c, 138a, 126a) and the two distal markers (7a, 126c) were not amplified in the putative tertiary recombinants either. This is to be expected, since these translocations resulted from recombination and combine the proximally shortest (299) and distally shortest (478) regions.

Fragment 126c distal was always produced in recombinant 4773 but was not consistently produced in 4772, suggesting that both has the locus but that 4772 segregated for the presence of the translocation. Segregation of 4772 was also evident with the endopeptidase analysis. It appears that the translocation in 4772 is proximally shorter than that in 4773, since a *Thinopyrum* fragment was not amplified by marker 126a in 4772 whereas a fragment was produced in 4773. The order of the loci in present results bins 12,15 and 16 could not be determined by Groenewald (2001). Present results (Table 2.3), confirmed that markers 7a and 126c are located distally from *Lr19* but proximally from *Wsp-D1c*. The data also suggest that marker 126a is the most proximal marker in bin 15 (Figure 3.25).

Table 3.1 Characterization of *Lr19* translocatioins and recombinant forms with 12 AFLP markers

Genotype	AFLP marker (see Table 2.2)												
	27a	137c	138a	126a	7b	27b	54a	56a	137a	138c	126c	7a	Wsp -D1c
'Indis'	+	+	+	+	+	+	+	+	+	+	+	+	+
'Inia-66'	-	-	-	-	-	-	-	-	-	-	-	-	-
'CS'	-	-	-	-	-	-	-	-	-	-	-	-	-
'CS'-*Lr19*-149	+	+	+	+	+	+	+	+	+	+	+	+	+
'CS'-*Lr19*-149-252	+	+	+	+	+	+	+	+	+	+	+	+	+
'CS'-*Lr19*-149-462	+	+	+	+	+	+	+	+	+	+	+	+	+
'CS'-*Lr19*-149-478	+	+	+	+	+	+	+	+	+	+	-	-	-
'CS'-*Lr19*-149-299	-	-	-	-	+	+	+	+	+	+	+	+	+
'CS'-*Lr19*-149	+	+	+	+	+	+	+	+	+	+	+	+	+
00M96-88	-	-	-	-	+	+	+	+	+	+	-	-	-
00M96-142	-	-	-	-	+	+	+	+	+	+	-	-	-
00M96-173	-	-	-	-	+	+	+	+	+	+	-	-	-
00M96-176	-	-	-	-	+	+	+	+	+	+	-	-	-
00M96-101	-	-	-	-	+	+	+	+	+	+	-	-	-
00M96-119	-	-	-	-	+	+	+	+	+	+	-	-	-
00M96-129	-	-	-	-	+	+	+	+	+	+	-	-	-
00M96-154	-	-	-	-	+	+	+	+	+	+	-	-	-
'CS'	-	-	-	-	-	-	-	-	-	-	-	-	-
4772	-	-	-	-	+	+	+	+	+	+	-/+	+	-
4773	-	-	-	+	+	+	+	+	+	+	+	+	-

+ *Thinopyrum* chromatin

\- Wheat chromatin

-/+ Fragment not consistently produced in all plants

Xwg380-7el$_1$ ◄- - - ► (27a–137c)

Lr19 ◄- - - ► (138a–138c)

↑ *Wsp-D1c*

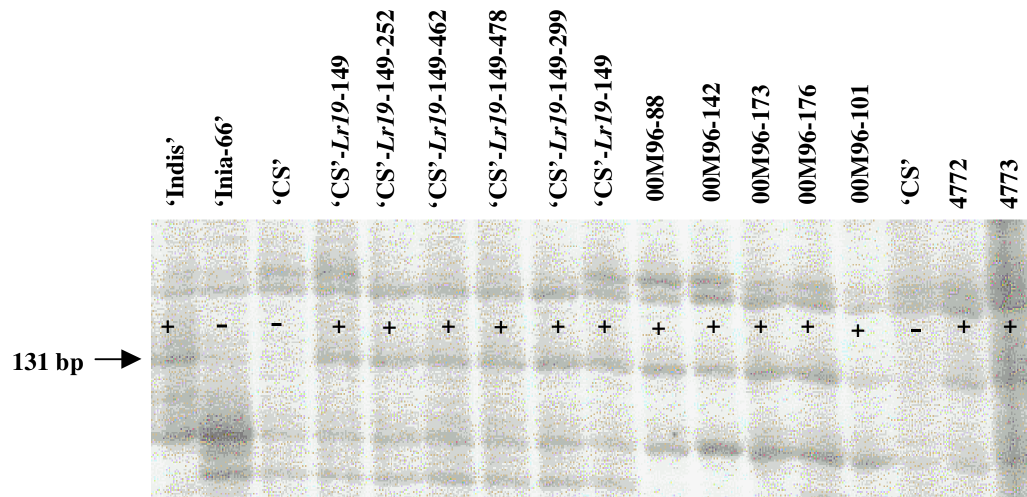


Figure 3.16 Partial autoradiograph of amplification products derived with 138c:S-T/M-TGT_{131}

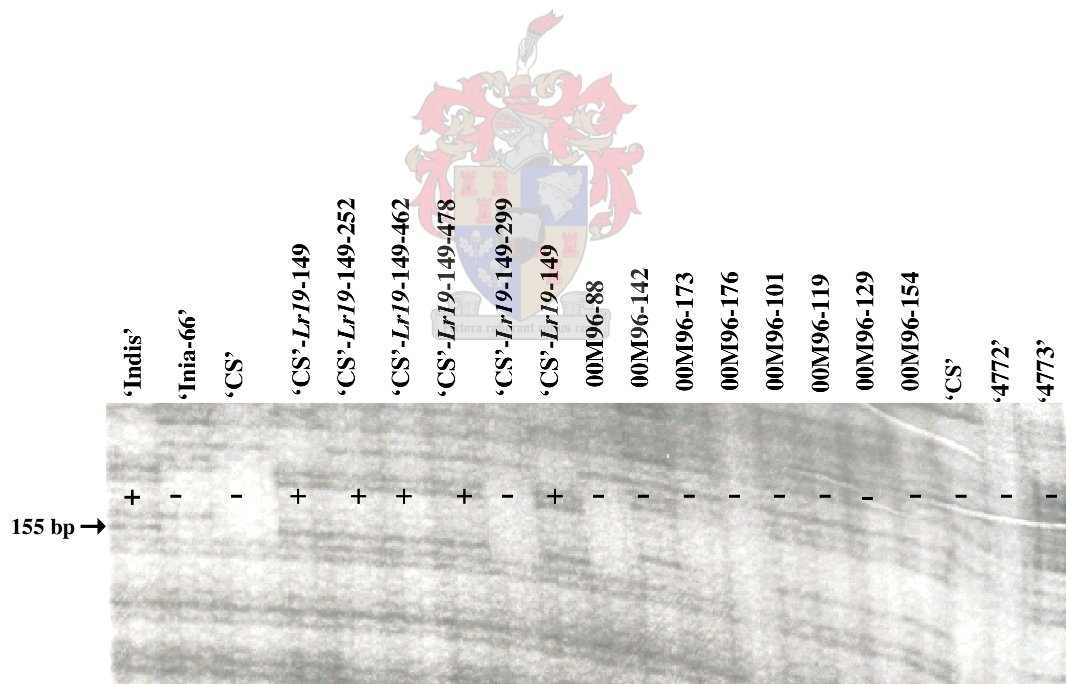


Figure 3.17 Partial autoradiograph of amplification products derived with marker 27a:S-AC/M-AT155

3.5 Sequence tagged site (STS) and sequence characterized amplified region (SCAR) analysis

The *Lr19* translocations and control genotypes were also tested for the presence of STS marker, STS*Lr19*$_{130}$ (Prins *et al*., 2001), and SCAR marker, SCS73$_{719}$ (Cherukuri *et al*., 2003). Marker STS*Lr19*$_{130}$ is derived from the AFLP fragment 12c of bin 16 (Table 2.3).

3.5.1 STSLr19$_{130}$

The 130 bp PCR fragment that constitutes this marker, was amplified in the four *Lr19*-149 recombinants and the control genotype,' Indis' (Figure 3.18).

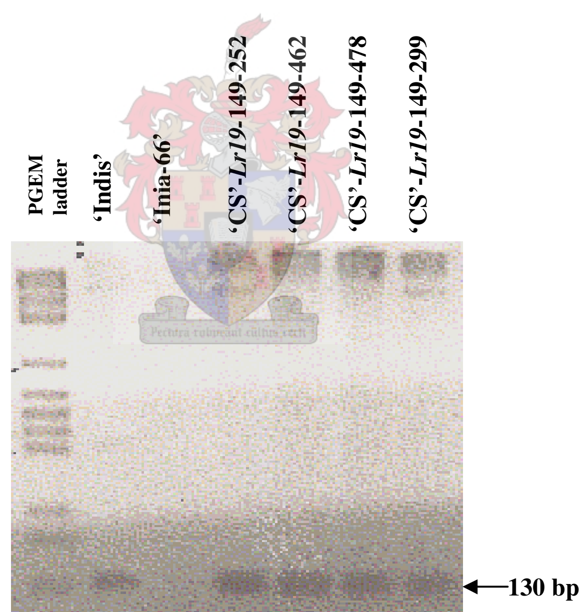


Figure 3.18 STS analysis of *Lr19* recombinants with marker STSLr19$_{130}$

The marker was not amplified in the control genotype, 'Inia-66'. Amplification of this product in the four secondary recombinants, means that it can not be used to differentiate between distal and proximal recombinants. However, if double recombinants of 'CS'-*Lr19*-149-299 and 'CS'-*Lr19*-149-478 can be selected, this STS can still be used as a marker of its presence.

The $STSLr19_{130}$ fragment was not produced by the deletion mutants 89M2-40, 87M23-103 and 87M23-225 (Figure 3.19).

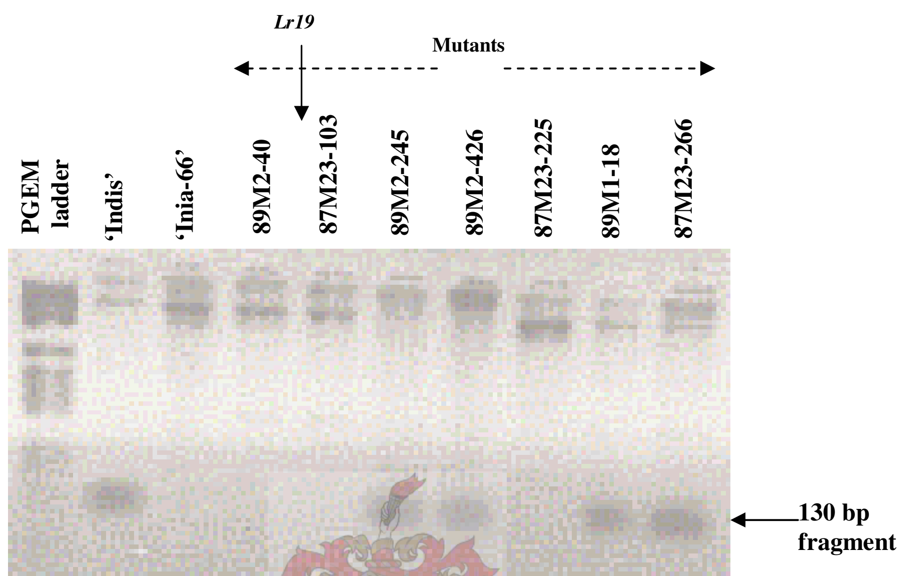


Figure 3.19 STS analysis of *Lr19* translocation terminal deletion mutants with marker $STSLr19_{130}$ (the deletions are ordered from the largest to the smallest)

Apart from its terminal deletion, mutant 87M23-225 also has an intercalary deletion that spans two AFLP loci as well as the *Wsp-D1c* locus (Groenewald, 2001). The results obtained with the deletion mutants, confirm that $STSLr19_{130}$ is located in bin 16. The results obtained with the recombinants similarly suggest that the locus is located distally *Lr19*, between *Wsp-D1c* and *Lr19* as *Lr19*-149-478 still expresses $STSLr19_{130}$ but does not produce a *Wsp-D1c* product (Prins *et al.*, 2001). The marker may be situated closer to *Wsp-D1c*, as both loci occur within an intercalary deletion (Figure 3.19) in mutant 87M23-225 which spans the 81b, 126c and *Wsp-D1c* loci.

The fact that $STSLr19_{130}$ does not map physically close to *Lr19* does not pose problems for its use in marker-assisted selection (Prins *et al.*, 2001), since the alien segment (both the original and recombined forms) does not recombine with its wheat homoeologue in the presence of the homoeologous pairing gene *Ph1* (Marais, 1992).

3.5.2 SCAR marker $SCS73_{719}$

The PCR protocol provided for marker $SCS73_{719}$ (Cherukuri *et al.*, 2003) did not result in amplification of a single pronounced band of 719 bp in the *Lr19*-carrier genotypes, 'Indis' and deletion mutant 87M23-266, or in the controls, 'CS' and W84-17, which lack *Lr19* (data not shown). Instead, multiple faint bands were amplified. Background smears were also observed. The results were uncharacteristic of a SCAR, which is a genomic DNA fragment that is specifically amplified at a single genetically defined locus by a pair of long oligonucleotide primers (Wechter *et al.*, 1998).

Optimisation of the PCR protocol was therefore attempted in order to obtain a single DNA fragment. However, a complex of faint bands was always observed during optimisation, even with the use of high stringency conditions. A polymorphic fragment with an average size of 250 bp was detected under certain PCR conditions. The fragment was best observed with 2 mM $MgCl_2$ and 200 μM of each dNTP used in the reactions, amplified with the PCR program provided, except for the annealing temperature which was 59°C (Figure 3.20).

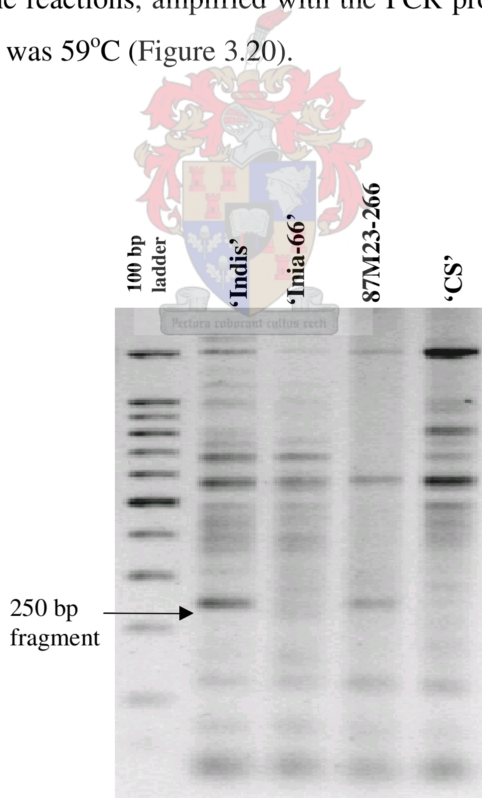


Figure 3.20 Amplification product of 250 bp amplified with marker $SCS73_{719}$ in two *Lr19* carriers ('Indis' and 87M23-266) and the controls 'Inia-66' and 'CS' which *lacks Lr19.*

As the polymorphic fragment was present in the two *Lr19* lines and absent in the two non-*Lr19* lines it was tested further in order to determine whether it is specific for the *Lr19* translocation. The marker ($SCS73_{719}$) was tested on five sets of near-isogenic lines with and without the *Lr19* translocation and developed in the genotypes 'CS', 'SST66', 'W84-17', '87M90' and'Inia-66' (Figure 3.21). The optimized conditions for detection of the 250 bp fragment were used. DNA was extracted using the carborandum protocol rather than the Doyle and Doyle (1990) procedure used before. The results listed in Figure 3.21 show that the fragment is not *Lr19*-specific. With the exception of the 'Inia-66' NILs, both members of each NIL-pair always expressed the band. Neither member of the 'Inia-66' NIL-pair produced a band.

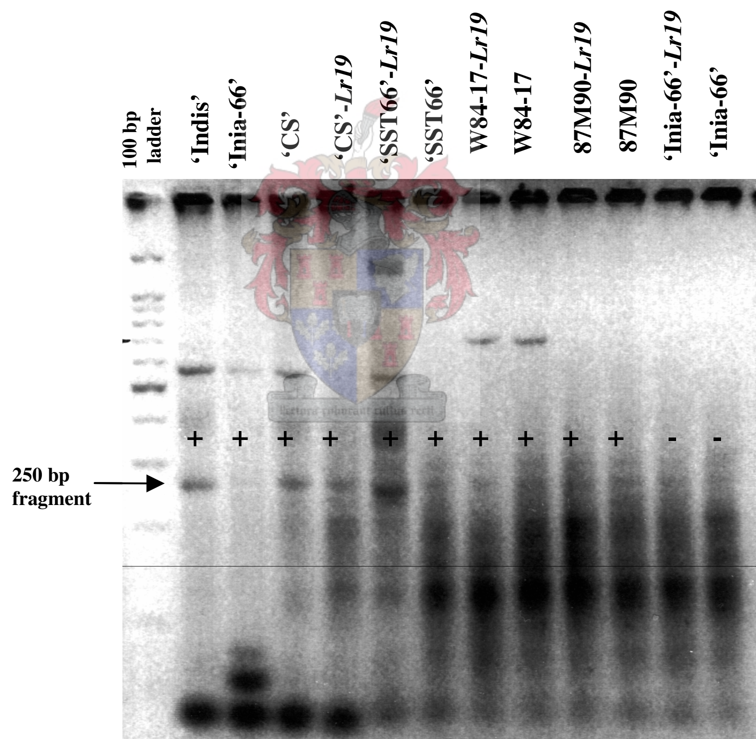


Figure 3.21 SCAR analysis (marker $SCS73_{719}$) of near-isogeneic lines with and without *Lr19.*

The majority of sequence-tagged-site primer sets developed from wheat clones generated reproducible amplifications in other laboratories (Vanichan *et al*., 2000). However, sometimes results from a particular primer pair may vary among laboratories (He *et al*., 1994). That is, a primer pair that produces a product marking a particular chromosome region for a laboratory may not produce the same product when the experiment is repeated in another laboratory. Factors that influence the repeatability of primer sets include different genetic backgrounds within the same species, annealing temperature and the quality of DNA preparation (Talbert *et al*., 1994; Blake *et al*., 1996).

According to Cherukuri *et al*. (2003) the donor of the leaf rust resistance gene *Lr19* in the material they used to develop the SCAR marker was Sunstar*6/C80-1, which apparently lacked the yellow flour pigment gene. The normal *Lr19* translocation, however, contains the yellow pigment gene (Sharma & Knott, 1966) and it is well-known that the *Lr19* translocation and wheat 7DL arms normally do not pair and recombine during meiosis (Marais *et al*., 1992). This raised doubts as to whether the authors did in fact study the *Lr19* translocation produced by Sharma & Knott (1966). Cherukuri *et al*. (2003) furthermore reported that marker $STSLr19_{130}$ developed by Prins *et al*. (2001) was non-polymorphic on the *Lr19*-carrying Indian bread wheats. This contradicted the clear-cut results of Prins *et al.* (2001) who developed and mapped this *Lr19*-specific marker. These observations suggested that $SCS73_{719}$ may in fact not be a *Lr19* marker.

In a recent report (Prabhu *et al*. 2004) it was confirmed that SCAR marker $SCS73_{719}$ did not tag *Lr19*, and that the reference stock used for its development carried the *Lr24*/*Sr24* translocation rather than *Lr19*. Monomorphic expression of the SCAR marker in 10 near-isogenic line (NIL) pairs for *Lr19* and *Lr24* revealed that each NIL pair in reality possessed the same gene, *Lr24.* The donor parents used in the NIL pairs for *Lr19* ('Sunstar*6/C80-1') and *Lr24* ('TR380-14*7/3Ag#14') yielded the same fragment. Non-segregation for leaf rust resistance in the F_2 population of the cross between the above donor parents confirmed the presence of the same gene (*Lr24*) in the two parents. Apparently, a genuine parent stock of 'Sunstar*6/C80-1' was not used in the development of the NIL pairs for *Lr19* due to improper maintenance either at source or destination which went undetected in the absence of signs of virulence for either gene in the region (India) (Prabhu *et al*., 2004).

In this study the SCAR marker was then tested on the variety 'Agent' which carries the gene *Lr24*. 'Agent' was also used in the study done by Prabhu *et al*. (2004). 'CS'-*Lr19,* as well as a negative control, containing no DNA were included as controls (Figure 3.22).

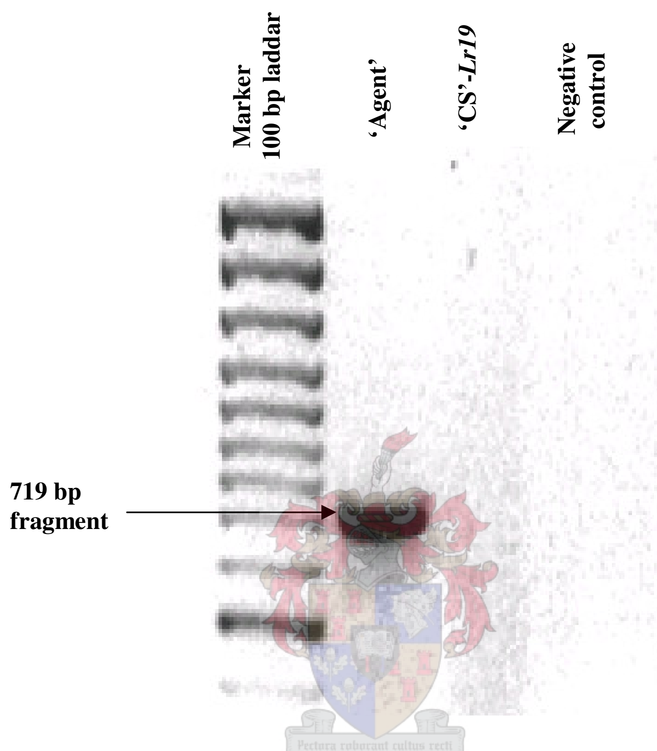


Figure 3.22 SCAR ($SCS73_{719}$) analysis of *Lr24*-carrier 'Agent' and *Lr19*-carrier 'CS'-*Lr19*.

Marker $SCS73_{719}$ amplified the characteristic 719 bp fragment associated with *Lr24* in 'Agent' but not in 'CS'-*Lr19* which confirms that the marker tags *Lr24* and not *Lr19* as was stated previously (Cherukuri *et al*., 2003).

3.6 Microsatellite analysis

Seven microsatellite markers, viz. *Xgwm68*, *Xgwm146*, *Xgwm344*, *Xgwm577*, *Xgwm611* (Röder *et al.,* 1998; Langridge *et al*., 2001), *Xcnl2 (*Langridge *et al*., 2001), *Xwmc216* (Gupta *et al.,* 2002) and *Xwmc276* (Gupta *et al.,* 2002; Sourdille *et al*., 2004) were tested on primary recombinant *Lr19*-149 and the four secondary recombinants derived from it. 'CS', 'CS'-*Lr19* and 'CS'-Nullisomic7B were included as controls.

Markers *Xgwm68*, *Xgwm344, Xcnl2* and *Xwmc216* amplified products in 'CS'-Nullisomic7B and were not polymorphic in any of the genotypes. They were therefore not specific for chromosome 7B and thus not useful. Although it is well known that microsatellites are often genome and chromosome-specific, it is possible that some primer sets may amplify two or more loci which can be mapped to homoeologous as well as to non-homoeologous sites (Röder *et al*., 1998; Harker *et al*., 2001; Gupta *et al*., 2002; Somers *et al*., 2004). Markers *Xgwm68*, *Xgwm344, Xcnl2* and *Xwmc216* should be chromosome 7B specific according to the literature sources from which they were derived. However, other reports indicated that markers *Xgwm68,* (Röder *et al*., 1998; Chalmers *et al*., 2001; Langridge *et al*., 2001; Somers *et al*., 2004; Sourdille *et al*., 2004), *Xgwm344* (Harker *et al*., 2001) and *Xcnl2* (Sourdille *et al*., 2004) detects multiple loci. Marker *Xgwm68* detects two loci which map to non-homoeologous chromosomes 7B and 5B (Röder *et al*., 1998; Chalmers *et al*., 2001; Langridge *et al*., 2001; Somers *et al*., 2004; Sourdille *et al*., 2004). Marker *Xgwm344* detects two homoeologous loci on chromosomes 7A and 7B (Harker *et al*., 2001), and marker *Xcnl2* detects three loci of which one occurs on chromosome 7B and the other two on the non-homoeologous chromosome arm 3D (Sourdille *et al*., 2004). In genetic mapping studies involving *Xwmc216*, it mapped on the long arm of chromosome 1D (Langridge *et al*., 2001; Somers *et al*., 2004; Sourdille *et al*., 2004) and not on chromosome 7B as was reported by Gupta *et al*. (2002). However, there are inconsistencies in the paper of Gupta *et al*., 2002. On the list of the microsatellites with corresponding mapped chromosomal loci the location is given as 7B whereas their molecular genetic linkage map shows it to be located on 1D.

Differences in the mapped positions reported for the same microsatellites in separate studies suggest that multiple loci exist and that different polymorphisms can be mapped in different populations (Röder *et al*., 1998; Harker *et al*., 2001; Gupta *et al*., 2002). If it is assumed that multiple loci in bread wheat should be either homoeoloci or duplicated loci, then the loci for the same microsatellite on non-homoeologous chromosomes may be either due to translocations or to duplications to non-

homoeologous chromosomes (Röder *et al*., 1998; Gupta *et al*., 2002). Many such translocations and duplications were detected in plants such as wheat in comparative genetic studies involving the use of heterologous probes for molecular mapping (Gale and Devos., 1998; Gupta *et al*., 2002).

No products were amplified in 'CS'-Nullisomic7B by markers *Xgwm146*, *Xgwm611* (Figure 3.23), *Xgwm577* and *Xwmc276* (Figure 3.24) which confirmed its specificity for this wheat chromosome arm.

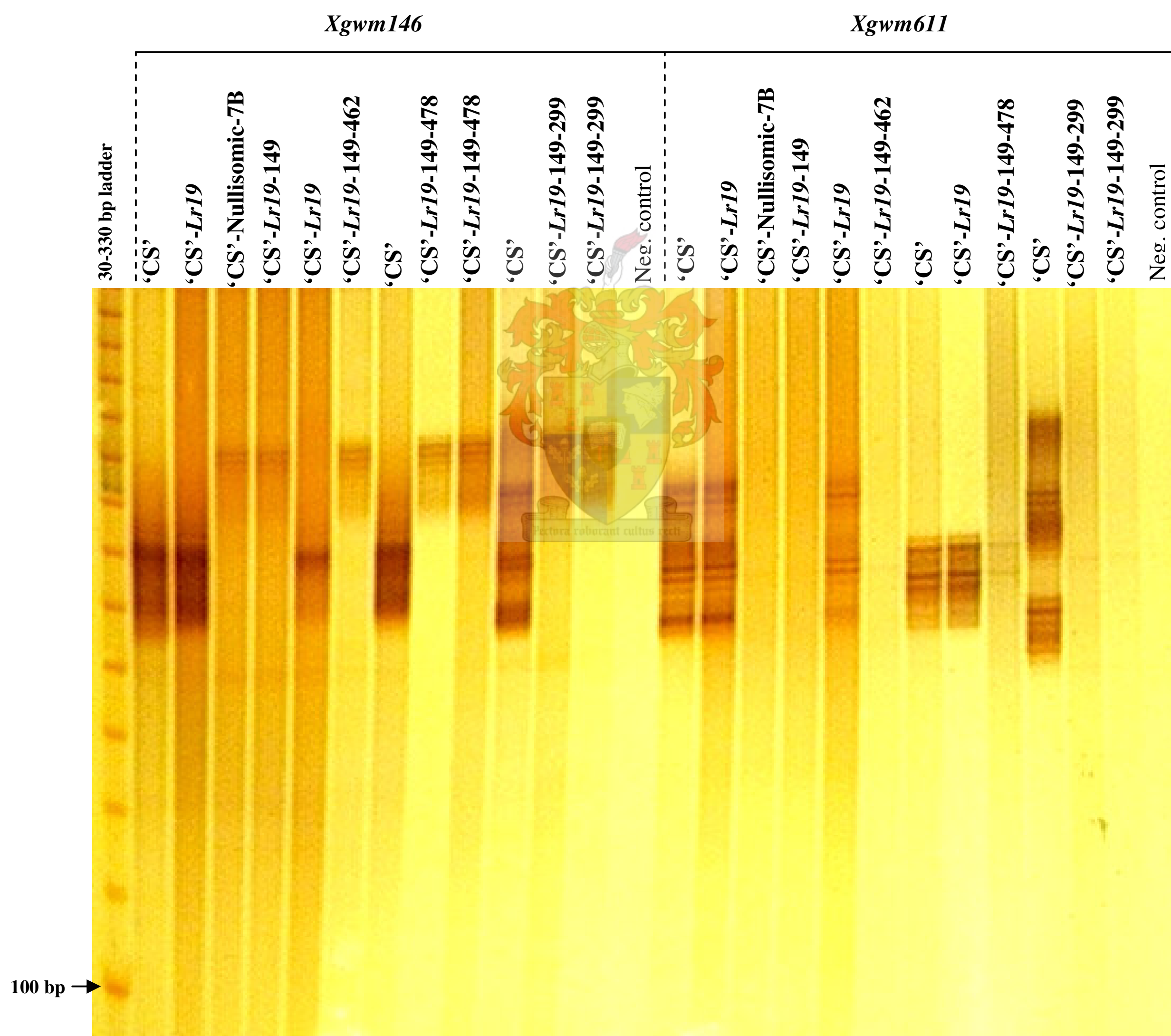


Figure 3.23 Partial silver-stained sequencing gel of amplification products generated using microsatellite *Xgwm146* and *Xgwm611* primer sets

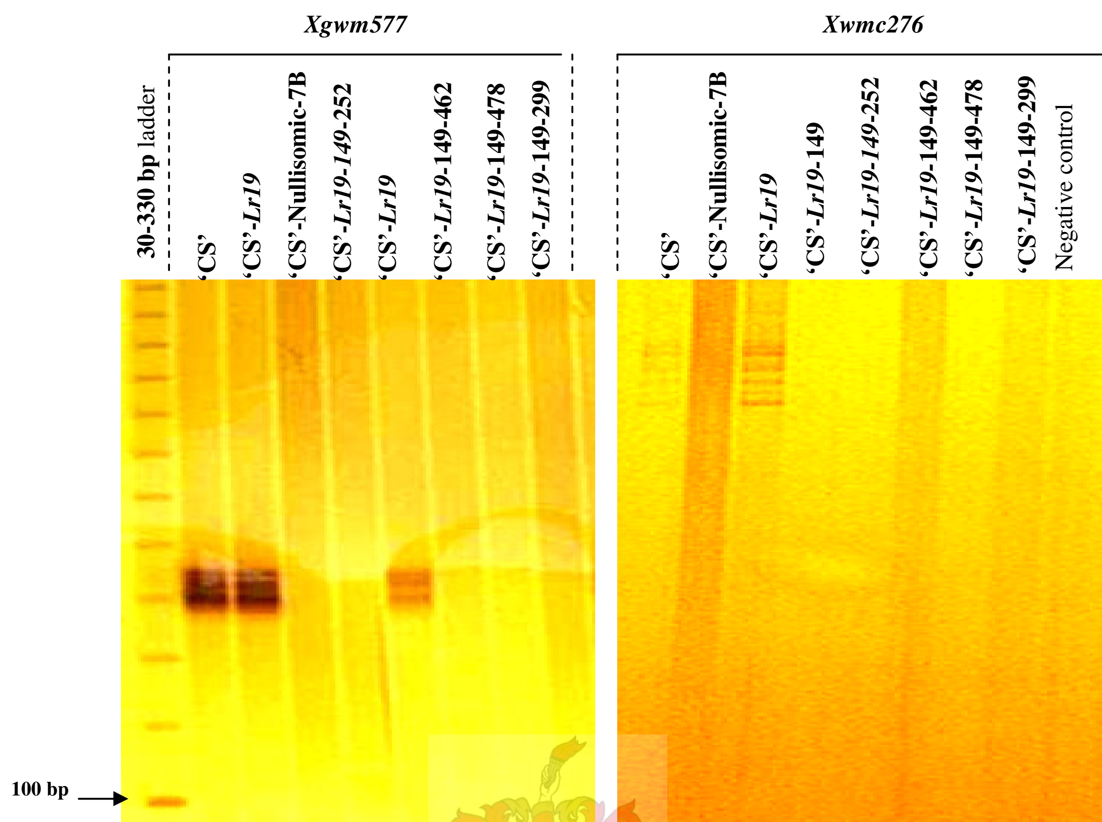


Figure 3.24 Partial siver-stained sequencing gel of amplification products generated using microsatellite marker *Xgwm577* and *Xwmc276* primers

Markers *Xgwm146*, *Xgwm611*, *Xgwm577* and *Xwmc276* produced amplification products in the genotypes tested. It is known that due to the large percentage of repetitive DNA in the wheat genome, it is likely that some primers will recognise multiple sites and may therefore vary considerably in their ease of use (Stephenson *et al*., 1998; Rampling *et al*., 2001). Stephenson e*t al.* (1998) devised a scale of one (best) to five (useful but only with care) for microsatellite markers, based on the degree of stuttering (the amplification of several artefactual fragments from a single locus, with both greater and lesser numbers of repeat units than the template fragment) observed on sequencing gels and the number of fragments produced from distinct loci, which is a particular problem in polyploids such as wheat. Stuttering can be particularly problematic in survey studies where a major band representing the microsatellite product needs to be assigned (Rampling *et al*., 2001). All markers appear to amplify multiple products at single loci as suggested by Stephenson *et*

al. (1998) and Harker *et al*. (2001). The four markers studied here produced products which were absent in primary recombinant *Lr19*-149 as well as the four secondary recombinants derived from it. 'CS'-*Lr19* (located on 7DL) produced bands since it has a normal 7B. Thus, the four loci occur within the wheat 7DL chromatin corresponding to the area of *Thinopyrum* chromatin that has remained in the shortest tertiary recombinants (Figure 3.25)

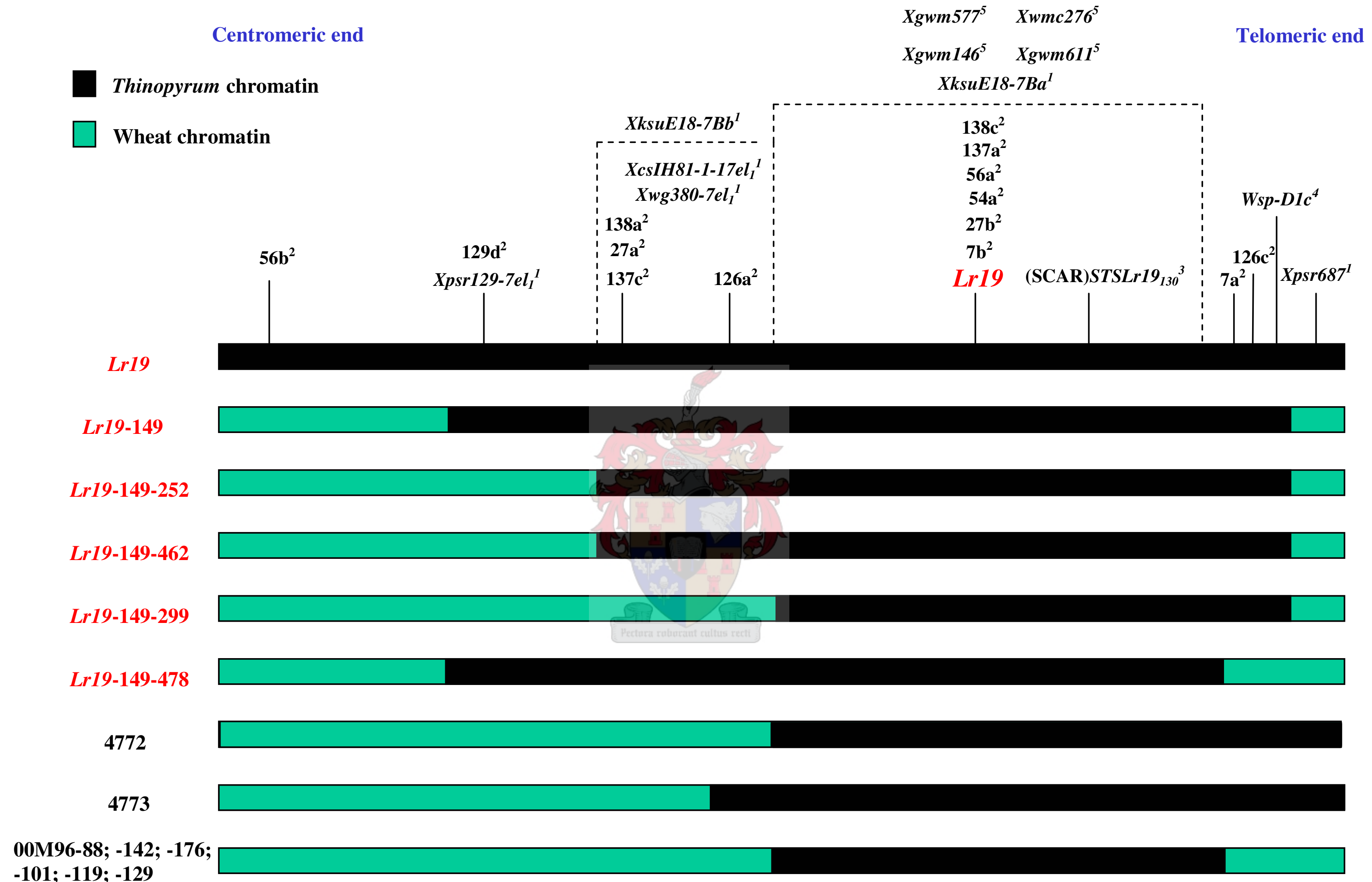


Figure 3.25 Physical map of the *Lr19* translocation and recombinants ([1]RFLP loci; [2]AFLP loci identified by Groenewald, 2001; [3]STS marker developed by Prins *et al*, 2001; [4]Water soluble protein locus; [5] Microsatellite loci).

3.7 Conclusions

One of the aims of this study was to identify and select tertiary double recombinants of the 'CS'-*Lr19*-149-299 and 'CS'-*Lr19*-149-478 translocations. Plants that were heterozygous for the latter translocations were testcrosssed with the susceptible tester, W84-17, to produce cross 00M96. It was expected that a certain proportion of the 00M96 progeny would be double recombinants, produced as a result of meiotic recombination in the *Thinopyrum*-derived regions of the two translocations. The *Thinopyrum*-derived water soluble protein locus, *Wsp-D1c*, served as marker for 'CS'-*Lr19*-149-299, and a cleaved amplified polymorphism (CAPS) marker based on the proximally located *Thinopyrum* locus, *Xpsr129-7el*$_1$, served as marker for recombinant 'CS'-*Lr19*-149-478. Resistant TF_1 00M96 progeny that lack both loci were putative double recombinants, 17 such plants were recovered. As the double recombinant translocations would be identical, only eight of them were subsequently confirmed.

The second study aim was to confirm the tertiary double recombinants obtained and to further characterize the primary, secondary and tertiary recombinants with new and existing molecular markers. The markers that were used included six RFLPs, 12 AFLPs, three STSs, seven microsatellites and the *Ep-B*$_1$ locus. The $7el_1$-alleles of RFLP markers *Xpsr129*, *XcsIH81-1* and *XmWg380* were replaced by corresponding wheat (7BL) alleles in 'CS'-*Lr19*-149-299 as well as in the 00M96 putative double recombinants. *Xpsr129-7el*$_1$ mapped closest to the centromere while *Xgw380-7el*$_1$ mapped closest to *Lr19*, as was reported by Prins and Marais (1998) and Marais *et al.* (2000). Two chromosome 7B loci are recognised by *TtksuE018*. The *XksuE18-7Bb* locus is present in recombinants 'CS'-*Lr19*-149, -252, -462 and –478 but absent in recombinant –299 as well as the double recombinants. This would suggest that it is situated proximally to *Lr19* but distally from *Xpsr129-7el*$_1$ or between the 'CS'-*Lr19*-252/462 and 'CS'-*Lr19*-149-299 break points (Figure 3.25). *XksuE18-7Ba* is expressed by all recombinants and therefore was retained on the *Thinopyrum* chromatin that remains. RFLP marker *Xpsr687* mapped distally of the bin 18 markers and *Wsp-D1c* and was not useful in characterising the primary, secondary and tertiary recombinants. Disappearance of the $7el_1$ band did not coincide with reappearance of a 7B band which may imply that the *Thinopyrum* and wheat chromatin may not be syntenic in this region. RFLP marker *Xcdo414* did not detect polymorphisms in the controls (except for 'CS'-Nullisomic-7D) and between the primary, secondary and tertiary recombinants. It was therefore not useful in characterising the recombinants and could not be physically mapped either.

The *Thinopyrum* fragments amplified with proximal AFLP markers 27a, 137c, 138a and 126a did not occur in 'CS'-*Lr19*-149-299, which confirmed that this translocation is proximally the shortest as was concluded by Marais *et al*. (2000). The distal markers 7a and 126c were not amplified in 'CS'-*Lr19*-149-478 which confirms that this is the distally shortest translocation as was also concluded by Marais *et al*. (2000). The *Thinopyrum* fragments of these six AFLP markers were also not amplified in the putative tertiary double recombinants which confirms that these derive from recombination between the proximally shortest (299) and distally shortest (478) translocations. A *Thinopyrum* fragment was not amplified by marker 126a in 4772 whereas a fragment was produced in 4773 and it therefore appears that the translocation in 4772 is proximally shorter than that in 4773. The remaining six AFLP markers (7b, 27b, 54a, 56a, 137a and 138c) amplified *Thinopyrum* fragments in all of the secondary, putative tertiary and the 4772 and 4773 recombinants.

Of the seven wheat specific microsatellite markers used, four were 7B specific which included *Xgwm146*, *Xgwm611*, *Xgwm577* and *Xwmc276*. However, the four markers did not amplify wheat fragments in the four secondary recombinants (-299, -252, -462, -478). This would suggest that the SSR loci occur within the 7B area that is replaced with *Thinopyrum* chromatin. The sequence tagged site (STS) marker, $STSLr19_{130}$, amplified a single *Thinopyrum* fragment of 130 bp in the four secondary recombinants and could not be used to confirm the putative tertiary double recombinants. This STS marker therefore cluster with the six AFLP markers 7b, 27b, 54a, 56a, 137a and 138c, the four microsatellite markers *Xgwm146*, *Xgwm611*, *Xgwm577* and *Xwmc276*, and the RFLP marker locus *XksuE18-7Ba* within the *Thinopyrum* region between the distal and proximal break points of recombinants 'CS'-*Lr19*-149–478 and 'CS'-*Lr19*-149–299. All of these markers are completely linked to *Lr19* and can be used as markers of its presence or can be used in further attempts to shorten the translocation (Figure 3.25). It could not be determined which of the two secondary recombinants, 'CS'-*Lr19*-149-252 and 'CS'-*Lr19*-149-462, are the shortest since they produced the same polymorphisms with all the markers used.

The presence of the *Ep-D1a* band in the primary recombinant 'CS'-*Lr19*-149, the four secondary recombinants and tertiary recombinants, confirmed the relocation of the translocation to 7BL in these recombinants. However, in the zymogram of recombinant 4772, the *Ep-D1b* band was absent in some of the extracts which suggested that the *Thinopyrum ponticum* chromatin carrying *Lr19* was translocated to 7DL of wheat in this line. In the zymograms of 4773 and 4774, however, an *Ep-D1a* band was always expressed. Thus, the translocation in 4773/4774 occurred to either 7AL or 7BL. It was confirmed by prof Lukaszewski (University of California-Riverside, Personal communication)

from whom these recombinants were obtained, that translocation in these lines occurred to 7AL. It was also confirmed by the endopeptidase results, that 4773 and 4774 are the same translocation, as was suggested by Lukaszewski. Since the 4772 and 4773/4774 translocations are not associated with 7BL, it will not be possible to combine any of these translocations with *Lr19*-149-478 in order to derive a shorter recombinant through meiotic crossover.

The SCAR marker $SCS73_{719,}$ developed by Cherukuri *et al*. (2003), did not produce a single *Thinopyrum* fragment of 719 bp in the *Lr19*-carrier genotypes as originally claimed by the authors. It was confirmed in this study that this marker tags *Lr24*, and not *Lr19*, as was subsequently shown by Prabhu *et al*. (2004).

It is possible that most of the markers used in this study can be used in the future to identify and select recombinants with shorter translocations than those in the tertiary double recombinants.

3.8 References

Aghaee-Sarbarzeh, M., Singh, -H., and Dhaliwal, H.S. (2001) A microsatellite marker linked to leaf rust resistance transferred from *Aegilops triuncialis* into hexaploid wheat. *Plant Breeding* 120:259-261.

Ahn, S., Anderson, J.A., Sorrels, M.E., and Tanksley, S.D. (1993) Homoeologous relationships of rice, wheat and maize chromosomes. *Molecular and General Genetics* 241:483-490.

Ainsworth, C.C., Doherty, P., Edwards, K.G.K., Martiensen, R.A., and Gale, M.D. (1985) Allelic variation at α-amylase loci in hexaploid wheat. *Theoretical and Applied Genetics* 70:400-406.

Ainsworth, C.C., Gale, M.D., and Baird, S. (1984) The genetic control of grain esterases in hexaploid wheat.1. Allelic variation. *Theoretical and Applied Genetics* 68:219-226.

Akem, C., Ceccarelli, S., Erskine, W., and Lenné, J. (2000) Using genetic diversity for disease resistance in agricultural production. *Outlook on Agriculture* 29(1):25-30.

Anderson, J.A., Ogihara, Y., Sorrells, M.E., and Tanksley, S.D. (1992) Development of a chromosomal arm for wheat based on RFLP markers. *Theoretical and Applied Genetics* 83:1035-1043.

Appels, R., Gerlach, W.L., Dennis, E.S., Swift, H., and Peacock, W.J. (1980) Molecular and chromosomal organisation of DNA sequences coding for the ribosomal RNAs in cereals. *Chromosoma* 78:293-311.

Aragón-Alcaide, L., Reader, S., Miller, T., and Moore, G. (1997) Centromeric behaviour in wheat with high and low homoeologous chromosomal pairing. *Chromosoma* 106:327-333.

Autrique, E., Singh, R.P., Tanksley, S.D., and Sorrels, M.E. (1995) Molecular markers for four leaf rust resistance genes introgressed into wheat from wild relatives. *Genome* 38:75-83.

Barnard, A. (2003) Items from the Republic of South Africa. Small Grain Institute, Bethlehem. *Annual Wheat Newsletter* 49:134-139.

Bennett, M.D., and Smith, J.B. (1976) Nuclear DNA amounts in angiosperms. Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences. B274:227-274.

Blake, T.K., Kadyrzhanova, D., Shepherd, K.W., Islam, A.K.M.R., Langridge, P.L., McDonald, C.L., Erpelding, J., Larson, S., Blake, N.K., and Talbert, L.E. (1996) STS-PCR markers appropriate for wheat-barley introgression. *Theoretical and Applied Genetics* 93:826-832.

Boshoff, W.H.P., Pretorius, Z.A., and van Niekerk, B.D. (2002) The impact of leaf rust on spring wheat in the winter rainfall region of South Africa. *South African Journal of Plant Soil* 19(2):84-88.

Botstein, D., White, R.L., Skonick, M., and Davis, R.W. (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* 32:314-331.

Pectora roborant cultus recti

Bournival, B., Obanni, M., Abad, A., Ohm, H., and Mackenzie, S. (1994) Isolation of a new species-specific repetitive sequence from *Thinopyrum elongatum* and its use in studies of alien translocations. *Genome* 37:97-104.

Boyko, E.V., Gill, K.S., Mickelson-Young, L., Nasuda, S., Raupp, W.J., Ziegle, J.N. *et al*. (1999) A high-density genetic linkage map of *Aegilops tauschii*, the D-genome progenitor of bread wheat. *Theoretical and Applied Genetics* 99:16-26.

Braun, H.J., Payne, T.S., Morgounov, A.I., van Ginkel, M., and Rajaram, S. (1998) Germplasm Development and Breeding. *In*: Proceedings of the 9th International Wheat Genetics Symposium, Saskatoon, Saskatchewan, Canada, Volume 1, p. 33.

Brettschneider, R. (1998) RFLP analysis. *In*: Molecular Tools for Screening Biodiversity: Plants and Animals, *Edited by* Karp, A., Isaac, P.G. and Ingram, D.S., Chapman and Hall, London, Volume 6, pp. 85-97.

Burr, B. (2001) Some concepts and new methods for molecular mapping in plants. *In*: DNA-Based Markers in Plants, 2nd Edition. *Edited by* Phillips, R.L. and Vasil, I.K., Dordrecht, Kluwer Accademic Plublishers, Volume 1, pp. 1-8.

Cadalen, T., Boeuf, C., Bernard, S., and Bernard, M. (1997) An intervarietal molecular marker map in *Triticum aestivum* L. em. Thell. and comparison with a map from a wide cross. *Theoretical and Applied Genetics* 94:367-377.

Campbell, A.W., Daggard, G., Békés, F., Pedler, A., Sutherland, M.W., and Appels, R. (2001) Targetting AFLP-DNA markers to specific traits and chromosome regions. *Australian Journal of Agricultural Research* 52:1153-1160.

Cantor, C.R., Smith, C.L. and Mathew, M.K. (1988) Pulsed-field gel electrophoresis of very large DNA molecules. *Annual Review of Biophysics and Chemistry* 17:287-304.

Chalmers, K.J., Campbell, A.W., Kretschmer, J., Karakousis, A., Henschke, P.H., Pierens, S., Harker, N., Pallotta, M., Cornish, G.B., Shariflou, M.R., Rampling, L.R., McLauchlan, A., Daggard, G., Sharp, P.J., Holton, T.A., Sutherland, M.W., Appels, R., and Langridge, P. (2001) Construction of three linkage maps in bread wheat, *Triticum aestivum*. *Australian Journal of Agricultural Research* 52:1089-1119.

Chao, S., Sharp, P.J., Worland, A.J., Warham, E.J., Koebner, R.M.D., and Gale, M.D. (1989) RFLP- based genetic maps of wheat homoeologous group 7 chromosomes. *Theoretical and Applied Genetics* 78:495-504.

Chen, Q., Conner, R.L., Laroche, A., and Thomas, J.B. (1998) Genome analysis of *Thinopyrum intermedium* and *Thinopyrum ponticum* using genomic *in situ* hybridization. *Genome* 41:580-586.

Cherukuri, D.P., Gupta, S.K., Charpe, A., Koul, S., Prabhu, K.V., Singh, R.B., Haq, Q.M.R., and Chauhan, S.V.S. (2003) Identification of a molecular marker liked to an *Agropyron elongatum*-derived gene *Lr19* for leaf rust resistance in wheat. *Plant Breeding* 122:204-208.

Cheung, W.Y., Chao, S., and Gale, M.D. (1991) Long-range physical mapping of the α-amylase-1 (*α-amy-1*) loci on homoeologous group 6 chromosomes of wheat. *Molecular and General Genetics* 229:373-379.

Cuadrado, A., and Schwarzacher, T. (1998) The chromosomal organization of simple sequence repeats in wheat and rye genomes. *Chromosoma* 107:587-594.

Cuadrado, A., Rubio, P., Ferrer, E., and Jouve, N. (1996) Sequential combinations of C-banding and *in situ* hybridization and their use in the detection of interspecific introgressions into wheat. *Euphytica* 89:107-112.

Curtis, C.A., and Lukaszewski, A.J. (1991) Genetic linkage between C-bands and storage protein genes in chromosome 1B of tetraploid wheat. *Theoretical and Applied Genetics* 81:245-252.

Curtis, C.A., Lukaszewski, A.J., and Chrzastek, M. (1991) Metaphase-I pairing of deficient chromosomes and genetic mapping of deficiency breakpoints in wheat. *Genome* 34:553-560.

DeLacy, I.H., Rajaram, S., Cooper, M., Fox, P.N., and Basford, K.E. (2000) The effect of the accumulation of disease resistance genes on the long-term association of a global sample of environments for testing spring bread wheat. *Theoretical and Applied Genetics* 101:1164-1172.

Delaney, D.E., Nasuda, S., Endo, T.R., Gill, B.S., and Hulbet, S.H. (1995a) Cytological based physical maps of the group-2 chromosomes of wheat. *Theoretical and Applied Genetics* 91:568-573.

Delaney, D.E., Nasuda, S., Endo, T.R., Gill, B.S., and Hulbet, S.H. (1995b) Cytological based physical maps of the group-3 chromosomes of wheat. *Theoretical and Applied Genetics* 91:780-782.

Demeke, T., Adams, R.P., and Chibbar, R. (1992) Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in Brassica. *Theoretical and Applied Genetics* 84:990-994.

Devey, M.E., and Hart, G.E. (1993) Chromosomal localization of intergenomic RFLP loci in hexaploid wheat. *Genome* 36:913-918.

Devos, K.M., and Gale, M.D. (1997) Comparative genetics in the grasses. *Plant Molecular Biology* 35:3-15.

Devos, K.M., Atkinson, M.D., Chinoy, C.N., Liu, C. and Gale, M.D. (1992) RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. *Theoretical and Applied Genetics* 83:931-939.

Doyle, J.J., and Doyle, J.L. (1990) Isolation of plant DNA from fresh tissue. *Focus* 12:13-15.

Dubcovsky, J., and Dvořák, J. (1995) Ribosomal RNA multigene loci: Nomads of the Triticeae genomes. *Genetics* 140:1367-1377.

Dubcovsky, J., Lukaszewski, A.J., Echaide, M., Antonelli, E.F., and Porter, D.R. (1998) Molecular characterization of two *Triticum speltoides* interstitial translocations carrying leaf rust and greenbug resistance genes. *Crop Science* 38:1655-1660.

Dubcovsky, J., Luo, M.C., and Dvořák, J. (1995) Differentiation between homoeologous chromosomes 1A of wheat and A1(M) of *Triticum monococcum* and its recognition by the wheat *Ph1* locus. *Proceedings of the National Academy of Sciences (USA)* 92:5547-6645.

Dvořák, J., and Appels, R. (1986) Investigation of homologous crossing-over and sister chromatid exchange in the wheat *NorB2* locus coding for rRNA and the *GliB2* locus coding for gliadins. *Genetics* 113:1037-1056.

Dvořák, J., and Chen, K.C. (1984) Distribution of non-structural variation between wheat cultivars along chromosome arm 6Bp: evidence from the linkage map and physical map of the arm. *Genetics* 106:325-333.

Dvořák, J., Chen, K.C., and Giorgi, B. (1984) The C-band pattern of a *Ph* mutant of durum wheat. *Canadian Journal of Genetic Cytology* 26:360-363.

Dvořák, J., McGuire, P.E., and Cassidy, B. (1988) Apparent sources of the A genomes of wheats inferred from polymorphism in abundance and restriction fragment length of repeated nucleotide sequences. *Genome* 30:680-689.

Dyck, P.L., Samborski, D.J., and Anderson, R.G. (1966) Inheritance of adult plant resistance derived from the common wheat varieties Exchange and Frontana. *Canadian Journal of Genetic Cytology* 8:665-671.

Eagles, H.A., Bariana, H.S., Ogbonnaya, F.C., Rebetzke, G.J., Hollamby, G.J., Henry, R.J., Henschke, P.H., and Carter, M. (2001) Implementation of markers in Australian wheat breeding. *Australian Journal of Agricultural Research* 52:1349-1356.

Eizenga, G.C. (1987) Locating the *Agropyron* segment in wheat-*Agropyron* transfer no. 12. *Genome* 29: 365-366.

Endo, T.R. (1988) Chromosomes mutation induced by gametocidal chromosomes in common wheat. *In*: Proceedings of the 7th International Wheat Genetics Symposium, Cambridge, England. pp. 259-265.

Endo, T.R. (1990) Gametocidal chromosomes and their induction of chromosome mutations in wheat. *Japanese Journal of Genetics* 65:135-152.

Endo, T.R., and Gill, B.S. (1996) The deletion stocks of common wheat. *Journal of Heredity* 87:295-307.

Evans, L.T. and Peacock, W.J. (1981) Wheat science-today and tomorrow, Cambridge University Press, Cambridge, UK, 5:75-91.

Faris, J.D., and Gill, B.S. (2002) Genomic targeting and high resolution mapping of the domestication gene *Q* in wheat. *Genome* 45:706-718.

Pectora roborant cultus recti

Faris, J.D., Fellers, J.P., Brooks, S.A., and Gill, B.S. (2003) A bacterial artificial chromosome contig spanning the major domestication locus *Q* in wheat and identification of a candidate gene. *Genetics* 164:311-321.

Faris, J.D., Haen, K.M., and Gill, B.S. (2000) Saturation mapping of a gene-rich recombination hot spot region in wheat. *Genetics* 154:823-835.

Feldman, M. (1993) Cytogenetic activity and model of action of the pairing homoeologous (*Ph1*) gene of wheat. *Crop Science* 33:894-897.

Feldman, M., Liu, B., Segal, G., Abbo, S., Levy, A.A., and Vga, J.M. (1997) Rapid elimination of low-copy DNA Sequences in polyploid wheat: A possible mechanism for differentiation of homoeologous chromosomes. *Genetics* 147:1381-1387.

Feuillet, C., Schachermayr, G., and Keller, B. (1997) Molecular cloning of a new receptor-like kinase gene encoded at the *Lr10* disease resistance locus of wheat. *The Plant Journal* 11:45-52.

Feuillet, C., Travella, S., Nils, S., Albar, L., Nublat, A., and Keller, B. (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. *Proceedings of the National Academy of Sciences* (USA) 100:15253-15258.

Flavell, R.B., Bennett, M.D., Seal, A.G., and Hutchinson, J. (1987) Chromosome structure and organisation. *In*: Wheat breeding, its scientific basis, *Edited by* F.G.H. Lupton, Chapman and Hall, London, pp. 211-268.

Flavell, R.B., Rimpau, J., and Smith, D.B. (1977) Repeated DNA relationships in four cereal genomes. *Chromosoma* 63:205-222.

Flor, H.H. (1971) Current status of gene-for-gene concept. *Annual Review of Phytopathology* 9:275-296.

Fox, M.E., Virgin, J.B., Metzger, J., and Smith, G.R. (1997) Position and orientation-independent activity of the *Schizosaccharomyces pombe* meiotic recombination hot spot *M26*. *Proceedings of the National Academy of Sciences* (USA) 94:7446-7451.

Friebe, B., Jiang, J., Knott, D.R., and Gill, B.S. (1994) Compensation indices of radiation-induced wheat-*Agropyron elongatum* translocations conferring resistance to leaf rust and stem rust. *Crop Science* 34:400-404.

Friebe, B., Jiang, J., Raupp, W.J., McIntosch, R.A., and Gill, B.S. (1996) Characterizations of wheat-alien translocations conferring resistance to diseases and pests: current status. *Euphytica* 91:59-87.

Friebe, B., Mukai, Y., Dhaliwal, H.S., Martin, T.J., and Gill, B.S. (1991) Identification of alien chromatin specifying resistance to wheat streak mosaic and greenbug in wheat germplasm by C-banding and *in situ* hybridization. *Theoretical and Applied Genetics* 81:381-389.

Furata, Y., Nishikawa, K., and Shimokawa, K. (1988) Relative DNA content of the individual telocentric chromosomes in Chinese Spring wheat. *In*: Proceedings of the 7th International Wheat Genetics Symposium, Cambridge, England, pp. 281-286.

Pectora roborant cultus recti

Gale, M.D., and Devos, K.M. (1998) Comparative genetics in the grasses. *Proceedings of the National Academy of Sciences* (USA) 95:1971-1974.

Gale, M.D., Sharp, P.J., Chap, S., and Law, C.N. (1989) Application of genetic markers in cytogenetic manipulation in wheat. *Genome* 31:137-143.

Gao, L.F., Jing, R.L., Huo, N.X., Li,Y., Li, X.P., Zhou, R.H., Chang, X.P., Tang, J.F., Ma, Z.Y., and Jia, J.Z. (2004) One hundred and one new microsatellite loci derived from ESTs (EST-SSRs) in bread wheat. *Theoretical and Applied Genetics* 108:1392-1400.

Geiger, H.H., and Heun, M. (1989) Genetics of quantitative resistance to fungal diseases. *Annual Review of Phytopathology* 27:317-341.

Gill, A.S., Gill, B.S., and Endo, T.R. (1993a) A chromosome region-specific mapping strategy reveals gene-rich telomeric ends in wheat. *Chromosoma* 102:374-381.

Gill, K.S., Gill, B.S., Endo, T.R., and Mukai, Y. (1993b) Fine physical mapping of *Ph1*, a chromosome pairing regulator gene in polyploid wheat. *Genetics* 134:1231-1236.

Gill, B.S. (1987) Chromosome banding methods, standard chromosome band nomenclature, and applications in cytogenetic analysis. *In*: Wheat and Wheat Improvement, 2nd Edition, American Society of Agronomy Inc, Madison, Wisconsin, USA. pp. 243-254.

Gill, B.S. (1993) Molecular cytogenetic analysis in wheat. *Crop Science* 33:902-908.

Gill, B.S. (1995) Molecular cytogenetics: integration of C-banding, *in situ* hybridisation, and RFLPs in genome mapping and crop improvement of wheat. *In*: Proceedings of the 8th International Wheat Genetics Symposium, Beijing, China, pp.41-47.

Gill, K.S., and Gill, B.S. (1994) Mapping in the realm of polyploidy: the wheat model. *Bioassays* 16:841-846.

Gill, B.S., and Gill, K.S. (1998) Molecular cytogenetic explorations into the genome of wheat. *In*: Proceedings of the 9th International Wheat *Genetics* Symposium, Saskatoon, Saskatchewan, Canada, Volume 1, pp. 29-32.

Gill, B.S., and Kimber, G. (1974). Giemsa C-banding and the evolution of wheat. *Proceedings of the National Academy of Sciences* (USA) 71:4086-4090.

Gill, B.S., Friebe, B., and Endo, T.R. (1991a) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). *Genome* 34:830-839.

Gill, K.S., Lubbers, E.L., Gill, B.S., Raup, W.J., and Cox, T.S. (1991b) A genetic linkage map *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). *Genome* 34:362-374.

Gill, K.S., Gill, B.S., Endo, T.R., and Taylor, T. (1996a) Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. *Genetics* 144:1883-1891.

Gill, K.S., Gill, B.S., Endo, T.R., and Boyko, E.V. (1996b) Identification and high-density mapping of gene-rich regions in chromosome group 5 of wheat. *Genetics* 143:1001-1012.

Grant, D., and Shoemaker, R. (1997) Molecular hybridization. *In*: DNA markers: Protocols, Applications and Overviews, *Edited by* Caetano-Anollés, G. and Gresshoff, P.M., New York, Wiley-VCH, Volume 2, pp. 15-16.

Gras, P.W., Anderssen, R.S., Keentok, M., Békés, F., and Appels, R. (2001) Gluten protein functionality in wheat flour processing: a review. *Australian Journal of Agricultural Research* 52:1311-1323.

Groenewald, J.Z. (2001)Tagging and mapping of prominent structural genes on chromosome arm 7DL of common wheat. Ph.D. thesis, Stellenbosch University, Stellenbosch, Republic of South Africa.

Gruenbaum, Y., Naveh-Many, T., Cedar, H., and Razin, A. (1981) Sequence specificity of methylation in higher plant DNA. *Nature* 292:860-862.

Gupta, P.K., Balyan, H.S., Edwards, K.J., Isaac, P., Korzun, V., Röder, M., Gautier, M.-F., Joudrier, P., Schlatter, A.R., Dubcovsky, J., De la Pena, R.C., Khairallah, M., Penner, G., Hayden, M.J., Sharp, P., Keller, B., Wang, R.C.C., Hardouin, J.P., and Jack, P. (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. *Theoretical and Applied Genetics* 105:413-422.

Gupta, P.K., Varhney, R.K., Sharma, P.C., and Ramesh, B. (1999) Molecular markers and their applications in wheat breeding. *Plant Breeding* 118:369-390.

Harker, N., Rampling, L.R., Shariflou, M.R., Hayden, M.J., Holton, T.A., Morell, M.K., Sharp, P.J., Henry, R.J., and Edwards K.J. (2001) Microsatellites as markers for Australian wheat improvement. *Australian Journal of Agricultural Research* 52:1121-1130.

Hart, G.H., and Langston, P.J. (1977) Chromosomal location and evolution of isozyme structural genes in hexaploid wheat. *Heredity* 39(2):263-277.

Hayden, M.J., Khatkar, S., and Sharp, P.J. (2001) Targeting microsatellites (SSRs) in genetic linkage maps of bread wheat. *Australian Journal of Agricultural Research* 52:1143-1152.

He, Q., Marjamäki, M., Soini, H., Mersola, J., and Viljanen, M.K. (1994) Primers are decisive for sensitivity of PCR. *Bio-Techniques* 17:82-87.

Helguera, M., Khan, I.A., and Dubcovsky, J. (2000) Development of PCR markers for the wheat leaf rust resistance gene *Lr47*. *Theoretical and Applied Genetics* 100:1137-1143.

Hernández, P., Hemmat, M., Weeden, N.F., Dorado, G., and Martin, A. (1999) Development characterization of *Hordeum chilense* chromosome-specific STS markers suitable for wheat introgression and marker-assisted selection. *Theoretical and Applied Genetics* 98:721-727.

Hoch, H.C., and Staples, R.C. (1987) Structural and chemical changes among the rust fungi during appressorium development. *Annual Review of Phytopathology* 25:231-247.

Pectora roborant cultus recti

Hohmann, U., Baclaeva, K., Busch, W., Friebe, B., and Gill, B.S. (1996) Molecular cytogenetic analysis of *Agropyron* chromatin specifying resistance to barley yellow dwarf virus in wheat. *Genome* 39:336-347.

Hohmann, U., Endo, T.R., Gill. K.S., and Gill, B.S. (1994) Comparison of genetic and physical maps of group 7 chromosomes from *Triticum aestivum* L. *Molecular and General Genetics* 2445:644-653.

Hohmann, U., Endo, T.R., Herrmann, R.G., and Gill, B.S. (1995a) Characterizations of deletions in common wheat induced by an *Aegilops cylindrica* chromosome: detection of multiple chromosome rearrangements. *Theoretical and Applied Genetics* 91:611-617.

Hohmann, U., Graner, A., Endo, T.R., Gill, B.S., and Herrmann, R.G. (1995b) Comparison of wheat physical maps with barley linkage maps for group 7 chromosomes. *Theoretical and Applied Genetics* 91:618-626.

Huang, L., and Gill, B.S. (2001) An RGA-like marker detects all known *Lr21* leaf rust resistance gene family members in *Aegilops tauschii* and wheat. *Theoretical and Applied Genetics* 103: 1007-1013.

Huang, L., Brooks, S.A., Li, W., Fellers, J.P., Trick, H.N., and Gill, B.S. (2003) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of wheat. *Genetics* 164:655-664.

Huang, X., Zeller, F.J., Hsam, S.L.K., Wenzel, G., and Mohler, V. (2000) Chromosomal location of AFLP markers in common wheat utilizing nulli-tetrasomic stocks. *Genome* 43:298-305.

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

Hutchinson, J., and Lonsdale D.M. (1982) The chromosomal distribution of cloned highly repetitive sequences from hexaploid wheat. *Heredity* 48:371-376.

Inagaki, M.N., Varughese, G., Rajaram, S., van Ginkel, M., and Majeeb-Kazi, A. (1998) Comparison of bread wheat lines selected by doubled haploid, single-seed descent and pedigree selection methods. *Theoretical and Applied Genetics* 97:550-556.

Kam-Morgan, L.N.W., Gill, B.S., and Muthukrishnan, S. (1989) DNA restriction fragment length polymorphisms: a strategy for genetic mapping of D genome of wheat. *Genome* 32:724-732.

Keller, B., Stein, N., and Feuillet, C. (2001) Comparative genetics and disease resistance in wheat. *Euphytica* 119:129-131.

Keller, B., and Feuillet, C. (2000) Colinearity and gene density in grass genomes. *Trends in Plant Science* 5:246-251.

Kent, N.L., and Evers, A.D. (1994) Kent's Technology of Cereals: An introduction for students of food science and agriculture, 4th Edition, Oxford, Pergamon.

Kibirige-Sebunya, I., and Knott, D.R. (1983) Transfer of stem rust resistance to wheat from an *Agropyron* chromosome having a gametocidal effect. *Canadian Journal of Genetics and Cytology* 25:215-221.

Kim, N.S., Amstrong, K., and Knott, D.R. (1993) Molecular detection of *Lophopyrum* chromatin in wheat-*Lophopyrum* recombinants and their use in the physical mapping of chromosome 7D. *Theoretical and Applied Genetics* 85561-567.

King, I.P., Purdie, K.A., Rezanoor, H.N., Koebner, R.M.D., Miller, T.E., Reader, S.M., and Nicholson, P. (1993) Characterization of *Thinopyrum bessarabicum* chromosome segments in wheat using random amplified polymorphic DNAs (RAPD) and genomic *in situ* hybridization. *Theoretical and Applied Genetics* 86:895-900.

Knott, D.R. (1980) Mutation of a gene for yellow pigment linked to *Lr19* in wheat. *Canadian Journal of Genetics and Cytology* 22:651-654.

Knott, D.R. (1986) Novel approaches to wheat breeding. *In*: Crop Science Society of America and American Society of Agronomy, 677 S. Segoe Rd., Madison, Wisconsin. Genetic improvement in yield of wheat. CSSA Special Plublication No. 13. pp. 25-40.

Knott, D.R. (1989a) The wheat rusts-breeding for resistance. *In*: Monographs on Theoretical and Applied Genetics 12, Springer-Verlag, Berlin, Heidelberg.

Knott, D.R. (1989b) The effect of transfers of alien genes for leaf rust resistance on the agronomic and quality characteristics of wheat. *Euphytica* 44:65-72.

Koebner, R.M.D., Miller, T.E., Snape, J.W., and Law, C.N. (1987) Wheat endopeptidase: genetic control, polymorphism, intrachromosomal gene location, and alien variation. *Genome* 30:186-192.

Koebner, R.M.D., Powell, W., and Donini, P. (2001) Contributions of DNA molecular marker Technologies to the genetics and breeding of wheat and barley. *Plant Breeding Reviews*, Volume 21, *Edited by* Janick, J., 5:181-220.

Kojima, T., Habu, Y., Iida, S., and Ogihara, Y. (2000) Direct isolation of differentially expressed genes from a specific chromosome region of common wheat: application of the amplified fragment length polymorphism-based mRNA fingerprinting (AMF) method in combination with a deletion line of wheat. *Molecular and General Genetics* 263:635-641.

Kolmer, J.A. (1996) Genetics of resistance to wheat leaf rust. *Annual Review of Phytopathology* 34:435-455.

Kota, R.S., and Dvořák, J. (1986) Mapping of a chromosome pairing gene and 5S rRNA genes in *Triticum aestivum* L. by a spontaneous deletion in chromosome arm 5Bp. *Canadian Journal of Genetics and Cytology* 28:266-271.

Kota, R.S., Gill, K.S., Gill, B.S., and Endo, T.R. (1993) A cytogenetically based physical map of chromosome 1B in common wheat. *Genome* 36:548-554.

Langridge, P., Lagudah, E.S., Holton, T.A., Appels, R., Sharp, P.J., and Chalmers, K.J. (2001) Trends in genetic and genome analysis in wheat: a review. *Australian Journal of Agricultural Research* 52:1043-1077.

Lawrence, G.J., and Appels, R. (1986) Mapping the nucleolus organizer region, seed protein loci and isozyme loci on chromosome 1R in rye. *Theoretical and Applied Genetics* 71:742-749.

Leitch, I.J., Leitch, A.R., and Heslop-Harrison, J.S. (1991) Physical mapping of plant DNA sequences by simultaneous *in situ* hybridization of two differently labelled fluorescent probes. *Genome* 34:329-333.

Lijavetsky, D., Muzzi, G., Wicker, T., Keller, B., and Wing, R., Dubcovsky, J. (1999) Construction and characterization of a bacterial artificial chromosome (BAC) library for the A genome of wheat. *Genome* 42:1176-1182.

Liu, B., Segal, G., Vega, J.M., Feldman, M., and Abbo, S. (1997) Isolation characterization of chromosome-specific DNA sequences from a chromosome arm genomic library of common wheat. *The Plant Journal* 11:959-965.

Liu, B., Vega, J.M., and Feldman, M. (1998) Differentiation of homoeologous chromosomes in polyploid wheat. *In*: Proceedings of the 9th International Wheat Genetics Symposium, Saskatoon, Saskatchewan, Canada, Volume 2, pp.70-71.

Liu, C.J., Chao, S., and Gale, M.D. (1989) *Wsp-1*, a set of genes controlling water-soluble proteins in wheat and related species. *Genetical Research, Cambridge* 54:173-181.

Liu, J.Q., and Kolmer, J.A. (1998) Molecular and virulence diversity and linkage disequilibria in asexual and sexual populations of the wheat leaf rust fungus, *Puccinia recondita*. *Genome* 41:832-840.

Liu, M.C., Dubcovsky, J., and Dvořák, J. (1996) Recognition of homeology by wheat *Ph1* locus. *Genetics* 144:1195-1203.

Liu, Y.G., and Tsunewaki, K. (1991) Restriction fragment length polymorphism (RFLP) analysis in wheat. II. Linkage maps of the RFLP sites in common wheat. *Japanese Journal of Genetics* 66:617-633.

Logojan, A.A., and Molhár-Láng, M. (2000) Production of *Triticum aestivum-Aegilops biuncialis* chromosome additions. *Cereal Research Communications* 28(3), pp. 221-228.

Lukaszewski, A.J., and Curtis, C.A. (1993) Physical distribution of recombination in B-genome chromosomes of tetraploid wheat. *Theoretical and Applied Genetics* 86:121-127.

Lukaszewski, A.J. (2000) Cell biology & Molecular genetics: Manipulation of the 1RS.1BL translocation in wheat by induced homoeologous recombination. *Crop Science* 40:216-225.

Lupton, F.G.H. (1987) Wheat breeding, its scientific basis, Chapman and Hall, London.

Ma, X.F., Ross, K., and Gustafson, J.P. (2001) Physical mapping of restriction fragment length polymorphism (RFLP) markers in homoeologous groups 1 and 3 chromosomes of wheat by *in situ* hybridization. *Genome* 44:401-412.

Ma, Z-Q, Röder, M., and Sorrels, M.E. (1996) Frequencies and sequence characteristics of di-, tri-, and tetra-nucleotide microsatellites in wheat. *Genome* 39:123-130.

Marais, G.F. (1990) Preferential transmission in bread wheat of a chromosome segment derived from *Thinopyrum distichum* (Thunb.) Löve. *Plant Breeding* 104:152-159.

Marais, G.F. (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, G.F. (1992b) The modification of a common wheat-*Thinopyrum distichum* translocated chromosome with a locus homoeoallelic to *Lr19. Theoretical and Applied Genetics* 85:73-78.

Marais, G.F., and Marais, A.S. (1990) The assignment of a *Thinopyrum distichum* (Thunb.) Löve-derived translocation to the long arm of wheat chromosome *7D* using endopeptidase polymorphisms. *Theoretical and Applied Genetics* 79:182-186.

Marais, G.F., and Pretorius, Z.A. (1996) Gametocidal effects and resistance to wheat leaf rust and stem rust in derivatives of a *Triticum turgidum* ssp. *durum/Aegilops speltoides* hybrid. *Euphytica* 88:117-124.

Marais, G.F., Marais, A.S., and Groenewald, J.Z. (2000) Evaluation and reduction of *Lr19*-149, a recombinant form of the *Lr19* translocation of wheat. *Euphytica* 28:1-7.

Marais, G.F., Roux, H.S., Pretorius, Z.A., and Pienaar, R. de V. (1988) Resistance to leaf rust of wheat derived from *Thinopyrum distichum* (Thunb.) Löve. *In*: Proceedings of the 7th International Wheat Genetics Symposium, Cambridge, England. pp. 369-373.

Marais, G.F., Wessels, W.G., Horn, M., and du Toit, F. (1998) Association of a stem rust resistance gene (*Sr45*) and two Russian wheat aphid resistance genes (*Dn5* and *Dn7*) with mapped structural loci in common wheat. *South African Journal of Plant and Soil* 15:67-71.

Marathée, J-P., and Gomez-MacPherson, H. (2001) Future world supply and demand. *In*: The World Wheat Book: A History of Wheat Breeding, *Edited by* Bonjean, A.P. and Angus, W.J., Londres, Intercept, Volume 43, pp. 1107-1119.

May, C.E., and Appels, R. (1987) The molecular genetics of wheat, toward a understanding of 16 billion base pairs of DNA. *In*: Wheat and Wheat Improvement, 2nd Edition, American Society of Agronomy Inc, Madison, Wisconsin, USA. pp. 165-198.

McIntosch, R.A., Hart, G.E., Devos, K.M., Gale, M.D., and Rogers, W.J. (1998) Catalogue of gene symbols for wheat. *In*: Proceedings of the 9th International Wheat Genetics Symposium, Saskatoon, Saskatchewan, Canada, Volume 5.

McIntosch, R.A., Wellings, C.R, and Park, R.F. (1995) Wheat Rusts; An Atlas of Resistance Genes. CSIRO: Australia, East Melbourne.

McIntosh, R.A. (1998) Breeding wheat for resistance to biotic stresses. *Euphytica* 100:19-34.

McMillin, D.E., and Tuleen, N.A. (1977) Determination of the linkage relationships and the gene-centromere genetic distances for endopeptidase structural genes in hexaploid wheat. *Genetics* 88 (Soppl):S44.

Messmer, M.M., Keller, M, Zanetti, S., and Keller, B. (1999) Genetic linkage map of a wheat x spelt cross. *Theoretical and Applied Genetics* 98:1163-1170.

Messmer, M.M., Seyfarth, R., Keller, M., Schachermayr, G., Winzeler, M., Zanetti, S., Feuillet, C., and Keller, B. (2000). Genetic analysis of durable leaf rust resistance in winter wheat. *Theoretical and Applied Genetics* 100:419-431.

Mickelson-Young, L., Endo, T.R., and Gill, B.S. (1995) Genetic linkage map of a wheat x spelt cross. *Theoretical and Applied Genetics* 98:1163-1170.

Moore, G. (1995) Cereal genome evolution: pastoral pursuits with 'Lego' genomes. *Current Opinions in Genetics and Development* 5:717-724.

Moore, G., Abbo, S., Cheung, W., Foote, T., Gale, M.D., Koebner, R.M.D. Leitch, A., Leitch, I., Stancombe, P., Yano, M., and Flavell, R. (1993) Key features of cereal genome organization revealed by the use of cytosine methylation-sensitive restriction endonucleases. *Genomics* 15:472-482.

Moore, G., and Gustafson, J.P. (1997) Comparative analysis of cereal genomes. *In*: DNA markers: Protocols, Applications and Overviews, *Edited by* Caetano-Anollés, G. and Gresshoff, P.M., New York, Wiley-VCH, Volume 17, pp. 259-270.

Moore, G., Cheung, W., Schwarzacher, T., and Flavell, R. (1991a) BIS 1, a major component of the cereal genome and a tool for studying genomic organization. *Genomics* 10: 469-476.

Moore, G., Lucas, H., Batty, N., and Flavell, R. (1991b) A family of retrotransposons and associated genomic variation in wheat. *Genomics* 10:461-468.

Moore, G., Devos, K.M., Wang, Z., and Gale, M.D. (1995) Cereal genome evolution: Grasses, line up and form a circle. *Current Biology* 5:737-739.

Morris, R., and Sears, E.R. (1973) The cytogenetics of wheat and its relatives. *In*: Wheat and Wheat Improvement, 2nd Edition, American Society of Agronomy Inc, Madison, Wisconsin, USA. pp.19-87.

Moullet, O., Zhang, H.B., and Lagudah, E.S. (1999) Construction and characterization of a large DNA insert library from the D genome of wheat. *Theoretical and Applied Genetics* 99:305-313.

Mukai, Y., Nakahara, Y., and Yamamoto, M. (1993) Simultaneous discrimination of the three genomes in hexaploid wheat by multicolour fluorescence *in situ* hybridization using total genomic and highly repeated DNA probes. *Genome* 36:489-494.

Müller-Starck, G. (1998) Isozymes. *In*: Molecular Tools for Screening Biodiversity: Plants and Animals. *Edited by* Karp, A., Isaac, P.G. and Ingram, D.S., Chapman and Hall, London, Volume 5.1, pp. 75-77.

Murray, T.D., Parry, D.W., and Cattlin, N.D. (1998) A colour handbook of diseases of small grain cereal crops, Mansion Publishing Ltd., London, pp.49-51.

Naranjo, T. (1990) Chromosome structure of durum wheat. *Theoretical and Applied Genetics* 79:397-400.

Naranjo, T., Roca, A., Goicoechea, P.G., and Giraldez, R. (1987) Arm homoeology of wheat and rye chromosomes. *Genome* 29:873-882.

Nelson, J.C., Singh, R.P., Autrique, J.P., and Sorrels, M.E. (1997) Mapping genes conferring and suppressing leaf rust resistance in wheat. *Crop Science* 37:1928-1935.

Nelson, J.C., Van Deynze, A.E., Autrique, E., Sorrels, M.E., Lu, Y.H., Atkinson, M., Bernard, M., Leroy, P., Faris, J.D., and Anderson, J.A. (1995) Molecular mapping of wheat: major genes and rearrangements in homoeologous groups 4, 5 and 7. *Genetics* 141:721-731.

Okubara, P.A., Anderson, P.A., Ochoa, O.E., and Michelmore, R.W. (1994) Mutants of downy mildew resistance in *Lactuca sativa* (lettuce). *Genetics* 137:867-874.

Pal, N., Sidhu, J.S., Singh, H., Singh, S., and Dhaliwal, H.S. (1998) Construction of molecular linkage map in *Triticum monococcum*. *Crop Improvement* 25(2):159-166.

Payne, P.I., Holt, L.M. Hutchinson, J., and Bennett, M.D. (1984) Development and characterization of a line of bread wheat, *Triticum aestivum*, which lacks the short-arm satellite of chromosome 1B and the *Gli-B1* locus. *Theoretical and Applied Genetics* 68:327-334.

Pienaar, R. de V., and Van Niekerk, H.A. (1973) The effect of gamma-irradiation on wheat gametophytes. *In*: Proceedings of the 4th International Wheat Genetics Symposium, Missouri Agricultural Experimental Station, Columbia, USA. pp. 289-294.

Pinkel, D., Straume, T., and Gray, J.W. (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proceedings of the National Academy of Sciences (USA)* 83:2934-2938.

Plaschke, J., Ganal, M.W., and Röder, M.S. (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theoretical and Applied Genetics* 91:1001-1007.

Powell, W. Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., and Rafalsky, A. (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* 2:225-238.

Prabhu, K.V., Gupta, S.K., Charpe, A., and Koul, S. (2004) SCAR marker tagged to the alien leaf rust resistance gene *Lr19* uniquely marking the *Agropyron elongatum*-derived gene *Lr24* in wheat: a revision. *Plant Breeding* 123:417-420.

Prins, R., and Marais, G.F. (1998) An extended deletion map of the *Lr19* translocation and modified forms. *Euphytica* 103:95-102.

Prins, R., and Marais, G.F. (1999) A genetic study of the gametocidal effect of the *Lr19* translocation of common wheat. *South African Journal of Plant Soil* 16:10-14.

Prins, R., Groenewald, J.Z., Marais, G.F., Snape, J.W., and Koebner, R.M.D. (2001) AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. *Theoretical and Applied Genetics* 103:618-624.

Prins, R., Marais, G.F., Janse, B.J.H., Pretorius, Z.A., and Marais, A.S. (1996) A physical map of the *Thinopyrum*-derived *Lr19* translocation. *Genome* 39:1013-1019.

Prins, R., Marais, G.F., Pretorius, Z.A., Janse, B.J.H., and Marais, A.S. (1997) A study of modified forms of the *Lr19* translocation of common wheat. *Theoretical and Applied Genetics* 95:424-430.

Qi, L.L., Echalier, B., Chao, S., Lazo, G.R., Butler, G.E., Anderson, O.D., Akhunov, E.D., Dvořák, J., Linkiewicz, A.M., Ratnasiri, A., Dubcovsky, J., Bermudez-Kandianis, C.E., Greene, R.A., Kantety, R., La Rota, C.M., Munkvold, J.D., Sorrels, S.F., Sorrels, M.E., Dilbirligi, M., Sidhu, D., Erayman, M., Randhawa, H.S., Sandhu, D., Bondareva, S.N., Gill, K.S., Mahmoud, A.A., Ma, X.-F., Miftahudin, Gustafson, J.P., Conley, E.J., Nduati, V., Gonzalez-Hernandez, J.L., Anderson, J.A., Peng, J.H., Lapitan, N.L.V., Hossain, K.G., Kalavacharla, V., Kianian, S.F., Pathan, M.S., Zhang, D.S., Nguyen, H.T., Choi, D.-W., Fenton, R.D., Close, T.J., McGuire, P.E., Qualset, C.O., and Gill, B.S. (2004) A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. *Genetics* 168(2):701-712.

Rampling, L.R., Harker, N., Shariflou, M.R., and Morell, M.K. (2001) Detection and analysis systems for microsatellite markers in wheat. *Australian Journal of Agricultural Research* 52:1131-1141.

Rayburn, A.L., and Gill, B.S. (1985) Use of biotin-labelled probes to map specific DNA sequences on wheat chromosomes. *Journal of Heredity* 76:78-81.

Reiter, R. (2001) PCR-based marker systems. *In*: DNA-Based Markers in Plants, 2nd Edition, *Edited by* Phillips, R.L. and Vasil, I.K., Dordrecht, Kluwer Accademic Publishers, Volume 2, pp. 9-29.

Riley, R., and Chapman, V. (1958) Genetic control of the cytological diploid behaviour of hexaploid wheat. *Nature* 182:713-715.

Röder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H., Leroy, P., and Ganal, W. (1998a) A microsatellite map of wheat. *Genetics* 149:2007-2023.

Röder, M.S., Korzun, V., Gill, B.S., and Ganal, M.W. (1998b) The physical mapping of microsatellite markers in wheat. *Genome* 41:278-283.

Röder, M.S., Plaschke, J., König, S.U., Börner, A., Sorrels, M.E., Tanksley, S.D., and Ganal, M.W. (1995) Abundance, variability and chromosomal location of microsatellites in wheat. *Molecular and General Genetics* 246:327-333.

Roelfs, A.P. (1989) Epidemiology of the cereal rusts in North America. *Canadian Journal of Plant Pathology* 11:86-90.

Sandhu, D., Champoux, J.A., Bondareva, S.N., and Gill, K.S. (2001) Identification and physical localization of useful genes and markers to a major gene-rich region on wheat group 1S chromosomes. *Genetics* 157:1735-1747.

Sarma, R.N., Fish, L., Gill, B.S., and Snape, J.W. (2000) Physical characterization of the homoeologous group 5 chromosomes of wheat in terms of rice linkage blocks, and physical mapping of some important genes. *Genome* 43:191-198.

Sarma, R.N., Gill, B.S., Sasaki, T., Galiba, G., Sutka, J., Laurie, D.A., and Snape, J.W, (1998) Comparative mapping of the wheat chromosome 5A *Vrn-A1* region with rice and its relationship to QTL for flowering time. *Theoretical and Applied Genetics* 97:103-109.

Sawhney, R.N., Sharma, J.B., and Kumar, R. (1998) Assessment and exploitation of genetic variation for resistance to *Puccinia recondita* for stabilizing wheat production. *Indian Journal of Genetics* 58:251-262.

Sayre, K., Singh, R.P., Huerta-Espino, J., and Rajaram, S. (1998) Genetic progress in reducing losses to leaf rust in CIMMYT-derived Mexican spring wheat cultivars. *Crop Science* 38:654659.

Schachermayr, G.M., Messmer, M.M., Feuillet, C., Winzeler, H., Winzeler, M., and Keller, B. (1995) Identification of molecular markers linked to the *Agropyron elongatum*-derived leaf rust resistance gene *Lr24* in wheat. *Theoretical and Applied Genetics* 90:982-990.

Schlessinger, D. (1990) Yeast artificial chromosomes: tools for mapping and analysis of complex genomes. *Trends in Genetics* 6:248-258.

Scoles, G.J., Gill, B.S., Xin, Z.Y., Clarke, B.C., McIntyre, C.L., Chopman, C., and Appels, R. (1988) Frequent duplication and deletion events in the 5S RNA genes and the associated spacer regions of the Triticeae. *Plant System Evolution* 160:105-122.

Seah, S. Bariana, H., Jahier, J., Sivasithamparam, K., and Lagudah, E.S. (2000) The introgressed segment carrying rust resistance genes *Yr17*, *Lr37* and *Sr38* in wheat can be assayed by a cloned disease resistance gene-like sequence. *Theoretical and Applied Genetics* 102:600-605.

Sears, E.R. (1954) The aneuploids of common wheat. Research Bulletin-Missouri Agricultural Experiment Station 572:1-59.

Sears, E.R. (1966) Nullisomic-tetrasomic combinations in hexaploid wheat. *In*: Chromosome Manipulation and Plant Genetics. *Edited by* R. Riley and K.R. Lewis, Oliver and Boyd, London. pp. 29-45.

Sears, E.R. (1972a) Chromosome engineering in wheat. Stadler Symposium 4:23-38.

Sears, E.R. (1972b) *Agropyron*-wheat transfers through induced by homoeologous pairing. *Canadian Journal of Genetics and Cytology* 14:736.

Sears, E.R. (1976) Genetic control of chromosome pairing in wheat. *Annual Review of Genetics* 10:31-51.

Pectora roborant cultus recti

Sears, E.R. (1977a) An induced mutant with homoeologous pairing in common wheat. *Canadian Journal of Genetic Cytology* 24:715-719.

Sears, E.R. (1977b) Analysis of wheat-*Agropyron* recombinant chromosomes. *In*: Interspecific Hybridization in Plant Breeding, Proceedings of the 8th Eucarpia Congress, Madrid, pp. 63-72.

Sears, E.R. and Sears, L.M.S. (1978) The telosentric chromosomes of common wheat. *In*: Proceedings of the 5th International Wheat Genetics Symposium, New Dehli, India. pp. 389-407.

Seyfarth, R., Feuillet, C., Schachermayr, G., Winzeler, M., and Keller, B. (1999) Development of a molecular marker for the adult plant leaf rust resistance gene *Lr35* in wheat. *Theoretical and Applied Genetics* 99:554-560.

Shapiro, H.S. (1976) *In*: Handbook of Biochemistry and Molecular Biology: Nucleic Acids, *Edited by* G.D. Fasman, CRC Press, Cleveland, Ohio, Volume 2, p.259.

Sharma, D., and Knott, D.R. (1966) The transfer of leaf rust resistance from *Agropyron* to *Triticum* by irradiation. *Canadian Journal of Genetics and Cytology* 8:137-143.

Sharp, P.J., Chao, S., Desai, S., and Gale, M.D. (1989) The isolation and characterization and application in the Triticeae of a set of wheat RFLP probes identifying each homoeologous chromosome arm. *Theoretical and Applied Genetics* 78:342-348.

Shizuya, H., Birren, B., Kim, U-J., Mancino, V., Slepak, T., Tachiiri, Y., and Simon, M. (1992) Cloning and stable maintenance of 300-kilobase pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. *Proceedings of the National Academy of Science (USA)* 89:8794-8797.

Singh, D., Park, R.F., Bariana, H.S., and McIntosch, R.A. (2001a) Cytogenetic studies in wheat XIX. Chromosome location and linkage studies of a gene for leaf rust resistance in Australian cultivar 'Harrier'. *Plant Breeding* 120:7-12.

Singh, D., Park, R.F., and McIntosch, R.A. (2001b) Inheritance of seedling and adult plant resistance to leaf rust of selected Australian spring and England winter wheat varieties. *Plant Breeding* 120 503-507.

Singh, G.P., Nagarajan, R.C.S., Singh, J.S.G., and Singh, S.K. (2003) Items from India. Directorate of Wheat Research, Karnal 132 001, India. *Annual Wheat Newsletter* 49:47-48.

Singh, R.P. (1992) Association betwen gene *Lr34* for leaf rust resistance and leaf tip necrosis in wheat. *Crop Science* 32:874-878.

Singh, R.P., Mujeeb-Kazi, A., and Huerta-Espino, J. (1998) *Lr46*: A gene conferring slow-rusting resistance to leaf rust in wheat. *Phytopathology* 88(9):890-894.

Smith, G.R. (1994). Hotspots of homologous recombination. *Experientia* 50:234-242.

Snape, J.W., Flavell, R.B., O'Dell, M., Hughes, W.G., and Payne, P.I. (1985) Intrachromosomal mapping of the nucleolar organiser region relative to three marker loci on chromosome 1B of wheat (*Triticum aestivum*). *Theoretical and Applied Genetics* 69:263-270.

Somers, D.J., Isaac, P., and Edwards, K. (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 109:1105-1114.

Sourdille, P., Singh, S., Cadalon, T., Brown-Guedira, G.L., Gay, G., Qi, L., Gill, B.S., Dufour, P., Murigneux, A., and Bernard, M. (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct. Intergr. *Genomics* 4:12-25.

Southern, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* 98:503-517.

Spielmeyer, W., Huang, L., Bariana, H., Laroche, A., Gill, B.S., and Lagudah, E.S. (2000) NBS-LRR sequence family is associated with leaf and stripe rust resistance on the end of homoeoelogous chromosome group 1S of wheat. *Theoretical and Applied Genetics* 101:1139-1144.

Staub, J.E., Serquen, F.C., and Gupta, M. (1996) Genetic markers, map construction, and their application in plant breeding. *HortScience* 31:729-741.

Stephenson, P., Bryan, G., Kirby, J., Collins, A., Devos, K.M., Busso, C., and Gale, M. (1998) Fifty new microsatellite loci for the wheat genetic map. *Theoretical and Applied Genetics* 97:946-949.

Stuber, C.W., Polacco, M., and Senior, M.L. (1999) Synergy of empirical breeding, marker-assisted selection, and genomics to increase crop yield potential. *Crop Science* 39:1571-1583.

Sutka, J., Galiba, G., Vaguifalvi, A., Gill, B.S., and Snape, J.W. (1999) Physical mapping of the *Vrn-A1* and *Fr1* genes on chromosome 5A of wheat using deletion lines. *Theoretical and Applied Genetics* 99:199-202.

Talbert, L.E., Blake, N.K., Chee, P.W., Blake, T.K., and Magyar, G.M. (1994) Evaluation of sequence-tagged-site PCR products as molecular in wheat. *Theoretical and Applied Genetics* 87:789-794.

Tang, K.S., and Hart, G.E. (1975) Use of isozymes as chromosome markers in wheat-rye addition lines in triticale. *Genetical Research, Cambridge* 26:187-201.

Timmermans, M.C.P., Das, O.P., Bradeen, J.M., and Messing, J. (1997) Region-specific *cis*-and *trans*-acting factors contributing to genetic variability in meiotic recombination in maize. *Genetics* 146:1101-1113.

Tsujimoto, H., and Noda, K. (1988) Chromosome breakage in wheat induced by the gametocidal gene of *Aegilops triuncialis* L.: Its utilisation for wheat genetics and breeding. *In*: Proceedings of the 7th International Wheat Genetics Symposium, Cambridge, England. pp. 455-460.

Tsujimoto, H., and Noda, K. (1990) Deletion mapping by gametocidal genes in common wheat: position of speltoid suppression (*Q*) and *β*-amylase (*β*-Amy-A2) genes on chromosome 5A. *Genome* 31:850-853.

Tsujimoto, H., Usami, N., Hasegawa, K., Yamada, T., Nagaki, K. *et al*. (1999) De novo synthesis of telomere sequences at the healed breakpoints of wheat deletion chromosomes. *Molecular and General Genetics* 262:851-856.

Van Deynze, A.E., Dubcovsky, J., Gill, K.S., Nelson, J.C., Sorrels, M.E., Dvořák, J., Gill, B.S., Lagudah, E.S., McCouch, S.R., and Appels, R. (1995) Molecular-genetic maps for group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. *Genome* 38:45-59.

Van Niekerk, H. (2001) South Africa wheat pool. *In*: The World Wheat Book: A History of Wheat Breeding, *Edited by* Bonjean, A.P. and Angus, W.J., Londres, Intercept, Volume 35, pp. 924-936.

Vander-Gaag, D.J., and Jacobs, T. (1997) Inheritance of host plant effect on latent period of wheat leaf rust in single-seed descent F_8 lines. *Euphytica* 93:67-72.

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

Wall, A.M., Riley, R., and Chapman, V. (1971) Wheat mutants permitting homoeologous meiotic chromosome pairing. *Genetic Research*. 18:311-318.

Wang, Z., Weber, J.L., Zhong, G., and Tanksley, D.S. (1994) Survey of plant short tandem DNA repeats. *Theoretical and Applied Genetics* 88:1-8.

Weber, D., and Helentjaris, T. (1989) Mapping RFLP loci in maize using B-A translocations. *Genetics* 121:583-590.

Wechter, W.P. and Dean, R.A. (1998) Development of a sequence-specific primer that amplify a 1.5 kb DNA marker for race 1 Fusarium wilt resistance in *Cucumis melo* L. *HortScience* 33:291-292.

Weining, S., and Langridge, P. (1991) Identification and mapping of polymorphisms in cereals based on the polymerase chain reaction. *Theoretical and Applied Genetics* 82:209-216.

Weng, Y., Tuleen, N.A., and Hart, G.E. (2000) Extended physical maps and a consensus physical map of the homoeologous group-6 chromosomes of wheat (*Triticum aestivum* L. em Thell). *Theoretical and Applied Genetics* 100:519-527.

Werner, J.E., Endo, T.R., and Gill, B.S. (1992) Toward a cytogenetically based physical of the wheat genome. *Proceedings of the National Academy of Sciences (USA)* 89:11307-11311.

Wiese, M.V. (1977) Compendium of Wheat Diseases, The American Phytopathological Society, Minnesota.

William, H.M., Hoisington, D., Singh, R.P., and González-de-León, D. (1997) Detection of quantitative trait loci associated with leaf rust resistance in bread wheat. *Genome* 40:253-260.

Wilson, R.K., Chen, C., Avdalovic, N., Burns, J., and Hood, L. (1990) Development of an automated procedure for fluorescent DNA sequencing. *Genomics* 6:626-634.

Yamamori, M. (1994) An N-band marker for gene *Lr18* for resistance to leaf rust in wheat. *Theoretical and Applied Genetics* 89:643-646.

Yan, L., Loukoianov, A., Tranquilli, G., Helguera, M., Fahima, T., and Dubcovsky, J. (2003) Positional cloning of the wheat vernalization gene *VRN1*. *Proceedings of the National Academy of Sciences (USA)* 100:6263-6268.

Yu, G.X., Bush, A.L., and Wise, R.P. (1996) Comparative mapping of homoeologous group 1 regions and genes for resistance to obligate biotrophs in *Avena*, *Hordeum* and *Zea mays*. *Genome* 39:155-164.

Zhang, H., Nasuda, S., and Endo, T.R. (2000) Identification of AFLP markers on the satellite region of chromosome 1BS in wheat. *Genome* 43:729-735.

Zhang, H.-B., and Dvořák, J. (1990) Characterization and distribution of an interspersed repeated nucleotide sequence from *Lophopyrum elongatum* and mapping of a segregation distortion factor with it. *Genome* 33:927-936.