Mapping genes for stem rust and Russian wheat aphid resistance in bread wheat (*Triticum aestivum*)

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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:

SUMMARY

Stem rust is considered the most damaging of the wheat rusts causing yield losses of more than 50% in epidemic years. Similarly, Russian wheat aphids (RWA) can be regarded as one of the most devastating insect pests of wheat. Yield losses due to RWA primarily result from a reduction in plant resources (sucking plant sap). Secondary losses are incurred by viruses transmitted during feeding. Mapping disease and insect resistance genes that are effective against prevailing pathotypes and biotypes of South Africa will optimize their utilization in breeding programmes.

The wheat line, 87M66-2-1, is homozygous for a single dominant stem rust resistance gene located on chromosome 1D. This stem rust resistance gene has been derived from *Triticum tauschii* accession RL5289 and is here referred to as *Srtau*. The aim of this study was to determine the chromosome arm involved. Following the chromosome arm allocation of *Srtau*, its possible linkage with the genes *Rg2*, *Lr21*, *SrX* and *Sr33* was studied.

A telosomic analysis has shown that *Srtau* is located on chromosome arm 1DS and is linked to the centromere with a recombination frequency of 21 ± 3.40%. Glume blotch and a heavy mildew infection of segregating families planted in the field in 1996 made the linkage study between *Lr21* (leaf rust resistance) and *Rg2* (glume colour) impossible. However, estimated linkages of 9 ± 1.9 map units between *Sr33* (stem rust resistance) and *Srtau*, ± 6 map units between *SrX* (stem rust resistance) and *Sr33* and ± 10 map units between *SrX* and *Srtau* suggested that *SrX*, *Sr33* and *Srtau* are closely linked on 1DS. Taking existing map data into consideration, it seems that the most likely order of the genes is: centromere — *Srtau* — *Sr33* — *SrX*.

A single dominant RWA resistance gene, *Dn5*, was identified in the *T. aestivum* accession 'SA 463' and is located on chromosome 7D. The aim of this study was to determine the chromosome arm involved. The possible linkage of *Dn5* with the endopeptidase locus, *Ep-D1b*, and chlorina mutant gene, *cn-D1*, was then studied. Endopeptidase zymograms of 'SA 463' revealed two unknown polymorphisms. F_2 monosomic analyses involving the chromosomes 7A, 7B and 7D were performed in an attempt to identify the loci associated with these polymorphisms.

Dn5 was mapped on chromosome arm 7DL. A recombination frequency of 60 ± 4.53% between *Dn5* and the centromere suggested the absence of linkage. Linkage between *Ep-D1* and *cn-D1* could not be calculated as a result of similar isoelectric points of the 7DL encoded endopeptidases of the parental material studied. Recombination frequencies of 32 ± 4.97% between *Dn5* and *Ep-D1* and 37 ± 6.30% between *Dn5* and *cn-D1* were, however, encountered. The two novel endopeptidase alleles encountered in 'SA 463' were designated as *Ep-D1e* and *Ep-A1d*.

A RWA resistance gene was transferred from the rye accession 'Turkey 77' to wheat and in the process the RWA resistant wheat lines 91M37-7 and 91M37-51 were derived. No rye chromatin could be detected in these plants following C-banding. The aim of this study was to determine (i) on which chromosome the gene(s) is located, and (ii) whether the resistance can be the result of a small intercalary translocation of rye chromatin.

A monosomic analysis of the RWA resistance gene in 91M37-51 has shown that a single dominant resistance gene occurs on chromosome 7D. The use of rye-specific dispersed probes did not reveal any polymorphisms between the negative controls and RWA resistant lines 91M37-7 and 91M37-51 which would suggest that it is unlikely that the resistance was derived from rye.

OPSOMMING

Stamroes word as die mees vernietigende graanroessiekte beskou en het in epidemiese jare oesverliese van meer as 50% tot gevolg. Russiese koringluise is eweneens een van die ernstigste insekplae van koring. Russiese koringluise veroorsaak oesverliese deurdat dit plantsap uitsuig en die plant van voedingstowwe beroof. Dit tree egter ook as 'n virusvektor op en kan so indirekte oesverliese veroorsaak. Kartering van siekte- en insekweerstandsgene wat effektief is teen die Suid-Afrikaanse patotipes en biotipes, sal hulle gebruik in teelprogramme optimiseer.

Die koringlyn, 87M66-2-1, is homosigoties vir 'n dominante stamroes-weerstandsgeen wat op chromosoom 1D voorkom. Hierdie weerstandsgeen is uit die *Triticum tauschii* aanwins, RL5289, afkomstig en word hierna verwys as *Srtau*. Daar is gepoog om te bepaal op watter chromosoom-arm *Srtau* voorkom, waarna sy koppeling met betrekking tot die gene *Rg2*, *Lr21*, *SrX* en *Sr33* bepaal is.

'n Telosoomanalise het getoon dat *Srtau* op chromosoom-arm 1DS voorkom en gekoppel is aan die sentromeer met 'n rekombinasie-frekwensie van 21 ± 3.40%. Segregerende populasies wat in 1996 in die land geplant is, is hewig deur aarvlek en poeieragtige meeldou besmet en dit het die moontlike bepaling van koppeling tussen *Lr21* (blaarroesweerstand) en *Rg2* (aarkaffie kleur) belemmer. Koppelingsafstande van 9 ± 1.9 kaart-eenhede tussen *Sr33* (stamroesweerstand) en *Srtau*, ± 6 kaart-eenhede tussen *SrX* (stamroesweerstand) en *Sr33* en ± 10 kaart-eenhede tussen *SrX* en *Srtau* is geraam en toon dat *SrX*, *Sr33* en *Srtau* nou gekoppel is. Die waarskynlikste volgorde van die gene op 1DS is: sentromeer — *Srtau* — *Sr33* — *SrX*.

'n Enkele dominante Russiese koringluis-weerstandsgeen, *Dn5*, is in die *T. aestivum* aanwins 'SA 463' geïdentifiseer en kom op chromosoom 7D voor. Die studie het ten doel gehad om te bepaal op watter chromosoom-arm *Dn5* voorkom, asook wat die koppeling van *Dn5* met die endopeptidase lokus, *Ep-D1*, en die chlorina mutante geen, *cn-D1*, is. Endopeptidase simogramme van 'SA 463' het twee onbekende polimorfismes getoon. Die gene wat kodeer vir hierdie twee polimorfismes is met behulp van F_2 monosoom-analises wat die chromosome 7A, 7B en 7D betrek, geïdentifiseer.

Dn5 is op chromosoom 7DL gekarteer. 'n Rekombinasie-frekwensie van 60 ± 4.53% is gevind vir die sentromeer en *Dn5* en dui op die afwesigheid van koppeling. Koppeling tussen *Ep-D1* en *cn-D1* kon nie bepaal word nie omdat die endopeptidase bande geproduseer deur die ouerlike materiaal wat in die studie gebruik is, nie met sekerheid in die nageslag onderskei kon word nie. Rekombinasie-frekwensies van 32 ± 4.97% tussen *Dn5* en *Ep-D1* en 37 ± 6.30% tussen *Dn5* en *cn-D1* is egter bereken. Dit word voorgestel dat daar na die twee onbekende endopeptidase-allele wat in 'SA 463' voorkom, verwys word as *Ep-D1e* en *Ep-A1d*.

'n Russiese koringluis-weerstandsgeen is uit die rog-aanwins, 'Turkey 77', oorgedra na koring en in die proses is die Russies koringluis weerstandbiedende lyne, 91M37-7 en 91M37-51, geproduseer. Geen rog-chromatien kon egter met behulp van C-bande in hierdie lyne waargeneem word nie. Die doel van die studie was om te bepaal (i) op watter chromosoom die geen(e) voorkom, en (ii), of die Russiese koringluis weerstandsgeen die gevolg kan wees van 'n klein interkalêre translokasie van rog- chromatien.

'n Monosoom-analise van die Russiese koringluis-weerstandsgeen in 91M37-51 het getoon dat 'n enkele dominante weerstandsgeen op chromosoom 7D voorkom. Rog-spesifieke herhalende peilers het geen polimorfismes tussen negatiewe kontroles en die Russiese koringluis weerstandbiedende lyne 91M37-7 en 91M37-51 getoon nie. Dit is dus onwaarskynlik dat die weerstand in die lyne uit rog verhaal is.

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1. LITERATURE REVIEW

1.1 INTRODUCTION

The high demand for food because of the rapidly growing world population is a cause for concern, particularly in the third world countries (Briggle and Curtis 1987). To meet increasing food demands crops are being grown to a greater extent in more marginal areas. This practice necessitates the development of cultivars that are better adapted to such conditions. Droughts, high levels of free aluminium in acid soils, diseases and insects are some of the more general causes of yield reductions (Briggle and Curtis 1987). There are, however, ways to reduce the impact of such stresses. Plants that have higher levels of salt and frost tolerance can for instance be selected, while diseases and insects can be controlled chemically, genetically and other biological means. Although spraying does provide effective pest control, it is not always an acceptable solution. Chemicals and their application are expensive, mostly harmful to the environment and often poses a health risk to those applying it or consuming treated products. The need exists therefore for cultivars that have natural resistance against diseases and insects. To achieve this goal, plant breeders have to use all the germplasm available, search for new genes and adapt their breeding programmes to address these problems.

Wheat has the broadest adaptation of all the cereal crop species and is the number one food grain consumed directly by humans. Furthermore, wheat provides on a global basis more nourishment for people than any other food source and is the most important source of carbohydrates in the majority of countries in the temperate zone (Briggle and Curtis 1987). Diseases and insects causing damage to wheat commonly occur in all the production areas. The main source of resistance genes is that present in wheat. This source has largely been depleted because new pathotypes and biotypes that acquire virulence to existing resistance genes continuously evolve. Monoculture, over-population and the destruction of natural habitats have led to extensive genetic erosion in the centres of germplasm diversity (Briggle and Curtis 1987). The broadening of the wheat gene pool is now based on the introgression of resistance genes from the wild relatives of wheat, induced mutations and genetic engineering.

Existing and newly acquired genes need to be mapped in order to optimize their utilization in breeding programmes. Mapping helps to define the spatial relationships among loci, the way in which they can be expected to segregate and possible instances of allelism. Well developed genetic maps may be particularly useful in attempts to find closely linked marker genes that may be used to select indirectly for a target resistance gene (Rafalski and Tingey 1993). Many disease and insect resistance genes are notoriously difficult to detect through biotesting in breeding populations. In these instances closely linked marker genes may allow for cheaper, more rapid and more accurate identification (Vahl and Müller 1991; Schachermayr *et al.* 1994). Many plant-parasite interactions are based on gene-for-gene mechanisms and it is generally recognized that a gene employed singly as resistance source may soon be overcome by the parasite (Flor 1942a,b). If several diverse and effective resistance genes can be pyramided in a single genotype, the resistance is buffered as the parasite can acquire virulence only if it mutates at several loci simultaneously. However, the effective application of the principle in breeding prerequires the availability of a reliable marker or markers for each resistance gene (Vahl and Müller 1991; Schachermayr *et al.* 1994).

1.2 MARKER ASSISTED SELECTION

Traditionally, important plant traits were selected on the basis of observable phenotypic differences. As a result, rapid genetic progress could be made for traits where the phenotype provides a reliable indication of underlying genotypic differences in, i.e., traits with high heritability. However, some traits (yield) e.g., are determined by multiple genes of which the effects are extensively modified by environmental effects. Such traits are said to have low heritability since the phenotype does not allow for an accurate assessment of the genotype (Ajmone-Marsan *et al.* 1995; Gimelfarb and Lande 1995). Sometimes it is possible to select indirectly for the presence of a gene of which the direct effect or product is difficult to detect. Morphological, biochemical and molecular markers are now used to simplify the introgression of a gene into the desired plant material (McIntosh 1988a).

The first applications of indirect selection were based on the presence of morphological marker genes closely linked to the target genes. Morphological markers, however, have low levels of

polymorphism, tend to have detrimental pleiotropic effects and are subject to variable expression in different genetic backgrounds (Sharp *et al.* 1989; Gale and Sharp 1988; Phillips and Vasil 1994). The number of markers that can be used in a single population is also limited because crosses have to be made to introgress each morphological marker into the material. Much work was therefore done to develop markers that display a high degree of polymorphism without the need to make extra crosses and that is not conditioned by the environment (Gale and Sharp 1988).

Saturated chromosome maps facilitate the mapping of new genes and the selection of markers closely linked to a gene of agronomic interest (Rafalski and Tingey 1993). As morphological markers are not sufficiently informative to construct these chromosome maps, much time is currently devoted to the development of appropriate biochemical and molecular markers. Each year new marker loci are mapped on the existing chromosome maps of all the cultivated crops (McIntosh 1988b). The chances of finding a marker gene that is closely linked to an agronomically important gene therefore continuously improve.

1.2.1 Plant genome mapping

Genome mapping is an important part of genetic research throughout the world. The Human Genome Project has the ultimate goal to map all the genes in the human genome and to determine the DNA sequence of the entire genome. Genome mapping contributes to the search for disease loci and possible genetic cures. Although this project is intensive and expensive, geneticists around the world are helping to saturate the human chromosome map with markers (Casey *et al.* 1992).

Morphological markers have low levels of polymorphism, are mostly dominant (heterozygotes can not be distinguished from homozygotes) and have pleiotropic effects on traits of agronomic significance (Gale and Sharp 1988). This hampers the application of such markers in the development of new cultivars. The development of molecular markers therefore added a powerful tool for selection in breeding programmes. It promises a major advantage in selecting the superior material not only for single genes, but also for multigenic traits (Rafalski and Tingey 1993).

Mapping can be done in two ways, namely physically or genetically. Physical mapping is based on the physical location relative to specific chromosome landmarks and involves techniques such as C-banding (Gill and Kimber 1974a,b), *in situ* hybridization (Rayburn and Gill 1985), fluorescence *in situ* hybridization (Nkongolo *et al.* 1993) and DNA-sequencing (Casey *et al.* 1992). Genetic mapping makes use of polymorphic markers and measures the distance between two markers in centimorgans. This strategy is based on the frequency of meiotic recombination and includes markers such as isozymes (McMillin *et al.* 1993), restriction fragment length polymorphisms (Chao *et al.* 1989), random amplified polymorphic DNA fragments (Devos and Gale 1992) and microsatellites (Devos *et al.* 1995).

Saturated molecular marker-based linkage maps of wheat will facilitate the mapping of newly discovered or introgressed genes to a specific chromosome region. Marker genes that are dispersed at regular intervals along the entire length of all the chromosomes will also be an enormous aid to indirect selection. This will ensure tight linkage between a selected marker gene and an agronomically important gene. For example, Singh (1992) reported close linkage (1.3%) between *Lr34*, an adult plant leaf rust resistance gene, and *Ltn*, a gene conferring leaf tip necrosis. Close linkage (<0.47%) was also observed between an endopeptidase isozyme, *Ep-D1b* and an eyespot resistance gene, *Pch-1* (McMillin *et al.* 1986; Worland *et al.* 1988; Summers *et al.* 1988). Screening for these marker genes is simple and less expensive than the respective disease scoring methods. It will speed up selection of resistant material in a segregating population and is especially of great value when more than one resistance gene is being used in breeding programmes. The chromosome linkage map of wheat is, however, poorly developed. This has largely resulted from the crop's polyploid nature and a relatively low level of useful polymorphism (Chao *et al.* 1989).

1.2.2 Comparative mapping

Bread wheat, *Triticum aestivum* L. em. Thell. (2n = 6x = 42, AABBDD), is an allohexaploid that has three independently derived genomes. It is believed to have resulted from a hybridization event that occurred about 8000 years ago between a tetraploid wheat, *T. turgidum* L. (2n = 4x = 28, AABB), and a diploid grass, *T. tauschii* (Coss) Schmal. (2n = 2x = 14, DD). The tetraploid,

T. turgidum, is in turn a descendant of the diploid grass *T. monococcum* L.var. *urartu* (2n = 2x = 14, AA) and at least one unknown diploid grass predecessor that contributed the B-genome (Gill and Gill 1994).

The hexaploid wheat genome is very large (16×10^{12} bp). Limited polymorphisms were observed among wheat cultivars with many molecular markers tested. Probable reasons for this are (i) the recent evolutionary origin of the hexaploid level and (ii) the fact that the genetic base has been extremely narrowed by the hybridization event that might have involved only a few plants (Chao *et al.* 1989). This complicates the construction of a genetic map of wheat which is based on polymorphisms. Since wheat is such an important agronomic crop, solutions must be found for this problem.

Gill *et al.* (1993) showed that the chromosomes of *T. tauschii*, the diploid D-genome progenitor of wheat, pairs completely with the D-genome chromosomes of wheat and the gene synteny relationship is conserved between the two species. The progenitors of wheat are diploids that contain higher levels of polymorphism and they are thus excellent sources which can be used to enrich the wheat linkage maps (Gill *et al.* 1991b). The order and relative location of molecular loci are largely conserved between *T. tauschii* and the D-, and also the A- and B-genomes of wheat. The *T. tauschii* map is therefore equivalent to the genetic linkage map of each genome of wheat barring some exceptions.

Due to the conserved gene synteny between the diploid progenitors and wheat, any molecular marker present in a certain region on the chromosomes of a diploid can serve to indicate the probable location of corresponding molecular markers on the wheat chromosomes. This will ensure that more markers will be available to plant geneticists for use in linkage studies. Much work has therefore been done to saturate the chromosome linkage maps of the *T. tauschii* genome (Gill *et al.* 1991b; Kam-Morgan *et al.* 1989). The reason for the selection of the D-genome for comparative mapping is that the chromosomes of the A- and B-genomes of the diploid ancestral species show reduced pairing with their homoeologues in polyploid wheat, and therefore have become modified during the long and parallel evolution at the diploid and polyploid levels (Gill and Kimber 1974b).

It has been known that gene synteny is conserved not only between diploid ancestors and the three genomes of wheat, but also among other diploid species of the Triticeae (Hart *et al.* 1987). When geneticists compared the hybridization of probes used to derive maps of barley, oats (Anderson *et al.* 1992), rye (Devos *et al.* 1993), maize and rice (Ahn *et al.* 1993) with that of wheat, they found that most of the probes cross-hybridized. This gene synteny will help to saturate the wheat linkage map even more rapidly.

1.2.3 Physical mapping

Physical mapping is usually done on metaphase chromosomes. These can be analysed to identify a certain chromosome or part of a chromosome, map a probe to a specific band or position on the chromosome or compare the relative binding sites of different probes. The distances between bands, markers or probes are measured directly. Physical mapping may be based on C-banding, *in situ* hybridization, fluorescence *in situ* hybridization, deletion analysis or sequencing. The different strategies followed in plant genome mapping differ from mapping in the human genome where finer resolutions are obtained (Casey *et al.* 1992). Physical mapping in plant genomes must be optimized to attain the best resolution for the specific crop.

1.2.3.1 C-banding

Although a number of banding techniques are available for chromosome identification in wheat, C-bands reveal the most information and all the chromosomes can be distinguished by a characteristic banding pattern (Endo 1986; Gill *et al.* 1991a). Giemsa C-band staining in cereal chromosomes is based on the denaturation-reassociation of DNA. The highly repetitive DNA (constitutive heterochromatin) reassociates faster and appears as dark bands. This ensures that the individual chromosomes of a metaphase cell develop characteristic C-band patterns. These features allow for the identification of each chromosome of a specific crop and for differentiation between the chromosomes of different species (Gill and Kimber 1974b). *In situ* hybridization of two repetitive rye DNA sequences (pSc119 and pSc74) produced banding patterns on rye chromosomes comparable to those resulting from C-banding. All the rye chromosomes and chromosome arms could be distinguished and the two techniques gave similar banding patterns

(Mukai *et al.* 1992).

Examples of C-band karyotypes and idiograms constructed within the Triticeae are those for *T. tauschii* (Friebe *et al.* 1992), *T. longissimum* (Friebe *et al.* 1993), *T. aestivum* (Gill and Kimber 1974b; Gill *et al.* 1991a) and *Secale cereale* (Gill and Kimber 1974a). The availability of detailed C-band karyotypes for many species allows for the detection of wheat-alien translocations or alien addition chromosomes in a wheat nucleus. Such an approach was used to detect wheat-rye translocation chromosomes following crosses to enrich the wheat gene pool (Lukaszewski and Gustafson 1983; Lukaszewski 1994).

1.2.3.2 *In situ* hybridization (ISH)

The *in situ* hybridization (ISH) technique localizes genes or DNA-sequences on chromosomes in cytological preparations. DNA probes used in this technique were first labelled with radioactive isotopes. The resolution was, however, poor and long exposure times were required to obtain signals (Jiang and Gill 1994). Rayburn and Gill (1985) were the first to use biotin-labelled probes to map the rye dispersed DNA probe, pSc119, by ISH to somatic metaphase chromosomes of a plant species. Compared to the use of isotopes, the sensitivity has been greatly improved and the hybridization sites could be detected within a few hours.

Detection of the presence of alien chromatin in a wheat nucleus is now possible by doing ISH with alien dispersed probes. This technique is useful in attempts to introgress resistance (or other) genes from different species to the gene pool of wheat (McIntyre *et al.* 1990; Mukai *et al.* 1993a). Chen and Gustafson (1995) determined the physical location of cDNA clones from a RFLP-based genetic map of wheat homoeologous group-7 chromosomes with the ISH technique. The results obtained confirmed that the gene order on the physical map was the same as the order derived through genetic mapping experiments. The distance of each marker from the centromere on the physical map was, however, different from that shown on the genetic map. Most of the markers were physically mapped in the middle of the chromosome arm, while genetic maps showed that the markers are evenly distributed along the chromosomes with markers also near the centromeres and telomeres. The results implied reduced recombination in the centromeric

and telomeric regions of the wheat homoeologous group-7 chromosomes. Markers on the physical map are located outside the C-banded regions and are therefore situated in the euchromatic regions.

1.2.3.3 Fluorescence *in situ* hybridization (FISH)

The strategy followed with fluorescence *in situ* hybridization (FISH) is basically the same as with the ISH technique, exept that fluorochromes are used for signal detection. Nkongolo *et al.* (1993) optimized the technique for the detection of rye chromatin in wheat. This ensured the reliable detection of small rye fragments in wheat chromosomes without the need for intensive analysis of hybridization patterns. FISH has an increased sensitivity over the enzymatic detection followed in the ISH protocol because hybridization signals can be amplified with the repeated use of anti-avidin-antibody and fluorescence-conjugated avidin. The technique was improved further by doubling the probe concentration, double exposing the cells during photography and using different filters.

Physical maps of wheat genomes were greatly improved with the simultaneous ISH of different labelled fluorescent probes. The order of the probes could be distinguished as well as their physical locations relative to each other (Leitch *et al.* 1991). Genomic *in situ* hybridization (GISH) in combination with multicolour FISH is a powerful tool for analysing genome organization and evolution. Mukai et al. (1993b) discriminated simultaneously between the three genomes in hexaploid wheat by labelling total genomic DNA of the possible diploid genome progenitors with different haptens while detection was made possible with the use of different fluorochromes.

Problems are, however, experienced with physical mapping in plant genomes due to the plant cell wall and cytoplasmic material present in metaphase cells. This produces background during detection that can result in wrong conclusions.

1.2.3.4 Deletion mapping

Linked polymorphic markers are not always available to map a gene to a certain locus. To bypass this problem, Endo (1990) induced chromosome mutations in wheat. Deletion mutants thus obtained were then used for physical mapping. The genome of wheat can tolerate the partial or complete loss of a chromosome due to its polyploid nature. Sears (1954) took advantage of this phenomenon and developed a complete series of aneuploids, in each of which a complete chromosome or chromosome arm is missing.

Endo (1990) developed deletion lines in the wheat cultivar 'Chinese Spring' (CS) making use of a chromosome of *T. cylindricum* that has gametocidal properties in a wheat background. Deletions occurred in hybrid zygotes when a monosomic addition line of the gametocidal chromosome was backcrossed as female parent to CS. The deletions arose in the egg cells lacking the alien gametocidal chromosome. Plants with deletions were identified through C-banding and were self-pollinated. Offspring homozygous for a deletion were again selected by C-banding. Through this strategy almost 400 deletion lines were recovered spanning all 21 wheat chromosomes. Deletion lines can now be used to allocate genes to a specific chromosome region. The gene is placed between the break point of the largest deletion showing the target trait and that of the least deletion not showing it.

Gill *et al.* (1993) constructed a consensus physical map of the wheat group-6 chromosomes with the aid of 26 chromosome deletion lines for this homoeologous group. Twenty-five chromosome 6D-specific DNA probes and six wheat homoeologous group-6 DNA probes were mapped on the deletion lines. This strategy is helpful to saturate the wheat chromosome maps with markers by ordering them physically on consensus maps.

Deletion mapping was also employed to map genes on a translocated chromosome segment (*Lr19*) derived from *Thinopyrum* (Thunb.) Löve (Marais 1992a). Deletion lines were produced by gamma irradiation of the germplasm line 'Indis' followed by its pollination with a susceptible cultivar and screening for mutants in the F_2 and F_3 generations using marker genes present in the translocated area. This technique helped to order the marker loci on the translocated chromosome

and will help to search for additional markers in this area.

1.2.3.5 Sequencing

The ultimate physical map of any genome is the complete DNA sequence. The rate, efficiency and reliability of the standard sequencing procedures must be improved to achieve this goal (Casey *et al.* 1992). Advances in sequencing included automation of the process, improvement of the electrophoresis technique to detect more base pairs per gel, modification of the labelling protocol to ensure that both strands can be sequenced simultaneously and the use of computer software to analyse the DNA sequence (Lundeberg and Uhlén 1995). Despite such improvements, the sequencing of the wheat genome is still relatively underdeveloped, mainly due to polyploidy of the genome.

1.2.4 Genetic mapping

Markers used in genetic mapping must be polymorphic, i.e., alternative alleles must exist among individuals. The strategy is based on meiotic recombination and detects how tightly two markers are linked with each other. Distances between markers are measured in centimorgans (Casey *et al.* 1992).

In wheat, the wide range of aneuploids that are available can be used to allocate genes to specific chromosomes or chromosome arms (Sears 1954). Nullisomic, monosomic, trisomic, tetrasomic and telosomic aneuploid chromosome stocks have been developed and are used extensively for genetic mapping in wheat breeding. Loci that have been mapped with the aid of aneuploid stocks include isozymes (McMillin *et al.* 1993), restriction fragment length polymorphisms (Chao *et al.* 1989), random amplified polymorphic DNA fragments (Talbert *et al.* 1994) and microsatellites (Rafalski and Tingey 1993).

1.2.4.1 Aneuploidy

Wheat is a polyploid species having three distinct but homoeologous genomes. The loss of a

chromosome or a part of a chromosome can therefore be buffered by the remaining homoeologous chromosomes (Sears 1944). Sears (1954) developed nullisomic, monosomic, trisomic and tetrasomic series for the 21 chromosomes of wheat in the cultivar 'Chinese Spring'. Sears also developed a complete series of telocentrics for all chromosome arms in 'Chinese Spring'.

Monosomic and telosomic aneuploids are most commonly used for genetic studies in wheat. A gene of interest is assigned to a particular chromosome by monosomic analysis, while telosomics are used to map a gene to a specific chromosome arm. The linkage between the gene and centromere is also determined during the telosomic analysis and is a valuable tool for the construction of linkage maps (Sears 1954; Sears 1966; Sears 1974).

1.2.4.1.1 Monosomic analysis

Monosomic analysis is done by crossing a line with the gene of interest as the male parent to each of the 21 'Chinese Spring' monosomics. The donor parent is usually (but not necessarily) homozygous for the unmapped gene. Monosomic F_1 plants are selected cytologically. If the gene of interest is recessive, the monosomic F_1 plants from the critical cross will be hemizygous for the locus being studied and will express the recessive phenotype. The monosomic F_1 plants produced from the 20 non-critical crosses will be heterozygous for the locus and will therefore express the dominant phenotype. If the unknown gene is dominant, all the monosomic F_1 plants will express the trait and need to be self-pollinated to produce a F_2. Segregation in the resulting F_2 populations will identify the critical chromosome and give an indication of the number of genes involved (Washington and Sears 1970; Law *et al.* 1987).

In a study of the breeding behaviour of the wheat monosomics, Kuspira and Unrau (1959) found that the F_2 population resulting from self-pollinated monosomic F_1 plants consists of approximately 24% disomics, 73% monosomics and 3% nullisomics. In the critical F_2 family of a monosomic analysis, the gene of interest occurs on the monosomic chromosome and reflects the segregation of this chromosome. Thus, if a single dominant gene is involved it will show disomic, 3:1, segregation in the 20 non-critical F_2 plants. However, in the critical F_2 monosomic

segregation will occur resulting in a 97:3 segregation ratio. When the gene of interest is recessive, 97% of the plants in the critical monosomic F_2 population will express the recessive phenotype, while in all the other monosomic F_2 populations only 25% of the plants will have the recessive phenotype. When two dominant genes on separate chromosomes are involved, a ratio of 15:1 is expected in the 19 non-critical families. The two critical families will each segregate in a ratio of 99:1 (Kuspira and Unrau 1959; Law *et al.* 1987).

In order to multiply a particular monosomic line, monosomic plants are self-pollinated to produce offspring consisting of approximately 73% monosomics. Person (1956) did cytogenetic studies on the selfed progeny of monosomic plants to verify that it is monosomic for the correct chromosome. He concluded that the offspring are not always monosomic for the same chromosome as the monosomic parent. This phenomenon became known as univalent shift and occurs because of partial asynapsis in the parental monosomics. If chromosome morphology reveals univalent shift, cytogenetic studies can be done to verify the monosomic progeny. If univalent shift cannot be detected by chromosome morphology, the monosomic plants must be crossed as the female parents with a 'Chinese Spring' plant that is ditelocentric for the chromosome under test. Univalent shift is deduced if plants occur in the progeny which produce pollen mother cells having 19 bivalents, a univalent and a heteromorphic bivalent, rather than 20 bivalents and a telocentric univalent. While maintaining monosomic stocks, this identity test should be done periodically to make sure that the correct material is being used (Morris and Sears 1967).

Numerous examples exist where a monosomic analysis was done to map a gene of agronomic importance to a specific wheat chromosome. Resistance genes are often assigned in this manner because of their simple inheritance, prominence in plant breeding and the need to broaden the wheat gene pool. Monosomic analyses were done with genes for Russian wheat aphid resistance (Marais and Du Toit 1993; Schroeder-Teeter *et al.* 1994), leaf and stem rust resistance (Piech and Supryn 1978; The *et al.* 1979; Kerber and Dyck 1978; Marais *et al.* 1988; Marais *et al.* 1994b), stripe rust resistance (Law *et al.* 1978) and eyespot resistance (Jahier *et al.* 1979).

1.2.4.1.2 Telosomic analysis

The donor parent used in a telosomic analysis can be either homozygous or heterozygous for the gene of interest. A telosomic analysis is done after the chromosome location of the gene has been determined, normally through monosomic analysis. Two aneuploid recipient parents are used in the primary crosses of a telosomic analysis, i.e., one that is ditelosomic long arm monotelosomic short arm (DTLMTS, $2n = 40 + 2t^{L} + t^{S}$) and one that is ditelosomic short arm monotelosomic long arm (DTSMTL, $2n = 40 + 2t^{S} + t^{L}$) for the critical chromosome. If the gene of interest is homozygous dominant or recessive in the donor parent, all the F_1 hybrids will have this gene and any of the monotelodisomic F_1 hybrids ($2n = 40 + I + t^{S}$ or $40 + I + t^{L}$) can be identified cytologically for further crosses. In the case where the gene of interest is dominant and the donor parent is heterozygote, the F_1 hybrids have to be screened to select only monotelodisomic plants expressing the trait. If the donor is heterozygous for a recessive gene of interest, verification will have to be made in the testcross progeny. Monotelodisomic F_1 hybrids are used as females in testcrosses with a disomic genotype carrying the recessive allele for the gene of interest. Root tip chromosome counts and screening for the trait of interest are done on the test- or backcross derived F_1 plants to determine possible linkage of the trait with the physical marker (telosomic condition).

The plants in the test- or backcross derived F_1 plants are classified as parental types or crossover products. Plants with a chromosome composition of $2n = 41 + t$ and not expressing the trait, and $2n = 42$ expressing the trait, are the parental types, while the plants with a chromosome composition of $2n = 41 + t$ expressing the trait and $2n = 42$ not expressing the trait, are the recombinant types. The gene of interest is located on the chromosome arm where recombinant types occur in the test- or backcross derived F_1 plants. An important feature of telosomic analyses is that the centromere serves as a physical marker. The recombination frequency between the gene of interest and the centromere can therefore be determined by dividing the number of recombinant types by the total number of progeny examined for the particular chromosome arm. This feature is very useful in the construction of genetic linkage maps (Sears 1966, 1974; The and McIntosh 1975; Law *et al.* 1987).

Many studies have employed telosomes to map a gene to a particular chromosome arm. The target genes have included resistance to the Russian wheat aphid (Du Toit *et al.* 1995), stem rust resistance (Sears 1962; The and McIntosh 1975; The *et al.* 1979; Kerber and Dyck 1978), leaf rust resistance (Rowland and Kerber 1974; Kerber 1988; Marais and Marais 1990) and endopeptidase structural genes (McMillin and Tuleen 1977).

1.2.4.2 Isozymes

The first genetic markers used as selection tools in wheat breeding programmes were biochemical markers. More polymorphisms were detected with biochemical markers as compared to morphological markers. Several kinds of biochemical markers exist in wheat, including isozymes such as alcohol dehydrogenase, esterase, endopeptidase, aminopeptidase, α- and β-amylase and storage proteins such as gliadins and glutenins (Tang and Hart 1975; Hart and Langston 1977).

Isozyme zymograms are obtained when an extract of plant tissue is subjected to electrophoretic separation and the enzymes visualized through specific staining procedures. The chromosome location of an isozyme locus can readily be detected with the aid of aneuploids (nulli-tetrasomics). Several alleles were found for many of the biochemical markers and facilitated the construction of more detailed genetic maps of the wheat genome (Tang and Hart 1975; Hart and Langston 1977). The isozyme zymograms also revealed the presence of homoeologous structural genes that are present on all three the wheat genomes (Hart and Langston 1977; Koebner *et al.* 1988) and also on the genomes of related Triticeae (Melz *et al.* 1992).

Isozymes can be used in marker assisted selection if they are linked to a trait of agronomic importance. They have a co-dominant mode of inheritance and it is therefore possible to distinguish between homozygotes and heterozygotes (Marais and Marais 1990; Koebner and Martin 1990). Koebner and Martin (1990) illustrated that the endopeptidase locus *Ep-D1* is closely linked to an eyespot resistance gene (*Pch1*) in wheat and can be used to select indirectly for homozygous resistant plants. Although many isozyme loci are found in wheat, they are insufficient to ensure overall close linkage with all important agronomic traits. Isozymes are therefore used along with other genetic markers to saturate plant genome maps.

Not all enzymes are monomeric. Polymeric enzymes produce complex zymogram patterns, often difficult to interpret (Hart 1987; Payne 1987). Furthermore, each enzyme requires a different extraction procedure, gel running conditions and staining procedures (Hart and Langston 1977). The use of isozyme polymorphism in genetic studies is therefore perhaps limited.

1.2.4.3 Restriction fragment length polymorphisms (RFLPs)

Genetic mapping of plant genomes has become more effective with the introduction of restriction fragment length polymorphism (RFLP) methodology. RFLPs are more abundant and have been used extensively to construct genetic maps in plants. The use of RFLP markers in wheat itself has been hampered by the limited polymorphism present (Chao *et al.* 1989; Kam-Morgan *et al.* 1989). However, saturated RFLP linkage maps may be obtainable in wheat by the construction of linkage maps in the diploid progenitors and other related diploid crops in which much more polymorphism is detectable since these species have a common gene synteny (Melz *et al.* 1992; Chao *et al.* 1989; Devos *et al.* 1993).

All genetic polymorphism is due to DNA base pair differences between individuals being studied. Such differences may arise from mutation, insertion, deletion and translocation. RFLP bands are co-dominant and are inherited in a Mendelian fashion. This makes it possible to select plants homozygous or heterozygous for a specific locus. DNA rearrangements are detected by using restriction endonucleases that recognize and cut at a specific base pair sequence. After the genomic DNA is digested with the appropriate restriction enzyme, the digested fragments are separated on an agarose gel. The fragments are transferred to a membrane and the polymorphism is then visualized by hybridization with a radioactively labelled probe. The probe may have originated from a cDNA or a genomic library. Recently, non-radioactive labelling protocols were introduced in RFLP analysis but this approach has some drawbacks that must be attended to before it can be used extensively in the RFLP protocol (Kochert 1994).

RFLP markers closely linked to leaf rust resistance loci make it possible to obtain durable leaf rust resistance in wheat through marker assisted selection. The advantages compared to conventional testing with rust pathotypes are: (i) all the relevant resistance genes in the progeny

can be determined and not only the gene with the strongest expression (Flor 1942a,b) and (ii) smaller numbers of progeny are required for testing with RFLP markers compared with conventional testing with rust pathotypes (Autrique *et al.* 1995). Autrique *et al.* (1995) reported close linkage between four leaf rust resistance genes and RFLP markers in wheat. Thus, it may be possible to pyramid leaf rust resistance genes and to determine accurately which of the genes are present in the progeny. Close linkage between RFLP and an agronomically important locus may therefore assist plant breeders during selection (Young and Tanksley 1989).

1.2.4.4 Random amplified polymorphic DNA fragments (RAPDs)

Another DNA fragment polymorphism identification is based on the polymerase chain reaction (PCR) technique in which short DNA sequences (primers) are used to recognize and bind with specific DNA sequences and for the amplification of the intervening sequence. During PCR, DNA polymerase uses single-stranded DNA as a template for the synthesis of a complementary new strand. These single-stranded DNA templates are produced by heating double-stranded DNA to temperatures near boiling. DNA polymerase also requires double-stranded primer regions from where it initiates synthesis, and a mixture of the four nucleotide tri-phosphates to synthesize the complementary strands. The amplification products are usually separated on agarose gels and visualized by ethidium bromide staining (Watson *et al.* 1992). Single primers, 8 to 10 nucleotides in length and low annealing temperatures, are used to ensure that many sites, randomly distributed throughout the genome are recognized and produce amplification products. Hence the term 'random amplified polymorphic DNA' (RAPD). The polymorphism is in the primer binding sites and the distance between primer binding sites (Burr 1994; Kochert 1994; Rafalski and Tingey 1993).

According to Devos and Gale (1992), RAPD markers have limited use in the construction of linkage maps in wheat due to the presence of homoeologous genomes. Certain areas of the homoeologous genomes are conserved so that fragments of all three genomes will be amplified. This hampers the construction of linkage maps in that a certain band cannot be mapped to a specific chromosome or chromosome region. RAPD markers are also dominant meaning that the loss of a primer binding site will result in no amplified fragment. Homozygous and heterozygous

plants for the RAPD marker can therefore not be distinguished. There is also no guarantee that a RAPD band linked to a target gene in one population is the same as a RAPD band with the same length in another population. Cloning of a band linked to a target gene and using it as a probe is also not always feasible. The reason being the presence of repeated DNA sequences in most of the amplified products. If such a sequence should be used as a probe in linkage studies, it may hybridize to numerous sites in the studied genome and may therefore not be a reliable marker.

RAPDs, however, provide markers possibly tightly linked with a target gene and the RAPD technique can be automated which makes the screening of large numbers possible.

In the search for molecular markers linked to a target gene, near-isogenic lines (NILs) or pooled DNA in a technique called bulked segregant analysis (BSAs) are used. NILs are created when two inbred lines, one line with the trait of interest (donor parent) and the other, normally exhibiting agronomically important traits (recurrent parent), are crossed with each other. The resulting F_1 plants are backcrossed to the recurrent parent. The backcross derived F_2 or F_2 generation is screened and those individuals exhibiting the trait of interest are again backcrossed to the recurrent parent. Backcrossing and selection are continued for several generations. The procedure rapidly restores the homozygous genotype of the recurrent parent at the majority of loci while a heterozygous allele for the trait of interest will also be present. This plant is selfed whereafter those plants homozygous for the trait of interest are selected. At this stage the NIL and recurrent parent will be identical and homozygous for most of the genes throughout the genome except those in the target area where the one line will have a gene homozygous for the trait of interest. These lines are then used to detect polymorphisms with RAPD primers because any polymorphisms observed will most likely be linked to the trait of interest (Kochert 1994; Tanksley *et al.* 1995).

BSAs are created when two lines, one line with the trait of interest and the other line without, are crossed with each other. The F_1 hybrids are selfed and the resulting F_2 generation is divided into two classes. The one class has at least one allele for the trait of interest while the other class does not have the trait of interest at all. Throughout the rest of the genome the plants in each class will

segregate for the loci of the two parents. The DNA of each class is pooled and will therefore only differ in the area of the trait of interest. Large numbers of F_2 plants must be obtained to get the best results. The bulked DNA can then be screened with RAPD primers to detect polymorphisms (Kochert 1994; Tanksley *et al.* 1995; Tinker *et al.* 1994).

Schachermayr *et al.* (1994) screened wheat NILs for a leaf rust resistance gene, *Lr9*, with 395 RAPD primers. Three primers showed polymorphisms between the NILs in that an additional band was found in the resistant lines. One of these polymorphic bands was sequenced and the data used to synthesize specific primers to be used in a PCR reaction. The primers amplified only one fragment in leaf rust resistant plants and no bands were detected in leaf rust susceptible plants, suggesting complete linkage between the *Lr9* gene and this marker. This has great significance in wheat breeding programmes, especially for pyramiding leaf rust resistance genes.

1.2.4.5 Microsatellite markers

Microsatellite loci consist of di-, tri- or tetranucleotides that are tandemly repeated. Di- and tetranucleotide repeats are evenly distributed throughout the genome while trinucleotide repeats tend to be located in the coding regions of many plant species (Wang *et al.* 1994). In plant genomes the AT repeat is more common than other repeats (Burr 1994).

Microsatellites can be analysed by PCR using specific primers to amplify the repeat sequence. The tandem repeats in microsatellites are highly polymorphic and reveal much more allelic variation at a specific locus than do the other molecular markers (Rafalski and Tingey 1993). Despite the advantages of microsatellite loci, they has not been used extensively as markers in wheat. Devos *et al.* (1995) reported on the first application of microsatellite sequences as molecular markers in wheat. These microsatellite loci were found to be genome-specific and displayed high levels of variation. However, much of their power as molecular markers is likely to be lost in interspecific introgression crosses where the primers derived from the cultivated species cannot predict the amplification of homoeoloci in the alien donor parent, or in intergenomic comparative mapping studies where microsatellite markers from one species will not guarantee a product in another species. Much more work must therefore be done to develop

linkage maps in wheat based on microsatellite markers.

1.2.5 Mapping with repetitive DNA sequences

Repetitive DNA sequences are abundant in higher plants and constitute on average 80% of their genomes (Flavell *et al.* 1974). These repetitive DNA sequences may be concentrated at specific loci or can be interspersed throughout the genome. Studies have shown that families of repetitive DNA sequences have formed during evolution (Bedbrook *et al.* 1980a; Bedbrook *et al.* 1980b). Some of these repetitive DNA sequences are specific to a certain species (Guidet *et al.* 1991; Zhang and Dvořák 1990a), while other repetitive DNA sequences share hybridization sites with other species as well (Xu *et al.* 1990; Cuadrado and Jouve 1994).

In order to broaden the gene pool of wheat, the incorporation of small fragments of alien DNA into the wheat genome is being done increasingly (Mukai *et al.* 1993a). The translocations produced must, however, be characterized to ensure that the minimum alien DNA is introduced and that the adaptability and quality of the recipient will not be adversely affected. Procedures for the detection of translocations often involves C-band polymorphisms, the expression of the relevant gene in the progeny or isozyme markers. These techniques have low resolution and often cannot distinguish small translocated fragments in a wheat background. Common features of wheat-alien translocations are the presence of detrimental genes associated with the target gene (Marais *et al.* 1988). To select those plants with the smallest translocation segment exhibiting the target gene, methods are required that are more efficient and sensitive. Procedures that measure polymorphism in repetitive DNA sequences may be the answer as they are species specific (Guidet *et al.* 1991; Zhang and Dvořák 1990a), abundant in the genomes of higher plants (Flavell *et al.* 1974) and can be used in ISH (Rayburn and Gill 1985), FISH (Nkongolo *et al.* 1993), as RFLP probes (Zhang and Dvořák 1990b) and in PCR analysis (Rogowsky *et al.* 1992b).

1.2.5.1 Species specific repeated DNA sequences

Several species specific repeated DNA sequences have been isolated which make it possible to detect alien chromatin in a crop. Repeated nucleotide sequences have been isolated in species

closely related to the genus *Triticum*. This includes the probes pLeUCD1 and pLeUCD2 that are derived from the wheatgrass *Lophopyrum elongatum* (Host) Löve (Zhang and Dvořák 1990a) and the rye (*Secale cereale* L.) repetitive DNA probes pSc119.1, pSc119.2 (McIntyre *et al.* 1990), pSc34 (Bedbrook *et al.* 1980a), pAW161, pAW173 (Guidet *et al.* 1991), pSc5.3H3 (Appels *et al.* 1986) and RXX630 (Mao *et al.* 1994). Probes pSc119.1 and pSc119.2 are subclones of pSc119 (Bedbrook *et al.* 1980a). Repeated nucleotide sequences were also found in wheat (*T. aestivum* L. em Thell.) and its D-genome progenitor *T. taushcii* (Coss) Schmal. The probes pTa1, pTa2, pTa7 and pTa8 were found to be wheat specific (Metzlaff *et al.* 1986) and the probe pAS1 is D-genome specific (Rayburn and Gill 1986). The latter probe makes it possible to identify the D-genome chromosomes in wheat due to the close relationship between the D-genome of wheat and the D-genome of *T. taushcii*.

1.2.5.2 Rye-specific repeated DNA sequences

Flavell *et al.* (1974) measured the proportion of repeated DNA sequences in plants and found that approximately 92% of the rye (*S. cereale*) genome consists of repeated DNA sequences. Bedbrook *et al.* (1980a) studied the distribution of six different repeated sequences in the rye genome and found that they were predominantly located within blocks of constitutive telomeric heterochromatin on all seven chromosome pairs. They furthermore defined four major repeated sequence families in the rye genome i.e. the 120 bp, 350-480 bp, 610 bp and the 630 bp families. Jones and Flavell (1982b) studied the structure, copy number and chromosomal location of these four families of repeated DNA sequences in different species of the genus *Secale*. They found that each species has a unique composition of the repeated sequences and that its distribution can be used in evolutionary studies or to distinguish between the different species.

Appels *et al.* (1986) reported a fifth major rye-specific repeated DNA sequence, the 5.3H3 family. This sequence is dispersed throughout non-heterochromatic and most heterochromatic regions and is therefore much more rye-specific than the previously mentioned probes. Guidet *et al.* (1991) cloned a repetitive DNA sequence unique to the rye genome and concluded that it is not related to the other repeated DNA families characterised in rye. This new family, the R173 family, is dispersed throughout the seven rye chromosomes and is not organised as blocks of

tandem arrays. Mao *et al.* (1994) cloned a new repetitive DNA sequence common to both the rye and wheat genomes. This 630 bp fragment, RXX630, is different from the families previously described in rye and is also not organized as blocks of simple tandem arrays.

1.2.5.2.1 Rye-specific probe pSc119.1

Restriction enzyme digestion of the clone pSc119 (Bedbrook *et al.* 1980a) with Hind III produced three fragments. These fragments were designated pSc119.1, pSc119.2 and pSc119.3, respectively. They were electro-eluted from an agarose gel and ligated into the plasmid pUC18. The 745 bp HindIII insert of pSc119.1 was sequenced and revealed no internal repetition of sequence relationship to pSc119.2. ISH with pSc119.1 showed that the sequence is highly dispersed over the entire length of all seven rye chromosomes except some telomeres and the nucleolar organiser region. Only weak cross-hybridization signals were observed to a few telomeric and centromeric regions of wheat (McIntyre *et al.* 1990).

Since pSc119.1 is such a rye-specific DNA probe, it can be used to detect chromosome segments introgressed from rye into wheat. ISH with pSc119.1 on rye chromosomes produced a more detailed banding pattern compared with C-banding and the hybridization sites were predominantly interstitial and in areas containing no C-bands. This will be useful in the identification of wheat-rye translocations (Jouve *et al.* 1991). Xu *et al.* (1990) used pSc119.1 in RFLP and ISH studies and found it to produce similar banding patterns on *Hordeum bulbosum* and rye chromosomes. No hybridization was revealed with barley DNA. Thus, pSc119.1 can also be used to detect chromosome segments transferred from *H. bulbosum* to barley.

1.2.5.2.2 Rye-specific probe pSc119.2

The probe pSc119.2 was cloned in the same manner as pSc119.1. It is 611 bp long and has a subrepeat of 118 bp that classifies it as a member of the 120 bp family of sequences (Bedbrook *et al.* 1980a). ISH studies with pSc119.2 revealed hybridization sites predominantly to the telomeric regions of rye chromosomes, with some interstitial sites. The probe also showed strong cross-hybridization signals with wheat chromosomes that limits its use as a probe to detect rye

Rogowsky *et al.* 1993). The R173 family is very useful in such studies because of its high copy number in rye, the fact that it is dispersed throughout the rye genome and that it does not cross-hybridize to wheat or barley DNA.

1.3 WHEAT RUSTS

The wheat rusts are the most important diseases of wheat worldwide (Knott 1989) because of their wide distribution, ability to acquire virulence to previously resistant cultivars, rapid dissemination by air and potential to cause a very rapid rate of disease increase and severe yield losses. McIntosh *et al.* (1995) compiled an overview of the yield losses due to cereal rusts worldwide, noting yield reductions of 30% or more in epidemic years. Such yield losses occurred since the domestication of wheat, but it was only in the 20th century that yield losses due to rust diseases received attention as a result of a better understanding of the host:pathogen interaction. Stem (black) rust and leaf (brown) rust are the most common rust diseases found in South Africa (De Jager and Le Roux 1979). Recently, stripe (yellow) rust was recorded for the first time in South Africa showing the devastating potential that wheat rusts have on the yield (Lochner 1996; Pretorius *et al.* 1996). This review will, however, concentrate on the primary rust diseases found in South Africa, i.e., stem and leaf rust.

1.3.1 The pathogens

The wheat rust pathogens belong to the genus *Puccinia* of the family Pucciniacaea in the order Uredinales of the class Basidiomycetes. Rust fungi are highly specialized parasites and have narrow host ranges. Stem and leaf rust fungi differ in morphology, life cycle and optimal conditions for growth.

1.3.1.1 Wheat stem rust

Wheat stem rust is caused by *Puccinia graminis* Pers. f. sp. *tritici* which parasitizes wheat, barley and many wheat relatives. It is also known as black rust because of the black teliospores it produces towards the end of the growing season (Knott 1989; McIntosh *et al.* 1995).

Stem rust is considered to be the most damaging of the wheat rusts. It attacks the stems, leaves, leaf sheaths, spikes and the awns where dark brownish-red, elongated pustules develop. The epidermis is raggedly ruptured by the pustules so that the diseased stems and sheaths have a ragged appearance. The pustules on the leaves can be of various sizes and shapes. Pustules on young leaves are often diamond shaped, while the pustules on the older leaves tend to be restricted to the veins. Sporulation occurs on both leaf surfaces but tends to be heavier on the lower surface.

The damage that stem rust causes to the plants results from the loss of photosynthetic area, the disruption of water and nutrient transport, reduction in root growth, lodging and stem breakage. Early stem rust attacks can cause various degrees of kernel shrivelling that results in yield losses. Lodging makes the crop difficult to harvest and results in lower yields. Severe and early attacks can therefore cause total loss of an unprotected crop (Schafer 1987; Knott 1989).

Free water must be present on wheat plants for the urediospores to germinate. Stem rust also requires relatively high temperatures to become epidemic and therefore mostly develops towards the end of a growing season. Warm conditions do, however, occur during the growing season from time to time which favour early stem rust infections and have serious yield losses as a result (Anderson 1978).

1.3.1.2 Wheat leaf rust

Wheat leaf rust, also known as brown rust, is caused by *Puccinia recondita* Rob. ex Desm. f. sp. *tritici*. Wheat leaf rust does not attack cereal rye but its host range includes the tetraploid wheats and *Aegilops* species (Knott 1989; McIntosh *et al*. 1995).

Leaf rust is the commonest and most widely distributed of the wheat rusts. Typical symptoms of leaf rust are small round, orange-red pustules that are mostly situated on the upper leaf surface. The epidermis is not so raggedly ruptured as compared to stem rust infections. Leaf rust primarily attacks the leaf blades and to a lesser extent leaf sheaths and glumes. In severe epidemic conditions the entire surface of the leaf blades can be covered with pustules, depriving

stem rust include common barberry (*Berberis vulgaris*) and species of *Mahonia* (Oregon grape), while the alternate hosts for leaf rust include species of *Thalictrum* (meadow rue), *Anchusa*, *Isopyrum* and *Clematis*. All five spore stages can be present during the life cycle. Leaf and stem rust are therefore described as being macrocyclic and heteroecious (Schafer 1987; Knott 1989; McIntosh *et al*. 1995).

The pustules of the fungi seen on wheat during most of its life cycle are called uredia and produce urediospores. Urediospores are the repeating spore stage on wheat and provide for successive infections as long as the host is present and environmental conditions are adequate. They are dikaryotic and reflect the distinctive colour of leaf and stem rust. Many spores are produced for several weeks that can remain within the crop canopy and cause reinfection, or may become airborne on a windy day and can be carried over long distances. Urediospores germinate only when free water is present on the wheat plants and the temperature conditions are optimal (Schafer 1987; Knott 1989; McIntosh *et al*. 1995).

The uredia turns black toward the end of the growing season as urediospore production ceases and black teliospores are produced in their place. Teliospores are two-celled, dikaryotic and borne on stalks. This is the overwintering stage of the rust in cold climates as teliospores are resistant to extremes of weather and germinate only after alternate periods of freezing and thawing or wetting and drying. In the spring each cell of a teliospore may germinate to produce a basidium. The nucleus undergoes meiosis and produce four haploid cells. These cells germinate and produce basidiospores that are forcibly discharged into the air and carried to the alternate host. Basidiospores germinate rapidly and infect leaves of the alternate hosts where pycnia are formed on the upper leaf surface. A viscous liquid (honeydew) containing pycniospores appears about 7-14 days after infection from open pycnia. The pycniospores function as male gametes while pycnia are of two mating types, + or -, and successful matings can occur only between opposite types. Insects are attracted to the honeydew and transfer the pycniospores from one pycnium to another. Raindrops and leaves rubbing together in the wind can also transfer the pycniospores from one pycnium to another. Flexuous hyphae, functioning as female gametes, are present in the pycnia and fuse with pycniospores of the opposite mating type. The nucleus of a pycniospore migrates after fusion through the flexuous hyphae to the

aecial primordium (Schafer 1987; Knott 1989; McIntosh *et al.* 1995).

The mycelium (dikaryotic) develops into an aecium on the underside of the alternate host's leaf. Since several fertilizations can occur in a pycnium, the resulting aecium will be genetically heterogeneous. A single aecium contains many aecial horns, while aeciospores from a single aecial horn usually are of one genotype. Aeciospores are produced in long chains and are forcibly discharged when aecia are wetted and dried. They are carried by the wind to nearby wheat fields where they can produce early infections. The infections on wheat develop into uredia, thus completing the life cycle (Schafer 1987; Knott 1989).

The alternate hosts of the rust fungi are not present in most wheat growing areas. They are therefore forced to survive stress periods, cold or heat in its asexual stage. The pathogen therefore remains on green tissues of volunteer plants such as wheat or barley and various grass species (McIntosh, 1976).

1.3.4 Host:pathogen interaction

Flor (1942a,b) was the first to point out the gene-for-gene relationship when he studied pathogenicity in the flax rust fungus, *Melampsora lini* (Pers.) Lév. and the rust reaction in the host, *Linum usitatissimum* L. F_2 cultures of the flax rust fungus were found to segregate into monofactorial ratios on varieties of flax that had one gene for resistance to the avirulent parental pathotype. On varieties having 2, 3 or 4 genes for resistance to the avirulent parental pathotype, the F_2 cultures segregated into bi-, tri- or tetrafactorial ratios, respectively (Flor 1955). He postulated that for each gene that conditions resistanc in the host there is a corresponding gene in the pathogen that conditions pathogenicity.

Flor (1955) crossed two rust pathotypes and obtained a 3:1 (avirulent:virulent) ratio when he inoculated the F_2 flax rust progeny on a flax host cultivar that showed resistance to one pathotype and susceptibility to the other. He therefore concluded that resistance in the host and avirulence in the pathotype are inherited as dominant characters and that susceptibility in the host and virulence in the pathotype are inherited as recessive characters.

1.3.4.1 Host specificity

Rust fungi are obligate parasites and are classified as biotrophic fungal pathogens of plants (Flor 1956; Pryor 1987). Biotrophs set out to control the host cells, steering it in directions favouring the growth and reproduction of the pathogen and keeping the plant cells alive. They are highly specific parasites with a limited host range and cannot be maintained in axenic cultures. Host resistance is inherited as a dominant character and susceptibility as a recessive character. Pathogen virulence on the other hand is inherited as a recessive character and avirulence as a dominant character.

Two types of resistance can be distinguished in rust systems, i.e., major gene and minor gene resistance. Major gene (vertical, pathotype-specific) resistance is mostly exploited by plant breeders (Sidhu 1987). A major gene results in distinct categories of resistance or susceptibility when a host plant is infected with rust pathogens. The pathogen must have the corresponding virulence gene to the major gene present in the host to be able to parasitize it. When the avirulence gene is present in the pathogen, the plant produces a hypersensitive response characterized by the rapid death of one or a few host cells in the area surrounding the pathogen (Sidhu 1987). This type of resistance is easily overcome by mutation in the pathogen and is therefore unstable (Flor 1955).

Minor gene (horizontal, non pathotype-specific) resistance is difficult to achieve but is durable (Sidhu 1987). The pathogens achieve only an intermediate level of infection when they infect the hosts. This partial resistance is mostly caused by minor resistance genes that have small individual effects on the pathogen and are difficult to distinguish. Mutations for virulence in the pathogen are therefore difficult to obtain and this type of resistance is durable (Sidhu 1987; Flor 1955).

The continuous breeding of resistant plants necessitates the pathogen to change its pathogenicity in order to survive. To become virulent on a host, the pathogen needs to avoid recognition by the host. This results in the development of new pathotypes that can infect plants that were once resistant to the specific pathogen. The most important mechanism for pathogenic adaptation is

mutation. Pathogen mutations can arise at a frequency as high as 1% and is generally 2-3 times higher than spontaneous mutation (Pryor 1987). These mutations are the result of deletions and genetic rearrangements. Virulence is, however, inherited as a recessive character and when a sexual stage does not occur, 2 mutations are therefore required in the dikaryotic urediospores to express this virulence (Pryor 1987; Flor 1971).

Hybridization between 2 pathotypes can also produce pathogenic variation. Flor (1942a,b, 1955) crossed 2 flax rust pathotypes that differed in pathogenicity on a range of hosts and found new pathotypes when he infected the hosts with F_2 cultures. This mode of pathogenic variation results from the recombination of the genes already present in the parental pathogens and is therefore not as effective as mutation. The sexual stage of the life cycle of wheat leaf and stem rust is absent in most countries which impairs pathogenic variation due to hybridization. Other sources of pathogen variability are somatic hybridization (the production of new pathotypes from existing pathotypes in the absence of a sexual phase), migration of the virulent pathotypes from other regions of host cultivation and selection of pathotypes that are virulent to the prevalent hosts but initially occurs at low frequencies in the rust population (McIntosh 1976).

The genetics of horizontal resistance are generally poorly understood. It is believed that some, but not all, of the minor genes may interact in a gene for gene manner with the pathogen (Wolfe and Gessler 1992). Much work has been directed to study the hypersensitive response. Although the hypersensitve response itself is relatively well understood, the mechanism whereby it is triggered is not well known.

1.3.4.2 Hypersensitive response (HR)

The hypersensitive response (HR) is a common feature of major-gene resistance and it is invoked when the resistant plant recognizes and responds rapidly to the presence of the pathogen. This results in an incompatible reaction between the plant and the pathogen. The HR is characterized by the rapid death of one or a few host cells in the region of infection. It prevents the pathogen from further colonization of healthy plant tissue and is observed visually as necrotic flecks (Hahn *et al.* 1989; Pryor 1987). Triggering and execution of the HR involve complex biochemical

reactions which seem to differ from plant to plant.

1.3.4.2.1 Course of the HR

Urediospores of leaf or stem rust germinate when free water is present on the wheat plants and the environmental conditions are favourable for the pathogen. Germ tubes grow laterally across the leaf surface until a stoma is reached. An appressorium is produced over the stomatal opening and an infection peg forces its way through the stoma (Schafer 1987; Knott 1989). At this stage the plant either recognizes the pathogen and triggers the HR, or does not recognize the pathogen and the pathogen completes its penetration of the cells and the consequential susceptible reaction.

Several physiological models were postulated to explain the gene-for-gene relationships involved in major gene resistance. These models were reviewed by De Wit (1986, 1987), but it is still not clear which is appropriate.

1.3.4.2.2 Elicitor activity

Elicitors can be defined as molecules produced by the fungus that trigger defense mechanisms in the host plant. These defense mechanisms (Fig. 2) are: (i) the formation of antimicrobial compounds that are toxic or produce stasis in the fungal environment, (ii) the development of physical barriers that prevent microbial spread to other cells, and (iii), the production of increased levels of enzymes that may attack the microorganism (Anderson 1989; Ebel and Scheel 1992).

Elicitors have been studied extensively and it was found that each elictor has a specific reaction in a host plant. Various types of elicitors will for instance induce the accumulation of phytoalexins. Phytoalexins are antimicrobial compounds of low molecular weight and are absent in healthy plants. The association of high phytoalexin production in plants infected with avirulent pathotypes makes it an obvious candidate for a role in the HR (Darvill and Albersheim 1984). Examples of elicitors of phytoalexins produced by the pathogens are glucans, proteins, glycoproteins and unsaturated fatty acids such as arachidonic and eicosapentaenoic acid. There

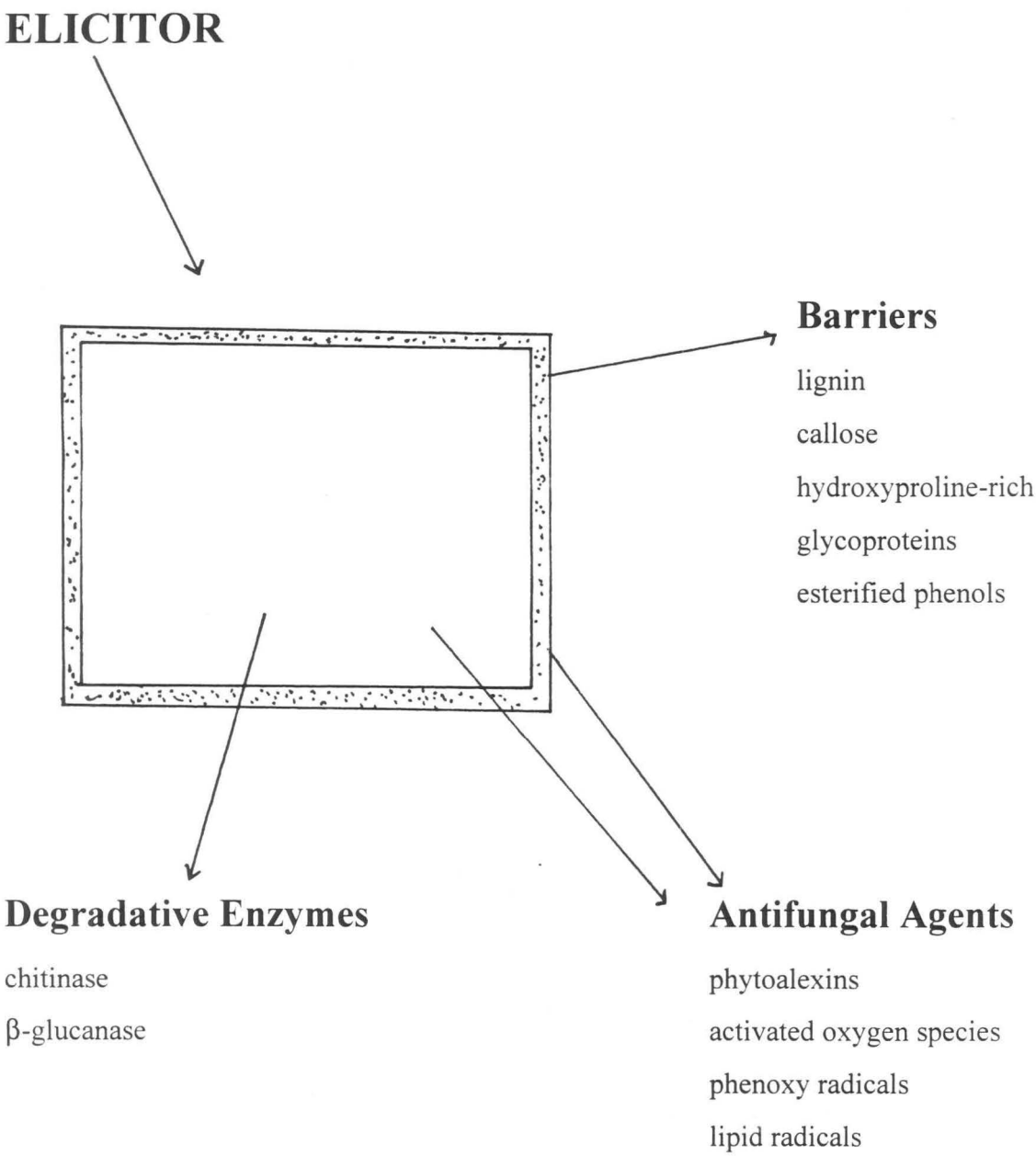

Fig. 2. The hypersensitive response defense strategies triggered by elicitors (Anderson 1989).

is also strong evidence that cell death gives rise to endogenous elicitors (of host origin) which in turn induce phytoalexin accumulation. Abiotic factors such as heavy metals, detergents and exposure to freezing or heating can also induce phytoalexin accumulation. Abiotic elicitors are, however, environmental factors that are not applicable to the host:pathogen interaction (De Wit 1986, 1987).

The major physical barrier to potential pathogens of plant tissues is the polysaccharide-rich cell wall. The barrier presented by the cell walls is rarely complete because natural openings, such as stomata and lenticels, and intercellular spaces do occur. These openings provide easy routes for pathogens to penetrate the host tissues and feed on their extracellular environment. Furthermore, the pathogens produce cell wall-degrading enzymes that include pectic-degrading enzymes, cellulases, arabinases, xylanases and galactanases. These enzymes ensure that the pathogen can feed on the nutrients present in the host tissues (Ride 1983; Hahn *et al.* 1989).

Structural alterations of the plant cell wall do occur in response to infection or attempted infection by fungi. The modifications of cell walls include processes like lignification, accumulation of hydroxyproline-rich glycoproteins (HRGPs) and callose deposition (papillae formation). HRGPs are involved in creating a more rigid wall structure through a cross-linking process that is catalysed by specific peroxidases. The chemical nature of inducers of HRGP accumulation remains unknown. Callose, a β-(1 $\rightarrow$ 3)-linked glucan, is produced on the internal surface of the cell wall when avirulent fungi attempt to penetrate the plant cells. This deposition of wall-like material results in the formation of papillae and is an effective barrier against pathogenic invasion. Callose deposition is possibly stimulated by chitosan (Hahn *et al.* 1989; Anderson 1989).

Lignin is a structurally complex phenolic polymer whose building blocks are hydroxycinnamyl alcohols and occurs naturally throughout the middle lamella, primary and secondary walls of xylem tissues and is often at its highest concentration in the middle lamella at cell corners. It is extremely resistant to microbial degradation and thus forms one of the most effective barriers against pathogenic invasion. Lignification could hinder fungal progress in several ways and is therefore an important resistance mechanism in plants: (i) the polymer may make the walls more

resistant to mechanical penetration, (ii) lignification may make the host walls more resistant to degradation by fungal enzymes, (iii) it limits the diffusion of enzymes and toxins from the fungus to the host and conversely nutrients and water from the host to the fungus, and (iv), it is conceivable that the tips of hyphae that are in close contact with lignifying host material might themselves become lignified and lose the plasticity necessary for growth (Ride 1983). Elicitors of lignification are chitin, chitosan and β-(3,6)-linked glucans. Moerschbacher *et al.* (1990) showed that the HR is correlated with cellular lignification when resistant wheat varieties are infected with an avirulent stem rust pathotype and the pathogen growth is therefore restricted by the rapid necrosis of host cells.

1.3.4.2.3 Receptors

Recognition factors in plants are, as are elicitors in the pathogen, considered as constitutively produced surface molecules. Their occurrence at the host and pathogen cell surfaces may facilitate recognition during the early stages of the host:pathogen contact. Lectins present on the cell walls of resistant plants may function as recognition factors for avirulent strains of rust pathotypes. Lectins are proteins or glycoproteins that can bind certain carbohydrate structures. They have hapten specificity that makes them acceptable candidates as receptors of elicitors (De Wit 1986, 1987).

The completion of the HR is based on complex biochemical reactions reviewed by Doke *et al.* (1987). The exact mechanisms are, however, still not clear and it is thought that each host:pathogen interaction has its own mode of biochemical reactions that leads to cell necrosis. Much work therefore still needs to be done to unravel this highly efficient mode of plant resistance.

1.3.5 Control of leaf and stem rust

Any factor that breaks the disease cycle of the wheat rust fungi or reduces the size of any part of the cycle acts as a control measure. The rusts are controlled primarily by either genetic resistance or the use of chemicals, and to a lesser extent, by cultural methods.

1.3.5.1 Cultural methods

Delayed planting of wheat is practised in many countries to prevent early infections from nearby infected plants. This prevents the rapid increase of inoculum during the favourable early stages of cultivation. The infected plants can be volunteer plants, or even late crops cultivated in another season. In areas where rust inoculum normally arrives late in the growing season, early planting may allow the crop to mature before rust infection becomes serious.

Susceptible hosts such as volunteer plants must be eliminated to help to control rust. Alternate hosts must also be eradicated. Such measures will prevent the overwintering of the rust fungi and somatic hybridization that can lead to the appearance of new pathotypes will not take place (Schafer 1987; Knott 1989).

1.3.5.2 Chemical control

The use of fungicides is only economical in areas where yields are high and where one fungicide controls several diseases. Chemicals are expensive and sometimes two or more applications are necessary to control the fungi. Throughout the world, there is growing concern about the use of chemicals and the potential danger of residues in food, contamination of water supplies and the danger to the environment and the people applying them. Instances have been reported where the pathogen has become resistant to the fungicides. For these reasons alternative methods of controlling rusts are preferable (Schafer 1987; Knott 1989).

1.3.5.3 Genetic resistance

The development of resistant cultivars is the most effective method of biological control of the rusts. No extra costs are incurred by farmers when resistant cultivars are used, the use of chemicals is limited and no additional cultural methods are necessary. Breeding for resistance is therefore a very cost-effective procedure. However, it also proves to be a never-ending task. When a resistant cultivar is released, the pathogen soon evolves new virulent pathotypes that are able to surmount the resistance present in the new cultivar. The breeding cycle then starts again.

Most of the wheat cultivars released to date have major genes for resistance to locally prevalent strains of the pathogen but susceptible to strains to which they might later be exposed. Wheat breeders are now increasingly interested in types of resistance and methods of using resistance genes that will result in long-lasting control of the rusts. Examples are the use of adult plant resistance genes such as *Sr2*, which provides adult plant resistance against stem rust pathogens, and *Lr12*, *Lr22* and *Lr34*, which provide adult plant resistance against leaf rust pathogens (McIntosh 1988a). Single genes such as *Sr2*, *Sr26*, *Sr31* and *Sr36*, against stem rust pathogens, and *Lr12*, *Lr13* and *Lr34*, against leaf rust pathogens have proven to be durable resistance genes and are of great value to plant breeders (Wolfe and Gessler 1992).

New sources of leaf and stem rust resistance genes are also needed to keep one step ahead of evolving new pathotypes. The relatives of wheat have proven to be of great value in this respect and several resistance genes have already been introduced into wheat from these species. Species from which such transfers have already been made include *T. turgidum* (Dyck 1994), *T. tauschii* (Innes and Kerber 1994; Kerber and Dyck 1969) and *Agropyron elongatum* (Knott 1961; Sharma and Knott 1966; McIntosh 1988a).

The pyramiding or stacking of resistance genes into a single cultivar is an important technique to obtain durable resistance. However, a drawback of this technique is that the resistance gene with the strongest resistance reaction will overshadow the other resistance genes and therefore the presence of more than one resistance gene in progenies cannot be detected (Flor 1942a,b). In order to overcome this problem, morphological, biochemical and molecular markers closely linked to each resistance gene will be of great help to screen for the presence of such resistance genes in progenies with more than one resistance gene. Singh (1992) reported close linkage (1.3%) between *Lr34*, an adult plant leaf rust resistance gene, and *Ltn*, a gene conferring leaf tip necrosis. Tight linkage with a recombination value of 1.0 ± 0.86% was obtained between *Lr19*, a leaf rust resistance gene, and the isozyme, *Ep-D1d* (McMillin *et al.* 1993). Schachermayr *et al.* (1994) synthesized specific primers from a polymorphic RAPD band that segregates with plants having the leaf rust resistance gene *Lr9*. When they used these primers in a PCR reaction on leaf rust resistant (*Lr9*) and susceptible plants, only one fragment was amplified in leaf rust resistant plants and no bands were detected in leaf rust susceptible plants. This suggests close

linkage between *Lr9* and this molecular marker.

The continued study of the host:pathogen interaction and the refinement of techniques to obtain durable plant resistance are of primary importance in attempts to genetically control the wheat rusts.

1.4 THE RUSSIAN WHEAT APHID

Aphids are the most cosmopolitan of the major insect groups and one of the world's major insect pests of wheat (Hatchett *et al.* 1987). They feed by sucking plant sap and may reduce plant resources to such an extent that total plant losses may occur under severe infestations. Secondary plant damage can also result from plant viruses transmitted by the aphids.

Since wheat is such an important cereal crop worldwide (Briggle and Curtis 1987), the recent spread of the Russian wheat aphid (RWA) *Diuraphis noxia* (Mordvilko) in wheat is quite alarming (Robinson 1992). Much effort has been directed towards the study of possible solutions to this new pest, but mixed successes have been the order of the day. Host resistance has proven to give the best results due to its durability and simplicity to employ once a resistant cultivar has been released. Much time is therefore spent on this area of research to control RWA damage.

1.4.1 Distribution

The RWA was first detected in 1900 in the Caucasus region of the southern Soviet Union. It is indigenous to Southern Russia, countries bordering the Mediterranean sea, Iran and Afghanistan. The first recording of RWA migration was in 1978 when they caused extensive damage to wheat yields in South Africa. It appeared in Mexico in 1980 and by 1986 it was recorded as a pest in Texas in the USA. It spread to Colorado, Kansas, New Mexico and Oklahoma (western USA), the provinces of Alberta, British Colombia and Saskatchewan in Canada and Chile and Argentina in Central and South America. Other countries to where it spread are the Middle East, Pakistan, northwestern China, Ethiopia and Mozambique (Walters *et al.* 1980; Robinson 1992).

Hughes and Maywald (1990) observed that Australia, Kenya and northeastern China face a serious threat as their climates provide suitable habitats for colonization by the RWA. Estimated potential wheat yield losses of up to 50% may occur when the RWA reaches these countries. Precautions must be taken timeously to prevent serious losses once the pest is introduced into these countries.

1.4.2 Biology and life cycle

The RWA belongs to the order Hemiptera, the suborder Homoptera, the superfamily Aphidoidea, the family Aphididea and the genus and species *Diuraphis noxia* (Mordvilko) (Hatchett *et al.* 1987). The RWA is less than 2 mm in length, pale green with an elongated, spindle-shaped body. It can be readily distinguished by its extremely short antennae, supracaudal process and absence of the prominent siphunculi (Walters *et al.* 1980; Robinson 1992).

The host plant range of the RWA is quite wide and includes wheat, barley and triticale, while rye and oats are infested to a lesser extent. Aphids are a serious threat to cereals during all their growth stages. They survive during the summer months on volunteer wheat, barley and triticale and on the alternate hosts such as rescue grass (*Bromus wildenowii* Kunth) and wild oats (*Avena fatua* L.) (Walters *et al.* 1980; Fouché *et al.* 1984). Kindler and Springer (1989) showed that the RWA can survive on a broad range of cool- and warm-season grasses that grow throughout the cereal production areas. This underlines their wide host range and the threat that they pose to wheat farmers.

The RWA in the mild climate of South Africa is anholocyclic, reproducing viviparously throughout the year. Nymphs are born live without the intervention of males (parthenogenesis). They begin to feed on susceptible plants and will mature in about 7 days time to reproducing wingless (apterous) females and begin to deliver about 3-4 nymphs per day during the remainder of their life span. Their life span is 25-30 days and 20-40 generations per year can occur under optimal conditions. On depletion of food sources and under adverse environmental conditions, winged (alate) females are produced. Winged aphids can fly only short distances on own power to nearby fields. Prevailing winds and convection currents spread the RWA over long distances

to infest other cereal fields. The winged aphids begin to feed on susceptible hosts and give birth to nymphs that grow quickly and develop into wingless females. The cycle thus starts over until the end of cereal production when the aphids survive on the alternate hosts and volunteer cereals (Walters *et al.* 1980; Dreyer and Campbell 1987; Robinson 1992).

The RWA is holocyclic in cold temperate areas such as Russia and survives the winter in the egg stage. No males have been found to date in the other places of RWA infestation. Butts (1992) reported that RWA nymphs and female adults can survive temperatures as low as -20°C. Nymph production increases when the temperature changes from 5 to 20°C and decreases until the temperature reaches 30°C. Extreme temperatures can contribute to keep the aphid populations in check (Robinson 1992).

1.4.3 Damage symptoms

The RWA feeds on new growth of host plants and is often found in the axils of the leaves or within curled-up leaves (Walters *et al.* 1980). Aphids probe intercellular into plants with a group of tongue- and groove-connected stylets and feed in the phloem. A lipoprotein sheath is secreted by the stylets during penetration that protects the stylets from plant wound reactions. The stylets wind their way between the mesophyll cells until they reach the vascular bundle sheath when penetration becomes intracelluar (Fouché *et al.* 1984).

Plant cells are held together by a layer of middle lamella. The middle lamella is composed chiefly of pectin and acts as a kind of cement that binds plant cells together. While probing, the aphid injects a watery saliva into the intercellular spaces in the plant. Aphids possess pectinase in their saliva that catalyses the digestion or depolymerization of the middle lamellar pectin. This mediates the rate of insect probing through the intercellular pectin. The aphid saliva also initiates a second biochemical process through the presence of a 1,3-glucosidase that depolymerizes the callose found in the pores of the phloem. This results in increases of the phloem flow of sugar and amino acids extracted by the aphids. It also proves to be advantageous to later generations of aphids born at the probing site as the feeding becomes easier as the number of aphids increases at a given site (Fouché *et al.* 1984; Dreyer and Campbell 1987; Belefant-Miller *et al.* 1994).

The RWA feeds in the micro-environment of rolled leaves that characteristically results from RWA infestation. Aphids also inject a toxin into plants that destroys the chloroplast membrane and results in white, yellow and purple to reddish-purple longitudinal streaks on the leaves that deprive the plant of photosynthetic area. This chlorosis eventually develops into necrosis and highly susceptible plants may die (Fouché *et al.* 1984). Spikes that develop are misformed as they are caught in the rolled flag leaves. RWA damage results in plant stunting that leads to yield losses (Burd *et al.* 1993). Heavily infested plants exhibit flattened, droughtlike symptoms with the tillers lying almost parallel with the ground. The RWA is also capable of transmitting virus diseases that have a secondary effect on plants (Walters *et al.* 1980; Robinson 1992).

1.4.4 Control of the RWA

The RWA has only recently become a serious pest of wheat and barley worldwide. Winter wheat yield losses of between 35-60% have been recorded in South Africa (Robinson 1992). Several practices may be integrated to restrict the damage done by the RWA. These include cultural practices, chemical control, biological control and host plant resistance.

1.4.4.1 Cultural practices

Alternate host plants and volunteer wheat, barley and triticale should be eradicated to restrict the oversummering of the RWA in South Africa. Farmers have the right to choose the cultivars that they will plant. It is up to them to choose those cereal crops that are least susceptible to the RWA in order to prevent any increases of this pest. Delayed planting may further allow the young crop to escape infestations as winged oversummering females are distributed by wind. Dense, well-fertilised crop stands growing under favourable soil moisture conditions are also more resistant to RWA damage and farmers are encouraged to ensure that their crops are in a healthy condition (Walters *et al.* 1980; Robinson 1992; Riedell 1990).

1.4.4.2 Chemical control

Chemical control of the RWA has been hampered by the aphid's habit of feeding inside rolled

leaves. This complicates the penetration of contact insecticides and protects the aphids from the chemicals. Systemic insecticides have proved to be more successful in controlling the RWA, but the cost of application is high (Hill *et al.* 1993). Concern is mounting regarding the use of chemicals to control crop parasites as it poses a threat to the people handling the chemicals and to the environment. It also kills beneficial insects such as crop pollinators and those insect predators involved in the biological control of crop plants. The risk also exists that the aphids may develop resistance against the insecticides which will result in an increased difficulty of pest management (Dreyer and Campbell 1987; Robinson 1992). Du Toit (1986) established economic thresholds for RWA control on winter wheat in South Africa in order to limit the unnecessary application of chemical insecticides.

1.4.4.3 Biological control

The RWA arrived in most of the cereal production areas without its natural predators and parasitoids. Some countries, like South Africa, do however have parasitoid wasps and aphidophagous coccinellid beetles that attack the RWA, but they are not totally effective in controlling the RWA. They do not become sufficiently active early enough and their population growth normally lag behind that of the aphid population so that control is seldom complete. The aphids are often protected inside tightly rolled leaves where they feed while the predators are generally too large to reach them (Robinson 1992).

Jooste (1995) reported on a parasitoid wasp, *Aphelinus hordei*, introduced to South Africa from Russia. *A. hordei* has proved to be highly efficient in controlling the RWA in preliminary experiments. The wasp is about 1mm in length and is black. Females lay one egg in a RWA from which a larva develops. The larva devours the RWA from inside resulting in its death within 7 days. The females also feed directly on the aphids. Other wasps that show promise for control of the RWA in South Africa are *Aphidius matricariae* and *Aphidius rhopalosiphi* from Turkey and *Aphelinus varipes* from Germany.

Fungal endophytes of the species *Erynia*, *Conidiobolus* and *Verticillium* were also found to attack and control aphids. The aphid lethal paralysis virus may be useful for the biological control of

the RWA. Much research is directed towards the use of endophytes and viruses in integrated control of RWA populations. However, it is still too early to apply any of the biological agents on a commercial scale to combat the RWA (Robinson 1992).

1.4.4.4 Host plant resistance

The sudden appearance of RWA in most of the wheat producing areas has led to the screening of germplasm collections to identify sources of host plant resistance. Wheat cultivars have virtually no resistance to RWA and severe yield losses were suffered (Souza *et al.* 1991). RWA resistance genes are also lacking in cultivated durum wheat cultivars (Butts and Pakendorf 1984b). Many studies were conducted to identify resistance genes against RWA in wheat collections from the aphid's region of origin, i.e. central Asia (Souza *et al.* 1991), Iran and the Soviet Union (Smith *et al.* 1991; Du Toit 1987), Bulgaria (Du Toit 1988), and in the germplasms of other wheat production areas (Webster *et al.* 1987; Quick *et al.* 1991; Nkongolo *et al.* 1991b). Resistance has been reported in triticale (Nkongolo *et al.* 1989; Tolmay *et al.* 1993), rye (Nkongolo *et al.* 1989, 1990b), oats (Webster *et al.* 1987), *Hordeum* (barley) species (Kindler and Springer 1991) and in the wild relatives of wheat, i.e. *Triticum monococcum*, *T. tauschii*, *T. ventricosum*, *T. dicoccoides* and *T. timopheevii* (Du Toit and Van Niekerk 1985; Nkongolo *et al.* 1990a, 1991a; Potgieter *et al* 1991; Butts and Pakendorf 1984b).

The gene-for-gene relationship postulated by Flor (1942a,b) in which a gene for resistance in the host is matched by a gene for virulence in the pathogen is also applicable to the host-parasite relationship between wheat and RWA (Butts and Pakendorf 1984a). Du Toit (1987) identified RWA resistance genes in the *T. aestivum* lines PI 137739 (from Iran) and PI 262660 (from USSR). He showed that resistance in each line is controlled by a single dominant gene that is independently inherited and assigned the gene symbols *Dn1* and *Dn2* for the RWA resistance genes in PI 137739 and PI 262660, respectively (Du Toit 1989). Schroeder-Teeter *et al.* (1990, 1994) mapped *Dn1* on chromosome 7D of common wheat. A recessive RWA resistance gene was identified by Nkongolo *et al.* (1991a) in *T. tauschii*, accession SQ24, and the gene symbol *dn3* was assigned to the gene. Du Toit (1988) reported on RWA resistance found in the *T. aestivum* line PI 294994 (from Bulgaria). Saidi and Quick (1992) and Marais and Du Toit (1993)

independently concluded that a single dominant gene segregated in the progeny of backcrosses using this source. Elsidaig and Zwer (1993) reported that resistance in the line PI 294994 is conferred by a dominant allele at one locus and a recessive allele at a second locus. Marais and Du Toit (1993) showed by monosomic analysis that chromosome 7D carries a single dominant gene for resistance against RWA and assigned the gene symbol *Dn5* to this gene. Du Toit *et al.* (1995) mapped *Dn5* on chromosome arm 7DL and found that it was not linked with the centromere.

Nkongolo *et al.* (1989) screened several wheat, rye and triticale lines for resistance against RWA. They found that the rye and triticale lines were all moderately resistant to resistant and that the wheat lines ranged from susceptible (PI 262660) to resistant (PI 137739, PI 294994, PI 372129). They also identified the rye cultivar 'Imperial' as having good RWA resistance. Nkongolo *et al.* (1990b) conducted a study to identify the rye chromosome(s) and chromosome arm(s) carrying the RWA resistance gene(s) using 'Imperial' wheat-rye addition lines. They found resistance genes on chromosomes 1R, 3R, 4R and 7R and concluded that chromosome 3R made the largest contribution. Genes for resistance were located on chromosome arm 1RL and on both the long and short arms of chromosomes 3R, 4R and 7R. The RWA resistance in 'Imperial' rye depends on the dosage of the various resistance genes suggesting that it is mostly controlled by minor genes located on different chromosomes. Marais *et al.* (1994a) succeeded in transferring a gene(s) for resistance to the RWA from the rye cultivar 'Turkey 77' to a common wheat that has the 'Veery' translocation. Chromosome arm 1RS was identified as carrying at least one RWA resistance gene.

Simply inherited dominant genes are important sources for vertical resistance. They express total resistance, but this type of defense is soon neutralized by the evolution of new RWA biotypes. Biotypes of aphid species are morphologically indistinguishable from one another but differ in their behaviour or performance in that they attack different hosts. New biotypes mostly arise through mutations such as translocations, duplications or point mutations. Sexual recombination can also results in new biotypes, but to date male aphids were not found in South Africa. A sexual phase may therefore occur only in Russia where the climate favours the development of male aphids and sexual recombination can take place. The process of selection favours those

biotypes that can feed on the prevailing cultivars while the avirulent biotypes are selected against (Dreyer and Campbell 1987; Robinson 1992).

The pyramiding of RWA resistance genes in wheat breeding programmes is important to obtain a cultivar that has multibiotypic resistance and is therefore durable (Nkongolo *et al.* 1991b). Horizontal resistance is conferred by polygenes with a cumulative effect. The advantage of horizontal resistance is that it has the effect of slowing the rate of the epidemic and thus limits the spread of the disease. This lengthens the time necessary for the RWA to reach population levels that are economically damaging and slows down the rate at which new biotypes develop (Butts and Pakendorf 1984a; Robinson 1992). Quick *et al.* (1991) reported that the RWA resistance gene present in *T. aestivum* line PI 372129 has several undesirable traits associated with it. Molecular markers will therefore be useful in identifying those RWA resistant progenies in a backcross programme with the least PI 372129 chromatin. In order to integrate several RWA resistance genes in a cultivar, all the RWA resistance genes should have a closely linked marker to designate the various genes present in the progeny.

Three types of plant resistance to insects are referred to in plant resistance literature, namely (i) antixenosis or non-preference, (ii) antibiosis and (iii) tolerance. Antixenosis describes the insuitability of a plant, due to the presence of morphological or chemical plant factors that adversely alter insect behaviour, to serve as a host to the pest insect. The potential pest insect is forced to select an alternate host plant on which it can feed. Physical barriers such as thickened plant epidermal layers, waxy coatings on leaves and stems or trichomes may force insects to abandon their efforts to feed on such plants and rather feed on an otherwise palatable host plant. RWA resistant wheat plants may be without, or lack sufficient levels of, phytochemicals that stimulate feeding or oviposition. Antixenosis may also be due to the possession of unique phytochemicals that repel or deter aphids from feeding or oviposition.

Antibiosis describes the negative effects of a resistant plant on the biology of an insect attempting to use that plant as a host. Both chemical and morphological plant defenses mediate antibiosis and antibiotic effects of resistant plants range from mild to lethal. Antibiosis occurs because of either the presence of plant allomones or the presence of plant kairomones. Resistant cultivars

may lack the proper quantities of basic insect nutrient or contain phytochemicals that are toxic to insects. Some plant trichomes can secrete adhesive components that trap insects attempting to feed on the leaf surface and cause their starvation. Antibiosis may also occur because of high concentrations of structural plant substances such as lignin and silica that reduce insect digestion (Smith 1989; Robinson 1992). Belefant-Miller *et al.* (1994) reported on RWA resistant barley plants that exhibit the hypersensitive response characteristic in incompatible host:parasite interactions. This response is useful in screening resistant barley plants at an early stage and will facilitate breeding programmes.

Tolerance is the ability of host plants to withstand, or to recover from, damage caused by insect infestations equal to those on susceptible cultivars. Tolerance is therefore the inherent genetic ability of a plant to outgrow an insect infestation or to recover and add new growth after the destruction or removal of damaged tissues. Plants of a tolerant cultivar produce a better yield than plants of a susceptible cultivar at comparable levels of infestation. Tolerance involves only plant characteristics and is not part of an insect-plant interaction like with antixenosis and antibiosis. It, however, often occurs in combination with antibiosis and antixenosis (Smith 1989).

Due to the absence of an insect-plant interaction, tolerance is generally seen as durable and very difficult to obtain. Antixenosis and antibiosis on the other hand generally result from major genes and are therefore easily overcome by the evolution of new biotypes. Tolerance, however, is modified by environmental conditions and this type of resistance may not hold under all the seasonal conditions (Robinson 1992).

1.5 LINKAGE MAPS OF SPECIFIC CHROMOSOME REGIONS OF WHEAT

The linkage map of wheat is poorly covered with genetic markers (McIntosh 1988b). This makes it difficult to search for genetic markers tightly linked to a gene of interest and hampers the application of marker assisted selection in wheat breeding programmes. The need exists therefore to construct saturated linkage maps of the wheat chromosomes, especially those that have proven to be more important from a breeding point of view.

1.5.1 Genetic linkage map of chromosome arm 1DS

T. tauschii has proven to be an important source of disease resistance genes for wheat improvement. Resistance genes against the fungal diseases, leaf and stem rust, are commonly found in this diploid D-genome progenitor of common wheat (Lagudah *et al.* 1993). A *T. tauschii*-derived leaf rust resistance gene (*Lr21*) and a stem rust resistance gene (*Sr33*) were mapped on chromosome arm 1DS of common wheat (Jones *et al.* 1990; Jones *et al.* 1991). Other genetic markers located in this region are *Gli-D1*, a gene encoding gliadin seed storage proteins, (Payne *et al.* 1982) and *Rg2*, conferring brown glume colour (Jones *et al.* 1990). A further stem rust resistance gene, here referred to as *Srtau*, was transferred from *T. tauschii* to wheat by Marais *et al.* (1994b) and was allocated to chromosome 1D. This gene has not been mapped. The current genetic map of chromosome arm 1DS of common wheat is given in Fig. 3 and a description of genes known to occur in this region, follows.

1.5.1.1 Stem rust resistance gene, *Srtau*, derived from *T. tauschii*, accession RL5289

T. tauschii accession RL5289 was found to be susceptible to the stem rust pathotypes of Western Canada (Kerber and Dyck 1978), but is highly resistant to the prevailing stem rust pathotypes of South Africa (Marais *et al.* 1994b). R. de V. Pienaar (see Marais *et al.* 1994b) crossed RL5289 with *T. turgidum* L. var. *durum* cultivar 'Cando'. An amphiploid was produced that was crossed with the common wheat line W107. Marais *et al.* (1994b) made three cycles of crosses of resistant derivatives to the common wheat cultivars 'Inia 66' and 'SST3' and resistant F_1 plants from the final crosses were planted and used to obtain F_2-derived F_3 and F_4 populations. F_4 populations were evaluated agronomically as well as for their resistance against the prevailing stem rust pathotypes. A superior true-breeding population, 87M66-2-1, homozygous for a single dominant stem rust resistance gene was selected. A monosomic analysis of 87M66-2-1 showed that the stem rust resistance gene is located on chromosome 1D. 87M66-2-1 produced ;1^- — ;$1^=$ infection types following inoculation with the stem rust pathotype 2SA4, has brown glumes and tough ears that are difficult to thresh. It is, however, not clear whether these characters are linked with the stem rust resistance gene (Marais *et al.* 1994b).

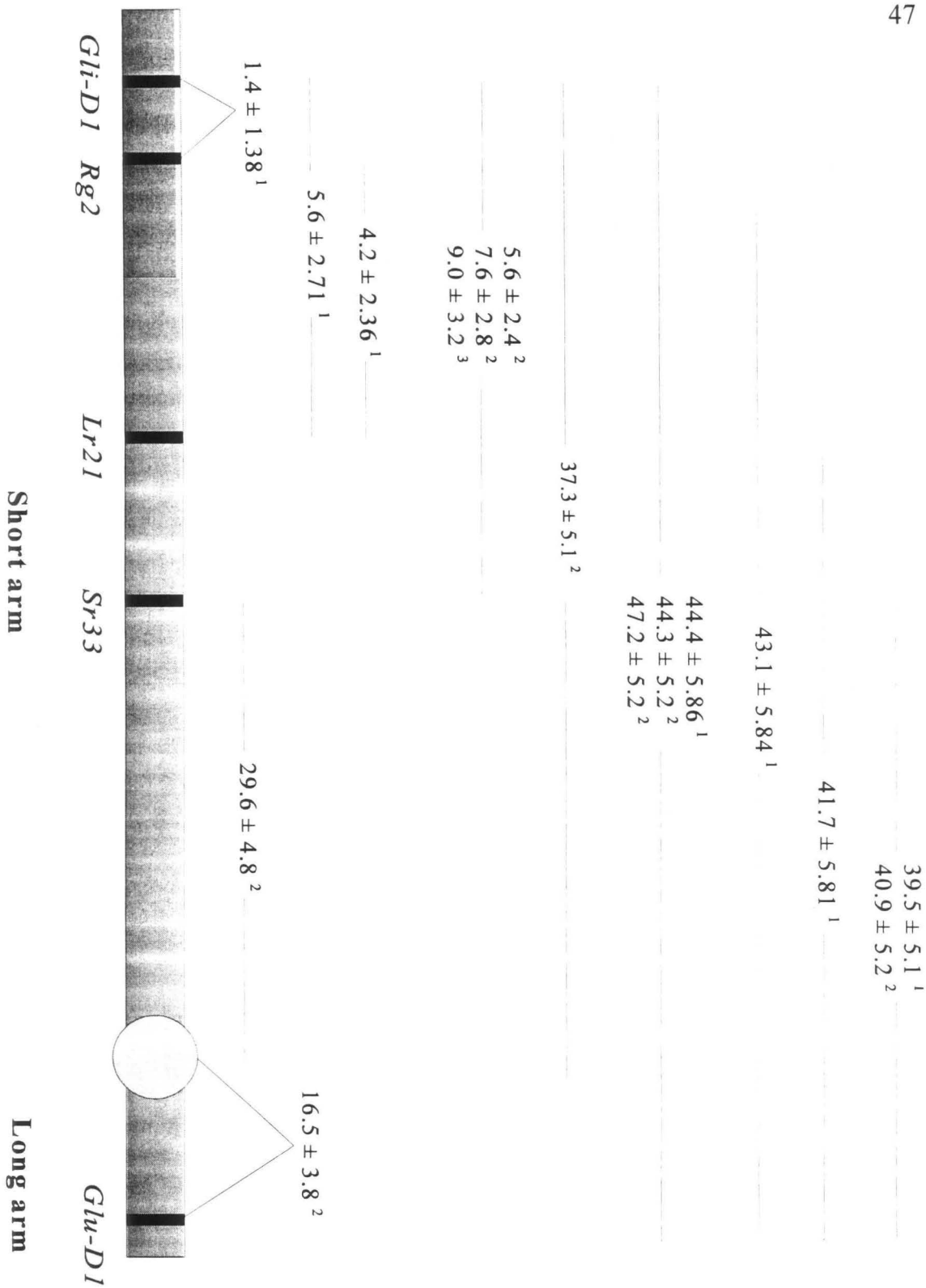

Fig. 3. Genetic linkage map of chromosome arm 1DS.

[1] Jones *et al.* 1990; [2] Jones *et al.* 1991; [3] Czarnecki and Lukow 1992

1.5.1.2 Stem rust resistance gene (*Sr33*)

Kerber and Dyck (1978) identified a dominant gene for seedling stem rust resistance in *T. tauschii* var. *strangulata* accession RL5288. The gene provided resistance to the prevalent stem rust pathotypes in Western Canada. They produced a synthetic hexaploid, RL5405, from a cross between the tetraploid 'Tetra Canthatch' and RL5288. The amphiploid was crossed with and backcrossed to common wheat to incorporate the stem rust resistance gene into wheat. The stem rust resistance gene gives an infection type of 2 in hexaploid wheat and ;1^{-} in diploid stocks.

Kerber and Dyck (1978) mapped this stem rust resistance gene on chromosome arm 1DL and gave it the temporary designation *SrSQ*. McIntosh (1988b) redesignated *SrSQ* as *Sr33*. Kerber and Dyck (1978) observed linkage between *SrSQ*, *Lr21* (a gene conferring leaf rust resistance) and *Rg2* (a gene conferring brown glume colour), and Jones *et al.* (1990) suggested that these genes, along with *Gli-D1* (a gene encoding gliadin storage proteins) are actually located on chromosome arm 1DS. This suggestion was confirmed by Jones *et al.* (1991) when they observed tight linkage between *Sr33* and *Gli-D1* (5.6 ± 2.4% and 7.6 ± 2.8% recombination in different studies) on chromosome arm 1DS and less tight linkage between *Sr33* and the centromere (29.6 ± 4.8% recombination) and also between *Sr33* and *Glu-D1*, a gene encoding subunits of high molecular weight glutenin storage proteins located on chromosome arm 1DL, (40.9 ± 5.2% and 39.5 ± 5.1% recombination in separate studies) (Fig. 3). Czarnecki and Lukow (1992) obtained a recombination value of 9.0 ± 3.2% between *Sr33* and *Gli-D1* on chromosome 1DS (Fig.3). Jones *et al.* (1991) concluded that *Sr33* is located on chromosome arm 1DS proximal to *Gli-D1* and that the order of the loci is *Glu-D1* — centromere — *Sr33* — *Gli-D1* (Fig. 3).

1.5.1.3 Leaf rust resistance gene (*Lr21*)

A dominant gene conferring seedling leaf rust resistance was identified in *T. tauschii* var. *meyeri* RL5289 (Kerber and Dyck 1969). Rowland and Kerber (1974) gave this leaf rust resistance gene the designation *Lr21*. A synthetic hexaploid, RL5406, was produced from the cross between the tetraploid 'Tetra Canthatch' and *T. tauschii* accession RL5289 and was used to incorporate *Lr21*

into common wheat (Kerber and Dyck 1969). Infection types of 0 — ;1 were observed when the introgression of the gene into common wheat was completed (Kerber and Dyck 1969; Kerber and Dyck 1978).

Rowland and Kerber (1974) and Kerber and Dyck (1978) suggested that *Lr21* is located on chromosome arm 1DL. Jones *et al.* (1990) however, showed that *Lr21* is tightly linked with *Gli-D1* and *Rg2* (recombination of 5.6 ± 2.71% and 4.2 ± 2.36%, respectively) on chromosome arm 1DS (Fig. 3). Recombination of 41.7 ± 5.81% was also observed between *Lr21* and *Glu-D1*, suggesting independent segregation between these two loci (Fig.3). The correct order of the three loci on chromosome arm 1DS is therefore centromere — *Lr21* — *Rg2* — *Gli-D1* with *Glu-D1* located on chromosome arm 1DL.

1.5.1.4 Brown glume colour (*Rg2*)

The gene conferring brown glume colour, *Rg2*, is dominant, it is expressed at maturity and the intensity of the glume pigmentation is influenced by environmental factors. This gene was originally mapped on chromosome arm 1DL (Kerber and Dyck 1969; Rowland and Kerber 1974; Kerber and Dyck 1978), but Jones *et al.* (1990) showed that *Rg2* is tightly linked with *Gli-D1* and *Lr21* on chromosome arm 1DS with recombination values of 1.4 ± 1.38% and 4.2 ± 2.36%, respectively (Fig.3).

1.5.1.5 Gliadin seed storage protein (*Gli-D1*)

Gliadins are the major storage protein fraction of wheat endosperm, of relatively lower molecular weight and do not have disulfide-bonds linking their subunits (Lafiandra and Kasarda 1985; Payne 1987). *Gli-D1* codes for ω- and γ-gliadins that can be fractioned by two-dimensional electrophoresis (Lafiandra and Kasarda 1985). Payne *et al.* (1982) mapped *Gli-D1* onto the short arm of chromosome 1D.

Several studies were conducted to detect the linkage relationships of *Gli-D1* with *Rg2*, *Lr21*, the centromere, *Glu-D1* and *Sr33* (Jones *et al.* 1990; Jones *et al.* 1991; Czarnecki and Lukow 1992).

Jones *et al.* (1990) showed that *Gli-D1* is tightly linked with *Rg2* and *Lr21* on chromosome arm 1DS with recombination values of 1.4 ± 1.38% and 5.6 ± 2.71%, respectively. They also showed that *Gli-D1* and *Glu-D1* segregate independently with a recombination value of 44.4 ± 5.86% (Fig. 3). The latter result was confirmed by Jones *et al.* (1991) when they observed recombination of 44.3 ± 5.2% and 47.2 ± 5.2% in separate studies. They furthermore found that *Gli-D1* is linked to *Sr33* on chromosome arm 1DS with a recombination value of 5.6 ± 2.4% and 7.6 ± 2.8% (in separate studies) and that *Gli-D1* and the centromere are less tightly linked (37.3 ± 5.1%) (Fig.3). A recombination value of 9.0 ± 3.2% was calculated between *Gli-D1* and *Sr33* showing that these two genes are linked (Fig. 3) (Czarnecki and Lukow 1992).

1.5.2 Linkage map of chromosome arm 7DL

Genetic studies of the known genes on chromosome arm 7DL were seldomly reported and as a result this region is poorly mapped (McIntosh 1988b). Several important disease resistance genes that were introgressed into cultivated wheat have been located to chromosome arm 7DL, including *Lr19* (leaf rust resistance gene), *Sr25* (stem rust resistance gene), *Pch1* (eyespot resistance gene) and recently *Dn5* (Russian wheat aphid resistance gene) (McIntosh 1988b; Du Toit *et al.* 1995). Biochemical markers such as *Ep-D1*, expressing the endopeptidase isozyme and *α-Amy-D2*, expressing the α-amylase isozyme, and a morphological marker, *cn-D1* (chlorina mutant, recessive) were also mapped to this area (McIntosh 1988b).

A summary of the present chromosome arm 7DL genetic map is given in Fig. 4 and descriptions of the genes known to occur on 7DL are given in the next section.

1.5.2.1 Russian wheat aphid resistance gene (*Dn5*)

Resistance to the Russian wheat aphid (RWA) was identified in the *T. aestivum* accession PI 294994 that was introduced from Bulgaria (Du Toit 1988). Elsidaig and Zwer (1993) conducted a study to determine the number of genes and mode of inheritance of RWA resistance in accession PI 294994. They concluded that the resistance is conferred by a dominant allele at one locus and a recessive allele at a second locus. Saidi and Quick (1992) and Marais and Du Toit

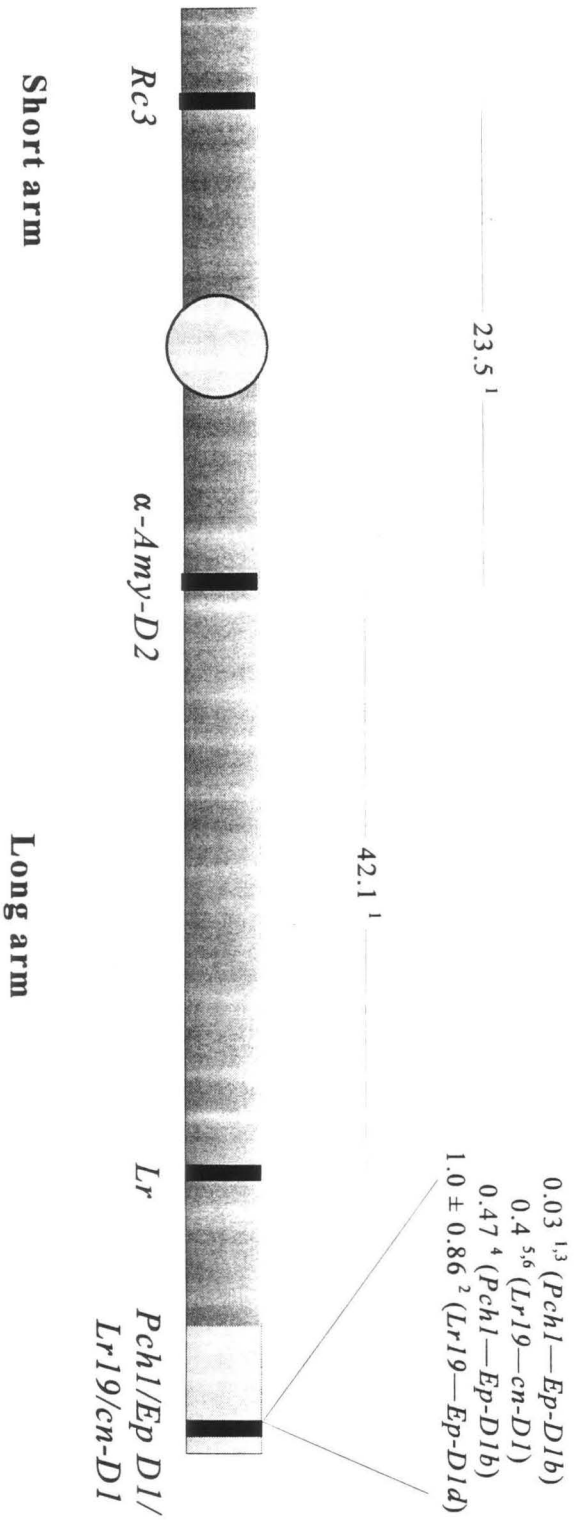

Fig. 4. Genetic linkage map of chromosome arm 7DL.

[1] Worland *et al.* 1988; [2] McMillin *et al.* 1993; [3] Law *et al.* 1988;

[4] Summers *et al.* 1988; [5] McIntosh *et al.* 1976; [6] Marais and Marais 1990

(1993) independently showed that a single dominant gene controls resistance to RWA in the wheat accession PI 294994. Marais and Du Toit (1993) showed by monosomic analysis that this resistance gene is located on chromosome 7D and designated it *Dn5*. A backcross breeding programme to transfer *Dn5* to four susceptible, advanced breeding lines was conducted.

1.5.2.2 Endopeptidase (*Ep-D1*)

Endopeptidase isozymes are coded by loci on the long arms of the group 7 chromosomes. These isozymes can be separated by isoelectric focusing of aqueous extracts from mature embryo tissues, immature kernels or etiolated seedlings (Tang and Hart 1975; Hart and Langston 1977; Koebner *et al.* 1988). Hart and Langston (1977) located the structural gene Ep-1 on chromosome arm 7DL following a study of the zymogram phenotypes produced by the group 7 aneuploids. They redesignated Ep-1 as *Ep-D1*. Koebner *et al.* (1988) screened a range of wheat varieties and found very limited polymorphism at the *Ep-D1* locus. The only polymorphisms detected in wheat derive from introductions of alien chromatin and were designated: *Ep-D1a* (CS and other common wheats), *Ep-D1b* (*T. ventricosum*) and *Ep-D1c* ('Synthetic', null allele). McMillin *et al.* (1993) reported a fourth *Ep-D1* allele which they designated *Ep-D1d* (*Thinopyrum ponticum*, null allele).

Endopeptidase genes linked to disease resistance genes have proven to be very useful markers. McMillin *et al.* (1993) observed very tight linkage between *Ep-D1d* and *Lr19* and calculated a recombination value of 1.0 ± 0.86% (Fig. 4). Several studies reported on the linkage between *Pch1*, a gene conferring resistance to eyespot, and the *Ep-D1* locus. Worland *et al.* (1988) and Law *et al.* (1988) reported a maximum recombination frequency of 0.03% between *Ep-D1b* and *Pch1*, suggesting very tight linkage between the two loci (Fig 4). Summers *et al.* (1988) confirmed these results when they obtained recombination of less than 0.47% between *Ep-D1b* and *Pch1* (Fig. 4). Worland *et al.* (1988) concluded that *Ep-D1* segregates independently from *Rc3*, a dominant gene conferring red coleoptile colour and located on chromosome arm 7DS, and found a recombination frequency of 18.4% between *Ep-D1* and *Lr*, an adult plant leaf rust resistance gene (Fig. 4).

It should be noted, however, that *Lr19*, *Sr25* and the null allele, *Ep-D1d*, occur on the T4 translocation that was derived from *Thinopyrum ponticum* by Sharma and Knott (1966). Pairing of the T4 region with homoeologous wheat chromatin on 7DL is believed to occur only in the absence of a *Ph*-gene (Marais 1992b). Similarly, both *Pch1* and the allele *Ep-D1b* was derived from *T. ventricosum* (Doussinault *et al.* 1983) and it is not known whether the introgressed region is completely homologous to the corresponding region on 7DL. Consequently, linkage data based on a study of recombination between wheat and species derived chromatin may largely underestimate the true genetic distances.

1.5.2.3 Chlorina mutant gene (*cn-D1*)

Spontaneous chlorophyll mutations are rare in hexaploid wheat due to double or treble doses of the genes controlling each step in normal chlorophyll synthesis (McIntosh and Baker 1968). Pettigrew *et al.* (1969) detected a spontaneous chlorophyll mutation in a F_2 population of the Cornell Wheat Selection 5075aB-2B-21/'Chinese Spring'. This plant was yellow and it is thought that the mutation arose as a spontaneous change at the *cn-A1* locus (McIntosh 1988b). Pettigrew and Driscoll (1970) showed that three homoeologous genes for chlorophyll production occur on the chromosome arms 7AL, 7BL and 7DL. Washington and Sears (1970) reported a recessive chlorophyll mutation at the *cn-D1* locus in the ethyl methanesulfonate (EMS) derived line, *chlorina*-214. Plants homozygous for the mutation are yellow, plants heterozygous for the mutation are normal and plant hemizygous for the chlorophyll mutation are intermediate. This recessive chlorina mutant gene, *cn-D1*, is located on chromosome arm 7DL (McIntosh 1973).

Marais and Marais (1990) showed that no recombination occurs between *cn-D1* and *Lr19* and the locus therefore falls within the translocated area. Recombination of 0.4% was reported for *cn-D1* and *Lr19* on chromosome 7DL (Fig. 4) (McIntosh *et al.* 1976).

1.5.2.4 Eyespot resistance gene (*Pch1*)

Eyespot disease, caused by the fungus *Pseudocercosporella herpotrichoides*, results in the lodging of wheat plants and as a consequence yield reductions (Doussinault *et al.* 1983). A high

level of resistance to this disease is found in the wild grass *Triticum ventricosum* and Doussinault *et al.* (1983) reported on the transfer of a dominant eyespot resistance gene from this wild grass to hexaploid wheat. Jahier *et al.* (1978) located the eyespot resistance gene (*Pch1*) on chromosome 7D, while Koebner *et al.* (1988) showed that *Pch1* is located on chromosome arm 7DL when they screened a range of wheat varieties to study polymorphism at the endopeptidase loci.

Worland *et al.* (1988) and Law *et al.* (1988) reported very tight linkage between *Pch1* and *Ep-D1b* with a maximum recombination frequency of 0.03% (Fig. 4). Summers *et al.* (1988) confirmed this very tight linkage when they obtained recombination of less than 0.47% between *Pch1* and *Ep-D1b* (Fig. 4). Independent segregation occurred between *Pch1* and *α-Amy-D2* and between *Pch1* and *Rc3* (Worland *et al.* 1988). They furthermore observed a recombination frequency of 18.4% between *Pch1* and *Lr*, an adult plant leaf rust resistance gene (Fig. 4)

1.5.2.5 Leaf rust resistance gene (*Lr19*)

Sharma and Knott (1966) found that the winter wheat cultivar 'Agrus' was a substitution line in which a pair of *Thinopyrum* chromosomes had replaced a pair of wheat chromosomes. 'Agrus' proved to be highly resistant to the prevailing leaf rust pathotypes due to the presence of a leaf rust resistance gene on the substituted *Thinopyrum* chromosome pair. Irradiation was used to produce translocations between the *Thinopyrum* chromosome carrying the leaf rust resistance gene and a 'Thatcher' chromosome. The translocation line, T4, was used to transfer the leaf rust resistance gene into a 'Thatcher' background by backcrossing. The derived line was named 'Agatha' (Sharma and Knott 1966). The latter authors concluded that the leaf rust resistance was conferred by a single dominant gene located on chromosome 7D. Browder (1972) designated this gene *Lr19*. Dvořák and Knott (1977) found that a substantial part of, or the complete 7DL arm, was replaced with homoeologous chromatin of the *Thinopyrum* chromosome $7el_1$ that carries the *Lr19* locus.

McMillin *et al.* (1993) reported very tight linkage between *Lr19* and *Ep-D1d* (recombination value of $1.0 \pm 0.86\%$) (Fig. 4). McIntosh *et al.* (1976) observed a maximum recombination of

0.4% between *Lr19* and *cn-D1* (Fig. 4). This tight linkage was confirmed by Marais and Marais (1990) when they detected no recombination between *Lr19* and *cn-D1* (Fig. 4).

1.5.2.6 α-Amylase (*α-Amy-D2*)

α-AMY-D2 is an acidic *α*-AMY2 isozyme (pIs 4.9-6.0) which is encoded by the α-amylase isozyme locus on chromosome arm 7DL (Niskikawa and Nobuhara 1971; Gale *et al.* 1983). α-AMY-D2 is expressed in the developing endosperm and in the germinating grain. Isozyme zymograms can be obtained by the induction of α-amylase production and the assay of these products by isoelectric focusing (Niskikawa and Nobuhara 1971).

Gale *et al.* (1984) reported independent segregation between *α-Amy-D2* and *Pch1* which was confirmed by Worland *et al.* (1988). Worland *et al.* (1988) observed a 23.5% recombination between *α-Amy-D2* and *Rc3* and a recombination frequency of 42.1% between *α-Amy-D2* and *Lr* (Fig. 4).

2. MATERIALS AND METHODS

2.1 Mapping of a stem rust resistance gene derived from *Triticum tauschii*

2.1.1 Telosomic analysis

The homozygous resistant line 87M66-2-1 carries a stem rust resistance gene that was derived from *Triticum tauschii* accession RL5289. This gene is known to be located on chromosome 1D (Marais *et al.* 1994b) and the purpose of the analysis was to determine the chromosome arm involved. 87M66-2-1 was therefore crossed as the male parent with (i) a 'Chinese Spring ditelo 1DS monotelo 1DL' plant and (ii) a 'Chinese Spring ditelo 1DL monotelo 1DS' plant. Stem rust resistant monotelodisomic plants were selected from each cross and test crossed with the susceptible cultivar 'Inia 66'. Root tip chromosome counts and seedling stem rust resistance tests (pathotype UVPgt50) were done on the test cross derived F_1 plants (Sears 1966, 1974). Standard errors for recombination frequencies were calculated according to The and McIntosh (1975) (Mather 1938; Allard 1956).

2.1.2 Linkage study

Following the chromosome arm allocation of the stem rust resistance gene, its possible linkage with the *Rg2*, *Lr21*, *SrX* and *Sr33* loci was studied. The designations and pedigrees of the wheats used in the linkage study are given in Table 1. The stem rust resistant line 87M66-2-1 has brown glume colour (*Rg2*) which was derived from *T. tauschii* and which could also be used as a marker gene. The parental lines listed in Table 1 were tested for their seedling resistance against the leaf rust pathotypes UVPrt2, UVPrt3, UVPrt8, UVPrt9, UVPrt13 and UVPrt16 and the stem rust pathotypes UVPgt50, UVPgt51, UVPgt52 and UVPgt53. The avirulence/virulence formulae of the respective pathotypes are given in Table 2. On the basis of the results obtained pathotypes were selected for analyzing segregation ratios in the progeny of specific parents. Also, it became evident that the resistance/susceptibility characteristics of the parents allowed for the study of linkage between only four of the possible 10 combinations of loci.

Table 1. List of the material used in the linkage study of a stem rust resistance gene derived from *T. tauschii*.

Designation	Pedigree
87M66-2-1	Cando/*T. tauschii*//W107//3/SST3/4/*2 Inia 66
W70	*Lr21-SrX*/*7 Condor
W72	Sr33/*4 Condor
—	Condor

Table 2. Avirulence/virulence formulas of the leaf and stem rust pathotypes used.

Pathotype	Avirulence/virulence formula
Leaf rust:	
UVPrt2[c]	*Lr1,2a,2b,3ka,11,15,17,20,24,26,30/2c,3a,3bg,10,14a,16*
UVPrt3[c]	*Lr3a,3bg,3ka,10,11,14a,16,17,20,26,30/1,2a,2b,2c,15,24*
UVPrt8[b]	*Lr3a,3bg,3ka,11,16,20,26,30/1,2a,2b,2c,10,14a,15,17,24*
UVPrt9[b]	*Lr2a,2b,3bg,15,16,17,26/1,2c,3a,3ka,10,11,14a,20,24,30*
UVPrt13[b]	*Lr3a,3bg,3ka,11,16,20,30,/1,2a,2b,2c,10,14a,15,17,24,26*
UVPrt16[c]	*Lr1,2a,2b,3bg,3ka,10,11,14a,15,16,17,20,26/2c,3a,24,30*
Stem rust:	
UVPgt50[a]	*Sr8b,9g,13,15,21,22,24,25,26,27,29,31,32,36/5,6,7a,7b,8a,9a,9b,9d,9e,9f, 10,11,12,14,16,17,19,23,30,37,Gt,Wd*
UVPgt51[a]	*Sr8b,9e,9g,13,15,21,22,23,24,25,26,27,29,30,31,32,37,Gt,Wd/5,6,7a,7b, 8a,9a,9b,9d,9f,10,11,12,14,16,17,19,36*
UVPgt52[a]	*Sr8b,9e,9g,13,15,21,22,25,26,27,29,30,31,32,36,37,Gt,Wd/5,6,7a,7b,8a, 9a,9b,9d,9f,10,11,12,14,16,17,19,23,24*
UVPgt53[c]	*Sr5,6,9b,9e,24,30,36/7b,9g,27*

References: [a] Le Roux (1986); [b] Marais *et al.* (1988); [c] Z.A. Pretorius (1996, personal communication)

W70, W72 and 87M66-2-1 were crossed in all possible combinations. F_1 seedlings from each cross were tested for resistance to leaf or stem rust using either of the pathotypes UVPrt8 and UVPgt50, respectively. Resistant F_1 plants were used as female plants and test crossed with the susceptible cultivar 'Inia 66'.

2.1.2.1 Brown glumes (*Rg2*) versus *Lr21*

In order to test for linkage between the brown glume character and *Lr21*, the F_1:W70/87M66-2-1 was test crossed with 'Inia 66' and test cross derived F_2 populations (TF_2) were tested for the presence of brown glume colour and *Lr21*. The leaf rust pathotype UVPrt8 was used to detect *Lr21*.

In order to confirm that *Lr21* (ex W70) was the only gene giving leaf rust resistance in this cross, the F_2:Condor/W70 was also tested with UVPrt8. Since Condor is susceptible to UVPrt8, a 3:1 segregation ratio was expected.

2.1.2.2 Stem rust resistance in 87M66-2-1 (*Srtau*) versus *Sr33*

The F_1:W72/87M66-2-1 was test crossed with 'Inia 66' to test for linkage between these two genes. The stem rust race UVPgt50 was used to screen for the presence of *Sr33* and the stem rust resistance gene derived from *T. tauschii*. The TF_1:W72/87M66-2-1//Inia 66 seedlings were scored as either resistant or susceptible. Both genes give very strong expression and their infection types could not be distinguished.

Marais *et al.* (1994b) concluded that 87M66-2-1 carries only one gene for resistance to UVPgt50. The F_2:Condor/W72 was tested against the stem rust pathotype UVPgt50 to verify that only one stem rust resistance gene is present in W72. A segregation ratio of 3:1 was therefore expected.

2.1.2.3 *SrX* versus *Sr33*

Apart from *Lr21*, W70 also carries a stem rust resistance gene, *SrX*. *SrX* was derived from *T.*

tauschii and has the same virulence/avirulence formula as *Sr21*, a stem rust resistance gene originally transferred from *T. monococcum* to hexaploid wheat and located on chromosome 2AL (McIntosh 1981). The relationship of *SrX* with *Sr21* is, however, still unknown. W70 was obtained from Dr B. Lombard (Sensako, Welgevallen Experimental Farm, Stellenbosch, 7600, South Africa).

In order to test for linkage between *SrX* and *Sr33*, the F_1:W70/W72 was test crossed with 'Inia 66', the TF_1 plants selfed and approximately 20 seedlings of the TF_2 families were tested for the presence of resistance to UVPgt50. The infection types produced by the two genes are also indistinguishable and TF_2 populations were scored as containing only resistant plants, segregating or susceptible.

The F_2:Condor/W70 was tested against the stem rust pathotype UVPgt50 to verify that only one effective stem rust resistance gene occurs in W70. A segregation ratio of 3:1 was expected.

2.1.2.4 *SrX* versus *Srtau*

Approximately 20 seedlings of the TF_2:W70/87M66-2-1//Inia 66 populations were inoculated with UVPgt50 in order to test for linkage between *SrX* and *Srtau*. The infection types produced by the two genes are indistinguishable and populations were scored as resistant, segregating or susceptible.

2.1.3 Leaf and stem rust seedling tests

2.1.3.1 Inoculation

Inoculation was done by spraying of seedlings at the 2-3 leaf stage (approximately 7-9 days old) with a suspension of spores in distilled water to which a few drops of a wetting agent, Triton, were added. The seedlings were then covered with a transparent plastic bag and incubated for 24h at 18-20°C for the rust to develop. Following incubation, the seedlings were moved to a growth chamber in which a temperature of 22-25°C and a day/night cycle of 16/8h were

maintained.

2.1.3.2 Disease assessment

Infection types (ITs) were scored according to the 0-4 scale proposed by Stakman *et al.* (1962) 7-10 days after inoculation for leaf rust and 14-16 days after inoculation for stem rust. ITs 0—2 were considered to be resistant, while ITs 3 and 4 were susceptible.

2.1.3.3 Spore maintenance

All the leaf and stem rust pathotypes tested were originally obtained from prof. Z.A. Pretorius, Department of Plant Pathology, U.O.F.S. They were maintained and increased in isolation on susceptible seedlings to avoid contamination. For long term storage, rust spores were collected from susceptible plants, dried under vacuum and stored in air tight containers at -80°C. Before use, the stored spores were heat shocked (37°C for 10 minutes) in order to break the cold-induced dormancy and were allowed to rehydrate slowly for two hours. For short term storage, infected leaves were air dried at room temperature and stored at room temperature for up to 2 weeks. If longer periods (2-6 weeks) were required before rust inoculation, the infected leaves were stored at 4°C in an open container. No special procedures were required before use of these spores and they were simply suspended prior to inoculation.

2.1.4 Glume colour

A row of each TF_2:W70/87M66-2-1//Inia 66 population was planted in the field in 1996. Glume colour (dark brown) was scored at maturity as either present or absent.

2.2 Mapping of a Russian wheat aphid resistance gene, *Dn5*, on chromosome 7DL of common wheat

2.2.1 Telosomic analysis

A near isogenic line of the cultivar 'Palmiet' (accession W574) carries the Russian wheat aphid (RWA) resistance gene, *Dn5*. W574 was crossed as the male parent with (i) a 'Chinese Spring' plant that was ditelosomic for chromosome arm 7DS, and (ii) a segregate from the cross: 'Chinese Spring ditelo 7DL monotelo 7DS'/'Inia 66' that was monotelodisomic for chromosome arm 7DL. The 'Chinese Spring ditelo 7DS' line used in cross (i) was obtained from the late professor E.R. Sears (Department of Agronomy, University of Missouri-Columbia, Columbia, Mo 65211). The telosome in cross (ii) was verified through C-banding and by testing for the presence of the *Ep-D1a* locus (Marais and Marais 1990). From each cross, monotelodisomic plants were selected and testcrossed with either the aphid susceptible cultivar, 'Inia 66', or a susceptible breeding line, 'W84-17'. Root tip chromosome counts and RWA resistance tests (according to Du Toit 1987) were done on the test cross derived F_1 plants (Sears 1966, 1974). Standard errors for recombination frequencies were calculated according to The and McIntosh (1975) (Mather 1938; Allard 1956).

2.2.2 Linkage study

Possible linkage of *Dn5* (dominant) with the marker loci *Ep-D1b* (co-dominant) and *cn-D1* (recessive) on chromosome 7DL was also studied. A description of the genetic material used and their genotypes regarding the marker genes is given in Table 3. The three sources were crossed in all possible combinations, reciprocals excluded (Table 3). F_1-seedlings from the crosses 93M167 and 92M166/W311 were used as females and were back crossed to W311. F_1-seedlings from the remaining cross (93M168) were used as females and test crossed with the susceptible cultivar 'Inia 66' or the susceptible breeding line 'W84-17'.

Table 3. Material used in the linkage study involving the Russian wheat aphid resistance gene, *Dn5*.

Number	Pedigree/Name	Marker genes
Parents:		
W574	SA 463/*6 Palmiet	Homozygous: ***Dn5***, *Cn-D1*, *Ep-D1a*
W311	Chinese Spring chlorofil mutant	Homozygous: *dn5*, ***cn-D1***, *Ep-D1a*
92M166	VPM-1/*3 W84-17	Homozygous: *dn5*, *Cn-D1*, ***Ep-D1b***
Crosses:		
93M167	W574/W311	***Dn5***/*dn5*, *Cn-D1*/***cn-D1***, *Ep-D1a*/*Ep-D1a*
93M168	W574/92M166	***Dn5***/*dn5*, *Cn-D1*/*Cn-D1*, *Ep-D1a*/***Ep-D1b***
—	W311/92M166	*dn5*/*dn5*, ***cn-D1***/*Cn-D1*, *Ep-D1a*/***Ep-D1b***

2.2.2.1 *Dn5* versus *cn-D1*

The F_1:93M167 was back crossed to W311 (Russian wheat aphid susceptible, chlorina mutant) in order to study the linkage between *Dn5* and *cn-D1*. B_1F_1:93M167/W311 seedlings were planted in a greenhouse and scored for the presence of *cn-D1*. The plants were allowed to grow to maturity and harvested separately. A sample of 15-20 seedlings per B_1F_2 family were then tested for the presence of *Dn5*.

2.2.2.2 *Dn5* versus *Ep-D1b*

To test for linkage between *Dn5* and *Ep-D1b*, the F_1:93M168 was test crossed with the Russian wheat aphid susceptible cultivar 'Inia 66' or the susceptible breeding line 'W84-17'. Both test cross parents express the *Ep-D1a* allele. Immature embryos from the TF_1:93M168/Inia 66 or the TF_1:93M168/W84-17 were excised 18-24 days after pollination and raised on an artificial medium. The endosperm was then used to screen for *Ep-D1a* and *Ep-D1b*. The plants derived from excised embryos were planted in a greenhouse after 3-5 weeks and allowed to mature

whereafter the TF_2 plants were harvested individually. Samples of 15-20 seeds per F_2 family (TF_2:93M168/Inia 66 and TF_2:93M168/W84-17) were then tested for the presence of *Dn5*.

2.2.2.3 *Ep-D1b* versus *cn-D1*

The linkage between *Ep-D1b* and *cn-D1* was studied by back crossing the F_1:92M166/W311 to W311 (chlorina mutant, *Ep-D1a)*. Immature embryos from the B_1F_1:92M166/*1 W311 were excised 18-24 days after pollination and cultured on an artificial medium. The endosperm was used to screen for *Ep-D1a* and *Ep-D1b*. The embryos were planted in a greenhouse after 3-5 weeks and scored for the presence of *cn-D1*.

2.2.3 RWA seedling resistance tests

Fifteen to twenty seedlings of each test cross F_2 family were kindly screened by Dr F. du Toit (PANNAR, PO Box 17164, 9388 Bainsvlei, South Africa) for the presence of *Dn5*. Aphids were cultured in a greenhouse on susceptible wheat seedlings while a day/night temperature of 22/15°C was maintained. Plants at the one-leaf stage were evenly infested with RWA of mixed instars. Twenty-one days after infestation plant damage was rated visually by scoring individual F_2 families as either susceptible or segregating for resistance according to Du Toit (1987). The aim of the RWA tests was to determine whether *Dn5* segregated in the test or back cross F_2 families and thus was present in the meiocyte being characterized.

2.2.4 Embryo culture

Immature kernels (18-24 days after pollination) were sterilized for 15 min in a 30% sodium hypochlorite solution and then rinsed three times in distilled water. The embryos were excised under a dissecting microscope in a laminar flow cabinet. The excised embryos were placed scutellar side down on embryo culture medium in petri dishes prepared according to Bajaj (1990). The petri dishes were placed in a growth chamber where cool-white fluorescent tubes provided 16h of daylight. A temperature of 25°C was maintained during this period. After approximately 3-5 weeks, the plants were moved to a greenhouse.

2.2.5 Identification of endopeptidase isozymes

2.2.5.1 Parental material

The endopeptidase zymograms of the parental material used were studied in order to associate the different endopeptidase bands in the linkage study with gene loci. The wheat material used for this purpose was 'CS', 'SA 463' with resistance to RWA, 'VPM-1', 'Inia 66' and 'W84-17'.

Hart and Langston (1977) assigned a homoeoallelic series of endopeptidase isozymes to the long arms of the group 7 chromosomes. Their results could be used to deduce some of the alleles present in the parental lines. In order to assign novel endopeptidase bands that were encountered in the study to wheat chromosomes, remnant seeds of the 7A, 7B and 7D derived F_2 material that Marais and Du Toit (1993) used in a monosomic analysis to find the chromosome location of the RWA resistance gene, *Dn5*, were used. Isoelectric focusing of endopeptidases was therefore done using the monosome derived F_2:CSM 7A/SA 463, F_2:CSM 7B/SA 463 and F_2:CSM 7D/SA 463. 'SA 463' and 'CS' were included as parental controls. The segregation of the individual bands was studied in each progeny to detect the critical chromosome carrying the locus for a specific endopeptidase band.

2.2.5.2 Isoelectric focusing

Following the dissection of embryos from immature seeds, the endosperm halves were kept at –4°C until the electrophoretic separations could be done. Crude enzyme extracts were obtained by macerating the endosperm halves in 90 μl of distilled water. Following centrifugation at 10 000 rpm for 20 min, 30 μl of the supernatant was loaded directly onto the gel surface at the cathodal end. Gels were prepared as described by Koebner *et al.* (1988), except that the ampholine solution consisted of 23.3% Pharmalyte 4.2-4.9 and 11.7% Pharmalyte 4-6.5. Electrophoresis was carried out at 4°C using a Hoefer Isobox unit. Gels were prefocused for 30 min at 13W and run for a further 2 hours at the same setting. Staining was done for 30 min according to Tang and Hart (1975). The stained gels were washed from the glass plates with running water, spread onto white paper and air dried.

2.2.6 Chlorina mutant

The presence of *cn-D1* was scored in a greenhouse during the seedling stages of the B_1F_1:93M167/W311 and B_1F_1:92M166/*1 W311. At approximately 3-4 weeks after planting, the chlorina plants had a light green/yellow appearance in contrast to the dark green colour of normal, chlorofil-producing plants.

2.3 Study of a RWA resistance gene that may have been derived from rye accession, 'Turkey 77'

2.3.1 Monosomic analysis

Marais *et al.* (1994a) reported the transfer of a gene for Russian wheat aphid resistance from the rye accession, 'Turkey 77'. This gene proved to be located on chromosome arm 1RS. Among the wheat-rye hybrid progeny derived in the course of their study, two euploid, RWA resistant plants were found. These were labelled 91M37-7 and 91M37-51, respectively. Following C-banding, no rye chromatin could be detected in the plants. An attempt was therefore made to determine (i) whether the resistance can be the result of a small intercallary translocation involving rye chromatin, and (ii) on which chromosome the gene(s) is located.

In order to determine the chromosomal location of the RWA resistance gene(s) in 91M37-51, a homozygous resistant selection from this cross was hybridized as the male parent to each of the 'CS' monosomics (Sears 1954). Monosomic F_1 plants were identified and raised in a greenhouse. F_2 populations derived from the respective monosomic F_1's were tested for their resistance against the RWA using the damage rating scale described by Du Toit (1987).

2.3.2 Molecular study

In an attempt to verify the presence of rye-chromatin in the RWA resistant lines 91M37-7 and 91M37-51, a RFLP study was conducted with the aid of rye specific dispersed probes.

2.3.2.1 Genetic material

The RWA resistant lines 91M37-7 and 91M37-51, the RWA susceptible cultivars 'CS' and 'Inia 66' and rye (*Secale cereale*) were used in this study. Also used were the cultivar 'Gamtoos' which has the 1BL.1RS ('Veery') translocation and the germplasm lines WRT238-5 (a 3RS.3AS translocation) and 90M128-10-BL (a 3RS.3BL translocation; Marais and Marais 1994). The latter lines served as positive controls for the presence of rye chromatin in a wheat background.

The rye-dispersed probes used included pSc119.1 and pSc119.2 (McIntyre *et al.* 1990) which are subclones of pSc119 (Bedbrook *et al.* 1980a) and pAW173 (Guidet *et al.*1991). The probes pSc119.1 and pSc119.2 were kindly provided by Dr Perry Gustafson (UMC, Columbia, Missouri, USA). They are, respectively, 745 and 611 bp long and ligated in the vector pUC18. The probe pAW173, kindly provided by Dr P. Langridge (Department of Plant Science, Waite Institute, University of Adelaide, Glen Osmond, South Australia, 5064), is 450 bp long and ligated in the vector pUC19.

2.3.2.2 Genomic DNA isolation

Fresh leaves were ground in a preheated 5% CTAB buffer using a mortar and pestle. The technique of Doyle and Doyle (1990) was used to obtain intact genomic DNA that was purified and precipitated according to Sambrook *et al.* (1989).

2.3.2.3 Isolation and labelling of probe DNA

About 20 ng of plasmid DNA was used to transform *E. coli* host JM109 (Promega). Recombinant colonies were selected whereafter plasmid DNA was isolated. Plasmid DNA was digested with HindIII and BamHI, respectively, to isolate the pSc119.1, pSc119.2 and the pAW173 inserts. The fragments were separated in 0.6% agarose gels and the inserts were eluted from gel slices by centrifugation through sterile siliconized glasswool (Heery *et al.* 1990), purified and precipitated according to Sambrook *et al.* (1989).

About 50 ng probe DNA was radioactively labelled with [α^{32}P]-dATP using the random primed DNA labelling kit supplied by Boehringer Mannheim. The labelled probes were separated from unincorporated nucleotides on a column containing Sephadex G50 (Sambrook *et al.* 1989).

2.3.2.4 Southern blots and hybridization

Approximately 10 μg of total plant DNA was digested to completion overnight with the restriction enzyme HindIII. The fragments were separated overnight at 35V in 0.8% agarose gels in TBE buffer (0.045M Tris-HCl pH 8.0, 0.045M boric acid, 0.001M EDTA) and capillary blotted onto a Hybond N^+ membrane (Amersham) with 0.4N NaOH.

The membranes were prehybridized overnight at 65°C in 15 ml buffer of 6x SSC, 0.05M Tris (pH8.0), 0.01M EDTA (pH8.0), 5x Denhardt's, 0.2% SDS and 2.55 mg denatured, sonicated herring sperm DNA. The prehybridization mix was discarded and the membranes were hybridized for 24 hours at 65°C in a prehybridization mix with the inclusion of 10% Dextran sulfate, 300 μg (instead of 2.55 mg) of denatured, sonicated herring sperm DNA and denatured radiolabelled probes (Appels *et al.* 1993).

Following hybridization, the membranes were washed 3 times for 20 min at 65°C in 2x SSC, 0.1% SDS after which they were autoradiographed at -70°C using intensifying screens.

2.3.2.5 Stripping of membranes

Radio-labelled probes were stripped from the membranes by washing the membranes for 30 min at 37°C in 0.2N NaOH, 0.1% SDS and rinsed with 2x SSC. The membranes were sealed and stored at 4°C for further use.

3. RESULTS AND DISCUSSION

3.1 Mapping of the stem rust resistance gene, *Srtau*

3.1.1 Telosomic analysis

The results of the telosomic analysis are summarized in Table 4. It appeared that a total of 19 plants from the test cross involving monotelodisomic 1DS plants and 2 plants from the test cross involving monotelodisomic 1DL plants had chromosome numbers that deviated from the expected 41 + t or 42. These chromosome numbers could have resulted from a degree of non-pairing between certain chromosomes of the parental genomes. R. de V. Pienaar has shown that the 'Inia 66' and 'CS' chromosome complements differ by one, or possibly two, reciprocal translocations (Marais and Marais 1990).

One aneuploid plant in the 1DL progeny proved to be resistant (Table 4). This was most likely not a recombinant plant but rather a misclassification. Recombined genotypes were more frequently observed in the 1DS progeny which would suggest that the stem rust resistance gene, *Srtau*, is located on chromosome arm 1DS (Table 4). It can furthermore be calculated that *Srtau* is linked to the centromere with a recombination frequency of 21 ± 3.40% (141 progeny tested). Refer to Table 4 for the Chi-square analysis of linkage.

3.1.2 Linkage study

The infection types that developed on the parental material following its inoculation with a collection of leaf and stem rust pathotypes prevalent in South Africa, are listed in Tables 5 and 6, respectively. Barring UVPrt2 and UVPrt8, W70 is susceptible to most leaf rust pathotypes. W70 is, however, strongly resistant to all the prevalent stem rust pathotypes (Table 6). This results from the presence of the gene *SrX* in W70. Condor is resistant to stem rust pathotypes UVPgt51 and UVPgt53 only.

W72 is susceptible to all the leaf rust pathotypes and resistant to all the stem rust pathotypes.

This confirms the absence of effective leaf rust resistance genes in the material and the presence of at least one effective stem rust resistance gene.

Table 4. Results of a telosomic analysis of a stem rust resistance gene derived from *T. tauschii*. Expected numbers for free recombination are given in parenthesis.

Chromosome arm	Root tip chromosome count	Number of plants		
		Resistant	Susceptible	Total
1DS	41; 42; 43	64[b] (35.25)	14[a] (35.25)	78 (70.5)
	41 + t; 42 + t	15[a] (35.25)	48[b] (35.25)	63 (70.5)
	Total	79 (70.5)	62 (70.5)	141
1DL	42	46[b]	0[a]	
	40 + t; 41 + t; 42 + t	1[a]	35[b]	

[a] Recombinants.

[b] Parental types.

1DS Chi-square analysis:

Source	Degrees of Freedom (df)	Chi-square (χ^2)	Probability (P)
Resistant vs Susceptible	1	1.60	0.207
Telo vs non-telo	1	2.05	0.152
Linkage	1	48.86	<0.001
Total	3	52.51	<0.001

87M66-2-1 is resistant to all the leaf rust pathotypes tested except UVPrt8 and UVPrt13, and is resistant to all the stem rust pathotypes. Marais *et al.* (1994b) concluded that 87M66-2-1 carries a gene for resistance to pathotype UVPgt50. A F_2 progeny that was supposed to have the pedigree: Condor/W72 proved to be susceptible to the stem rust pathotype UVPgt50. This evidently resulted from the absence of *Sr33* in the F_1 material and it was therefore not possible to confirm that *Sr33* is the only effective resistance gene in W72.

Table 5. Results of the leaf rust resistance tests of the parental lines used in the linkage study.

Genetic material	Leaf rust pathotypes					
	UVPrt2	UVPrt3	UVPrt8	UVPrt9	UVPrt13	UVPrt16
87M66-2-1	1^{cn}	$;1^{cn}$	4	$;1^{+cn}$	3^{+}	$;1^{=cn}$
W70	; — ;1	$2^{+}3$	;2 — $;2^{+}$	$2^{+}3$	$2^{+}3$	$2^{+}3$
W72	3	3^{+}	4	3	3^{+}	3
Condor	3	3	4	3	3^{++}	3

Table 6. Results of the stem rust resistance tests of the parental lines used in the linkage study.

Genetic material	Stem rust pathotypes			
	UVPgt50	UVPgt51	UVPgt52	UVPgt53
87M66-2-1	;	2	$;^{c}$	;
W70	;	;	;	0;
W72	;	;	;	0;
Condor	3^{+}	$;1^{+c}$	2^{+}	;

The presence or absence of *Lr21* in the TF_2:W70/87M66-2-1//Inia 66 populations (20 seedlings each) was easy to score as distinct resistant and susceptible classes were obtained following screening with the leaf rust pathotype UVPrt8 in each TF_2 population. Glume pigmentation associated with the presence of *Rg2*, the gene conferring brown glume colour at maturity, is highly influenced by environmental factors (Kerber and Dyck 1969; Rowland and Kerber 1974). Due to the occurrence of glume blotch and a heavy mildew infection in 1996, it was not possible to unambiguously distinguish populations that had *Rg2* from those without *Rg2*. Linkage

between *Lr21* and *Rg2* on chromosome arm 1DS could therefore not be determined.

The recombination frequency between *Sr33* and *Srtau* cannot be determined accurately as the two genes give similar reactions with the stem rust pathotype UVPgt50 (Table 6). Screening of the TF_1:W72/87M66-2-1//Inia 66 seedlings with the stem rust pathotype UVPgt50 revealed a total of 233 resistant and 11 susceptible plants. The presence of susceptible plants suggests that recombination did occur between these two loci and that *Sr33* and *Srtau* are therefore not allelic. Gametes lacking both *Sr33* and *Srtau* were formed at a frequency of 0.045 ± 0.013. Since this represents half of all recombinants possible, the estimate of linkage is 9 ± 1.9 map units provided gametes and seedlings are equally viable, i.e., there are no other causes of segregation distortion.

Possible linkage between *SrX* and *Sr33* was studied when approximately 20 seedlings each of TF_2:W70/W72//Inia 66 populations were tested for resistance to the stem rust pathotype UVPgt50. Four plants of the F_1:W70/W72 were test crossed with 'Inia 66' and the TF_1 plants were selfed to produce TF_2 families. The results of the linkage study are summarized in Table 7. Twenty-two populations had only resistant plants, however, this is probably due to chance and the small numbers of progeny that were tested in each case (20 seedlings per TF_2 population). One hundred and four populations segregated for resistance, whereas four populations contained only susceptible plants and are believed to be recombinant. The frequency of susceptible F_2's are therefore = 0.03 and the distance between *SrX* and *Sr33* can be estimated as being ± 6 map units.

Approximately 20 seedlings of each TF_2:W70/87M66-2-1//Inia 66 population were tested for resistance to the stem rust pathotype UVPgt50 in order to study the linkage between the stem rust resistance genes *SrX* and *Srtau*. Five plants of the F_1:W70/87M66-2-1 were test crossed with 'Inia 66' and the TF_1 seeds of these plants were kept separately. The results obtained following seedling resistance testing are summarized in Table 8. A total of 7 recombinant populations were encountered out of a total of 140 populations (Table 8), thus a proportion = 0.05. Since this represents only half the recombinants expected, the estimated recombination frequency between *SrX* and *Srtau* is ± 0.10 or 10 map units.

The evidence from these experiments suggest that *SrX*, *Sr33* and *Srtau* are closely linked on 1DS.

Since only half the recombinant gametes could be identified during mapping and relatively small numbers of progeny were involved, the estimated distances should be regarded as approximate and it must be stressed that they are based on the assumption that no other causes of segregation distortion occured. Since a three-point mapping experiment was not possible, the relative positions of the genes could not be determined. However, taking existing map data into consideration (Figure 5) it seems that the most likely order of the genes is: centromere — *Srtau* — *Sr33* — *SrX*.

Table 7. Results of a linkage study involving the stem rust resistance genes *SrX* and *Sr33* in the TF_2:W70/W72//Inia 66 families of 10-20 plants each.

F_1:W70/W72 plant test crossed with 'Inia 66'	Number of TF_2 families			Total
	Resistant	Segregating	Susceptible	
Plant 1	2	14	0	16
Plant 2	1	19	0	20
Plant 3	16	55	1	72
Plant 4	3	16	3	22
Total	22	104	4	130

Table 8. Results of the linkage study between the stem rust resistance genes *SrX* and *Srtau* in the TF_2:W70/87M66-2-1//Inia 66 families of 10-20 plants each.

F_1:W70/87M66-2-1 plant test crossed with 'Inia 66'	Number of populations			Total
	Resistant	Segregating	Susceptible	
Plant 1	1	1	2	4
Plant 2	15	29	3	47
Plant 6	5	48	2	55
Plant 7	2	16	0	18
Plant 8	2	14	0	16
Total	25	108	7	140

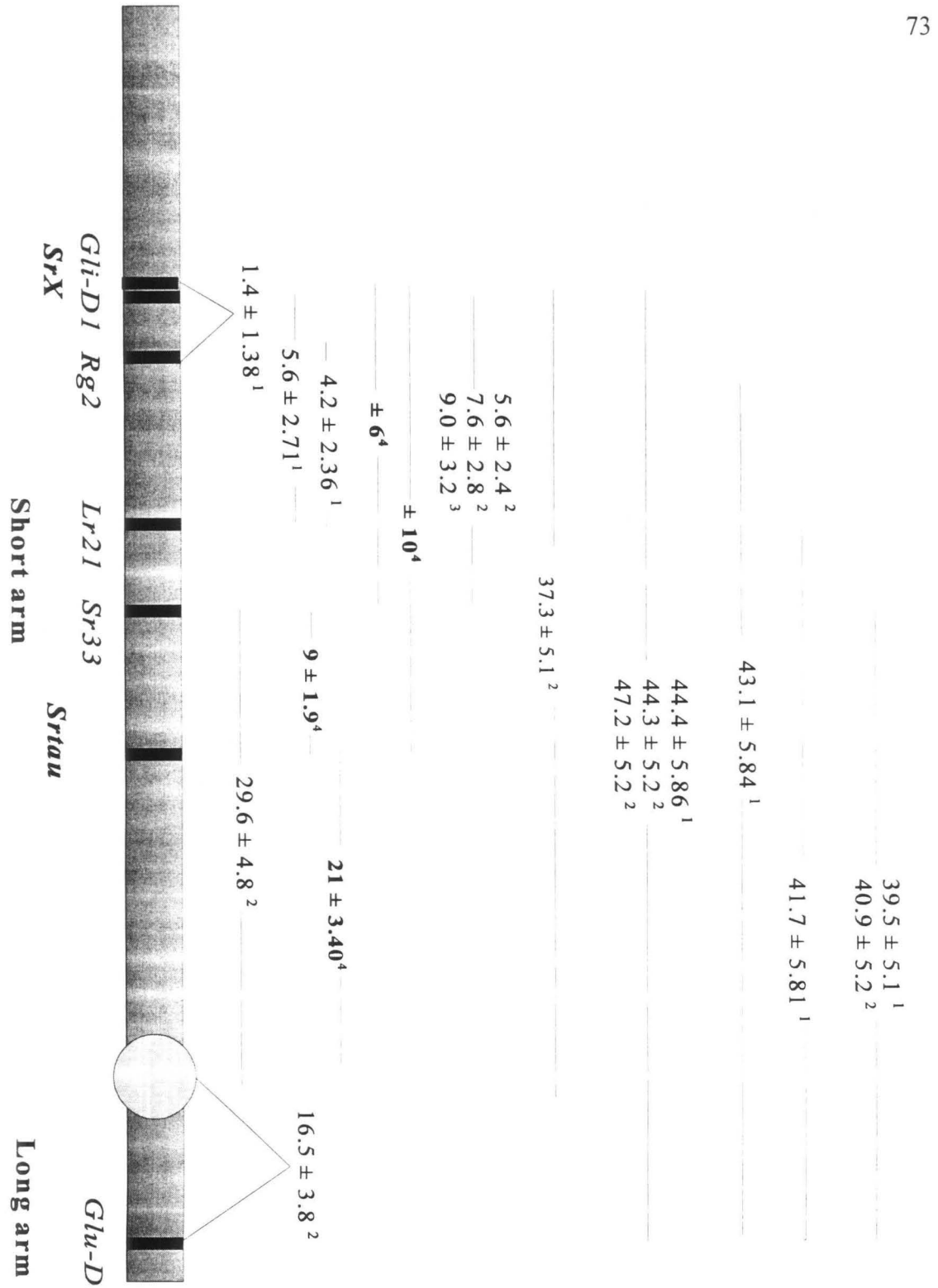

Figure 5. Revised genetic linkage map of chromosome arm 1DS.

[1] Jones *et al.* 1990; [2] Jones *et al.* 1991; [3] Czarnecki and Lukow 1992;

[4] Present study

3.2 Mapping of the Russian wheat aphid resistance gene, *Dn5*

3.2.1 Telosomic analysis

The results of the telosomic analysis are summarized in Table 9. It appeared that one plant from the test cross progeny involving 7DS had 43 chromosomes whereas 4 plants from the test cross progeny involving 7DL had 41 chromosomes. The unexpected chromosome numbers probably resulted from a degree of non-pairing between certain chromosomes of the parental genomes. R. de V. Pienaar has shown that the 'Inia 66' and 'CS' chromosome complements differ by one and possibly two, reciprocal translocations as reported by Marais and Marais (1990). The source of the 7DL telosome used in the study was a segregate from a 'Chinese Spring ditelo 7DL monotelo 7DS'/'Inia 66 cross'.

In the crosses involving chromosome arm 7DS, both the resistance and the telosomic condition segregated normally in the monotelodisomic female parent. However, in the 7DL material the susceptible condition was transmitted preferentially for both the parental type chromosomes and the recombinant chromosomes (86 vs 31). Parental types with the telosome were recovered more frequently than parental types having the normal chromosome (31 vs 16), thus, it is unlikely that the loss of 7DS *per se* reduced the viability of the gametes significantly. It would therefore seem that the chromosome region in 'CS' that is homologous to, or occurs opposite to *Dn5* and its surrounding chromatin, was transmitted preferentially. Preferential transmission of chromosome regions is well documented in the Triticeae (Marais 1992b), an example of which is the *Lr19* translocation. The latter translocation, derived from *Thinopyrum ponticum*, is homoeologous to 7DL of wheat and is transmitted preferentially in hybrids with many wheat genotypes (Marais 1992b). The mechanism that results in segregation distortion appears to have a complex, multigenic basis (Marais 1992c). *Dn5* was identified in a landrace (SA 463 = PI 294994) that originated in Bulgaria (Du Toit 1988). Parent 'W574' used in the mapping experiment has the pedigree SA 463/*6 Palmiet. In this material, *Dn5* is therefore probably associated with considerable SA 463 chromatin that was retained as a result of linkage drag.

Regarding the 7DS progeny, 3 plants that were euploid, yet apparently susceptible, were obtained

(Table 9). We believe that they were not recombinants but rather instances of misclassification that resulted from weak seedlings. Recombined genotypes occurred frequently among the 7DL-derived progeny which would suggest that *Dn5* is located on chromosome arm 7DL (Table 9). A recombination frequency of 60 ± 4.53% between *Dn5* and the centromere was calculated suggesting the absence of linkage.

Table 9. Results of the telosomic analysis of the Russian wheat aphid resistance gene, *Dn5*. Expected numbers for free recombination are given in parenthesis.

Chromosome arm	Root tip chromosome count	Number of plants		
		Resistant	Susceptible	Total
7DS	41 + t	0[a]	50[b]	
	42, 43	50[b]	3[a]	
7DL	41 + t	15[a] (29.25)	31[b] (29.25)	46 (58.5)
	41, 42	16[b] (29.25)	55[a] (29.25)	71 (58.5)
	Total	31 (58.5)	86 (58.5)	117

[a] Recombinants.

[b] Parental types.

7DL Chi-square analysis:

Source	Degrees of Freedom (df)	Chi-square (χ^2)	Probability (P)
Resistant vs Susceptible	1	25.8547	<0.001
Telo vs non-telo	1	5.3419	0.021
Linkage	1	4.5214	0.033
Total	3	35.7179	<0.001

3.2.2 Identification of endopeptidase isozymes

Diagrammatic and photographic representations of the endopeptidase bands produced by 'Chinese Spring', 'SA 463', 'VPM-1', 'Inia 66' and 'W84-17' are given in Figures 6 and 7. Bands 1—4, and 7 were faint in all the material studied, while band 8 was faint only in 'CS'. All the other bands were of the same intensities and very easy to distinguish. In order to assign these bands to their respective positions, mixtures were made of embryos, i.e. 'Inia 66' with 'SA 463'; 'CS' with 'SA 463' and 'SA 463' with 'VPM-1'. These mixtures were extracted and run together with the parental material and the relative positions of the endopeptidase bands were deduced from the zymograms (Data not shown).

Hart and Langston (1977) observed four endopeptidase bands in the zymogram of 'CS' which they designated as EP-D1, EP-A1, EP-B1 and EP1. Koebner *et al.* (1988) screened a range of wheat varieties and detected three alleles at the *Ep-A1* locus, five at the *Ep-B1* locus and three at the *Ep-D1* locus. McMillin *et al.* (1993) reported a fourth *Ep-D1* (null) allele. The latter endopeptidase allele is linked to the leaf rust resistance gene *Lr19* and they designated it *Ep-D1d*. The relationship of the endopeptidase bands observed in this study to the bands named by Koebner *et al.* (1988) and McMillin *et al.* (1986) can be seen in Figure 6. The identities of the bands in 'CS' and 'Inia 66' were deduced from the data of Marais and Marais (1990). 'W84-17' proved to produce identical polymorphisms to 'Inia 66' at the three homoeoloci. Like 'Inia 66' and 'W84-17', 'VPM-1' also has the *Ep-A1b* allele, however, it produces *Ep-D1b* instead of *Ep-D1a* (McMillan *et al.* 1986). *Ep-D1b* in 'VPM-1' focuses very close to *Ep-B1a* in 'CS' (Figure 6). Band 8 of 'SA 463' cofocuses with *Ep-B1c* in 'CS', 'Inia 66' and 'W84-17', but it appears to be encoded by a homoeoallele on a different chromosome. 'SA 463' produces a further unknown polymorphism (band 9). An attempt was therefore made to assign the two unknown alleles in 'SA 463' to the group 7 chromosomes by monosomic analysis.

In order to determine the chromosome locations of the genes encoding bands 8 and 9 of 'SA 463', F_2 monosomic analyses involving chromosomes 7A, 7B and 7D were performed. The F_2 results are given in Table 10. Bands 6 and 10 of 'CS' and bands 8 and 9 of 'SA 463' were expressed consistently and were easy to study in the plants tested. Other bands were also encountered, but

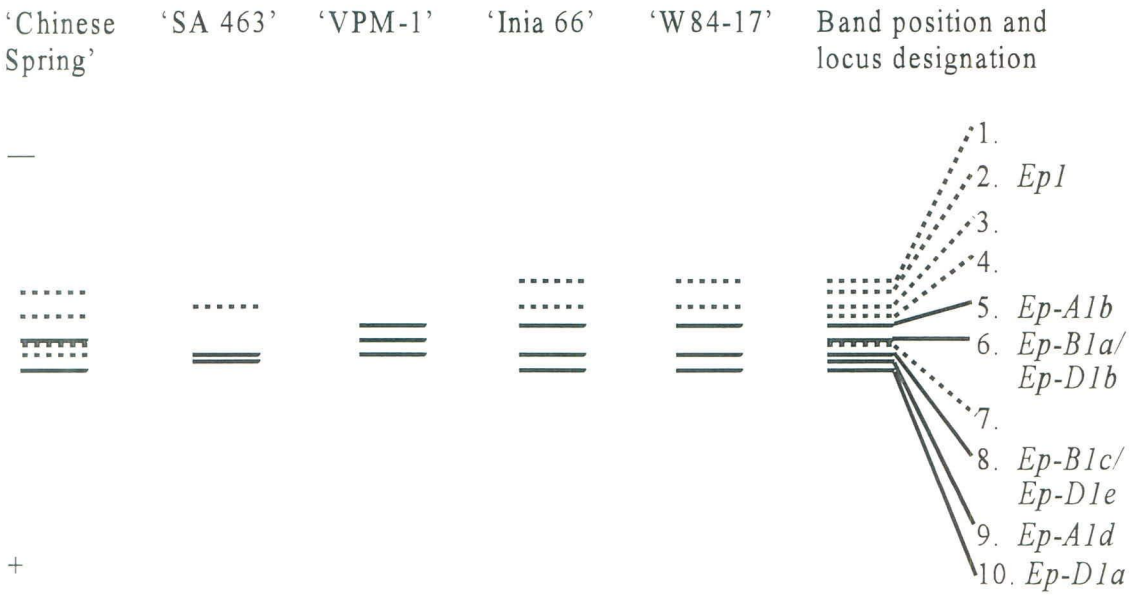

Figure 6. Relative positions of the endopeptidase bands encountered in the linkage and segregation studies.

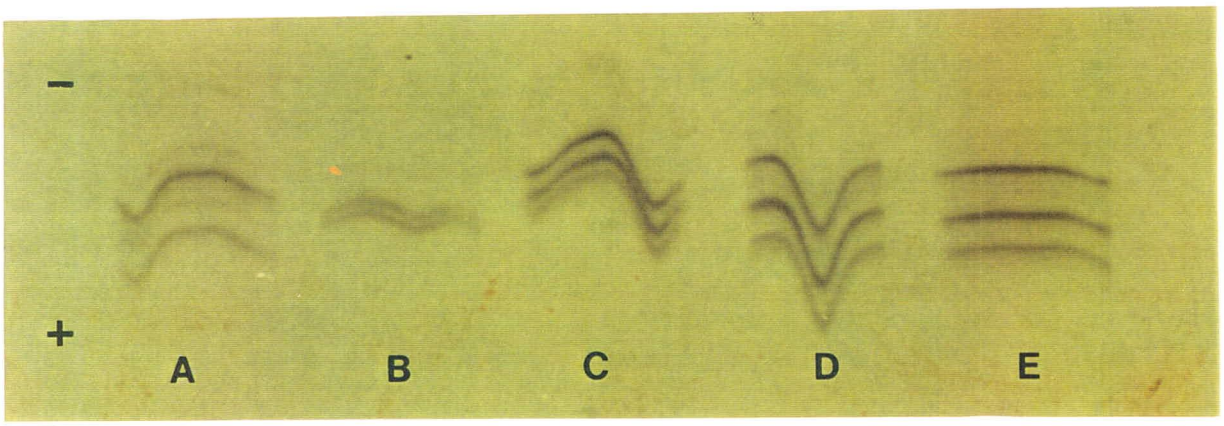

Figure 7. Expression of endopeptidases in embryos. **A**: 'Chinese Spring', **B**: 'SA 463', **C**: 'VPM-1', **D**: 'Inia 66', **E**: 'W84-17'

Table 10. Segregation of endopeptidase isozymes in the F_2 derived from monosomic F_1 plantsof the crosses: CSM7A/SA 463, CSM7B/SA 463 and CSM7D/SA 463.

F_2 population	Number of individuals having endopeptidase bands									
	6,9,10	6,8,10	6,8,9,10	6,8,9	8,9,10	8,10	6,8	6,9	8,9	8
CSM 7A/SA 463	3	1	5	2	1	-	-	-	-	-
CSM 7B/SA 463	4	1	-	-	3	2	1	-	-	-
CSM 7D/SA 463	-	-	-	13	-	-	4	1	2	2

their low intensities and irregular expression did not allow consistent detection. These included bands in positions 5 and 7 that were sometimes observed in the F_2:CSM 7B/SA 463 population. Some endopeptidases appear to be expressed less regularly (Marais and Marais 1990).

Band 8 of 'SA 463' appears to co-segregate with band 10 (*Ep-D1a*) (Table 10) and is here designated as *Ep-D1e* as it evidently represents a novel allele. When the monosomic F_1 derived F_2 data for chromosomes 7A and 7B are pooled, the following phenotypic classes are observed: *Ep-D1e* (3): *Ep-D1a*/*Ep-D1e* (13): *Ep-D1a* (7) [$\chi^2_{1:2:1} = 1.78$, P = 0.411]. In the monosomic F_1 of the cross CSM7D/SA 463, *Ep-D1e* occurred on the monosomic chromosome. Consequently, the F_2 of the latter cross, *Ep-D1e* was expressed by 21 plants and was absent in 1 plant. The latter plant was probably nullisomic for chromosome 7D and comprises 4.54% of the population of 22 plants which is in accord with the findings of Sears (1954) and Kuspira and Unrau (1959).

Band 9 of 'SA 463' does not co-segregate with band 6 (*Ep-B1a*) of 'CS' (or with bands 8 and 10) and is probably an *Ep-A1* allele. In the pooled F_2 derived from the monosomic F_1's: CSM7B/SA 463 and CSM7D/SA 463, band 9 was expressed by 23 embryos whereas 10 embryos exhibited the *Ep-A1* null allele [$\chi^2_{3:1} = 0.49$, P = 0.484]. In the F_2 derived from monosomic F_1:CSM7A/SA 463, 11 plants expressed band 9 while 1 plant lacked it. The latter plant was probably nullisomic for chromosome 7A. Band 9 does not correspond to any of the alleles described in the gene catalogue and is therefore designated as *Ep-A1d*.

In the F_2 progeny of cross CSM7B/SA 463, *Ep-B1a* does not show monosomic inheritance. Band 6 was expressed by 6 embryos whereas 5 embryos exhibited the *Ep-B1* null allele. This may be the result of (i) false identification of the F_1 monosome, (ii) monosomic shift as described by Person (1956) or (iii) stronger expression of a normally faintly expressed or null allele as was observed by Marais and Marais (1990).

3.2.3 Linkage study

Endopeptidase bands were obtained for the B_1F_1:92M166/*1 W311 in which it was attempted to determine the recombination frequency between *Ep-D1* and *cn-D1*. An attempt was made to characterize the progeny for segregation of the *Ep-D1a* and *Ep-D1b* bands. It was found, however, that *Ep-D1b* ('VPM-1') and *Ep-B1a* ('W311') could not be distinguished because of their very similar isoelectric points and classification of the progeny was therefore impossible (Figure 6). The linkage between *Ep-D1* and *cn-D1* could therefore not be calculated. The absence or presence of the *cn-D1* loci was, however, easy to score as the chlorina and normal plants contrasted in plant colour. McIntosh *et al.* (1976) reported a recombination frequency of 0.4% between *cn-D1* and *Lr19* on chromosome 7DL. McMillin *et al.* (1993) similarly reported very tight linkage between *Ep-D1d* and *Lr19* with a recombination value of 1.0 ± 0.86%. However, their data are misleading as the *Lr19* translocation encompasses almost the total length of chromosome arm 7DL and does not pair with wheat chromatin during meiosis.

Linkage between *Dn5* and *Ep-D1* was studied in the TF_2:93M168/Inia 66 and TF_2:93M168/W84-17 families. RWA seedling resistance tests revealed two classes, i.e., those segregating for resistance and those having only susceptible plants. The presence or absence of *Dn5* could therefore be easily determined. Endopeptidase zymograms of the TF_1:93M168/Inia 66 and TF_1:93M168/W84-17 revealed easily identifiable bands, and the absence or presence of the *Ep-D1* alleles was easy to score. The results of the linkage study are summarized in Table 11 and show that *Dn5* and *Ep-D1* are linked with a recombination frequency of 32 ± 4.97%. In the telocentric mapping experiment of *Dn5*, its 'CS' allele was transmitted preferentially. 93M168 has the pedigree: SA 463/*6 Palmiet//VPM-1/*3 W84-17. The data of Table 11 (39 TF_2 families segregating for resistance and 49 TF_2 families susceptible to RWA) are indicative of a lessened

or no segregation distortion effect ($\chi^2_{1:1}$ = 1.14, P = 0.286) which may relate to a different *Dn5* allele or a different genetic background effect (Nkongolo *et al.* 1990a). A significant distortion in *Ep-D1b* segregation (55 TF_2 families segregating for *Ep-D1b* and 33 TF_2 families without *Ep-D1b*) was experienced ($\chi^2_{1:1}$ = 5.50, P = 0.019) as seen in Table 11.

Table 11. Results of the linkage study between the genes *Dn5* and *Ep-D1*. Expected numbers for free recombination are given in parenthesis.

Genotype		Number of plants
Dn5	*Ep-D1b*	
+	—	22[a] (22)
—	+	38[a] (22)
—	—	11[b] (22)
+	+	17[b] (22)
		Total plants = 88

\+ Presence of loci; — Absence of loci

[a] Parental types; [b] Recombinants

Chi-sqaure analysis for linkage study:

Source	Number of times loci		Degrees of	Chi-square	Probability
	Present	Absent	Freedom (df)	(χ^2)	(P)
Dn5	39	49	1	1.1364	0.286
Ep-D1b	55	33	1	5.500	0.019
Linkage	-	-	1	11.6364	0.001
Total	-	-	3	18.2728	<0.001

Screening of the B_1F_1:93M167/W311 seedlings for the presence of the *cn-D1* loci gave distinct classes of normal and chlorina plants. RWA seedling resistance tests on the B_1F_2:93M167/W311 families also revealed distinct classes regarding the presence or absence of *Dn5*. A total of 24 plants had the chlorina mutant phenotype opposed to the 35 plants with a normal phenotype ($\chi^2_{1:1}$ = 2.05, P = 0.152). As the chlorina mutant phenotype is the result of a deletion, its lower transmission to the test cross progeny was expected. The segregation of RWA resistant:susceptible plants was on the other hand 43:16 and also deviated from the expected 1:1 ratio ($\chi^2_{1:1}$ = 12.36, P < 0.001). Serious distortion in the *Dn5* segregation was therefore experienced. This result is in contrast with the telocentric mapping experiment in which the transmission of *Dn5* was impaired. It is possible that the transmission of *Dn5* is also affected by genetic factors in the deletion area. The results of the linkage study between *Dn5* and *cn-D1* are summarized in Table 12 and show that *Dn5* and *cn-D1* are linked with a recombination frequency of 37 ± 6.30%.

Recombination frequencies of 60 ± 4.53% between *Dn5* and the 7DL centromere, 32 ± 4.97% between *Dn5* and *Ep-D1* and 37 ± 6.30% between *Dn5* and *cn-D1* were derived in this study. These recombination frequencies were integrated with existing map data for 7DL to produce Figure 8.

Table 12. Results of the linkage study between the genes *Dn5* and *cn-D1*. Expected numbers for free recombination are given in parenthesis.

Genotype		Number of plants
Dn5	*cn-D1*	
+	—	28[a] (14.75)
—	+	9[a] (14.75)
—	—	7[b] (14.75)
+	+	15[b] (14.75)
		Total plants = 59

+ Presence of loci; — Absence of loci

[a] Parental types; [b] Recombinants

Chi-sqaure analysis for linkage study:

Source	Number of times loci		Degrees of	Chi-square	Probability
	Present	Absent	Freedom (df)	(χ^2)	(P)
Dn5	43	16	1	12.3559	<0.001
cn-D1	24	35	1	2.0508	0.152
Linkage	-	-	1	3.8135	0.051
Total	-	-	3	18.2202	<0.001

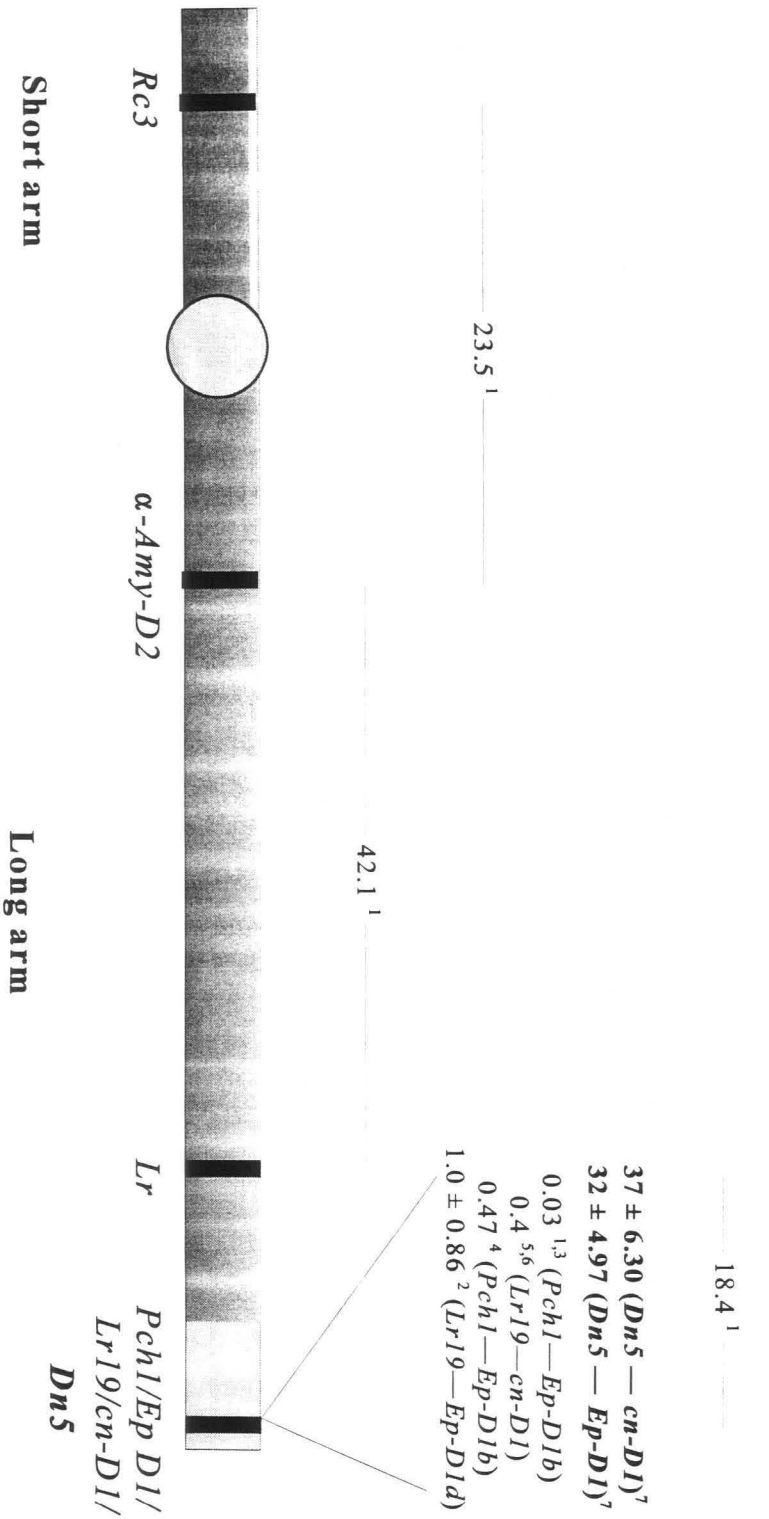

Figure 8. Revised genetic linkage map of chromosome arm 7DL.

[1] Worland et al. 1988; [2] McMillin et al. 1993; [3] Law et al. 1988;

[4] Summers et al. 1988; [5] McIntosh et al. 1976; [6] Marais and Marais 1990;

[7] Present study

3.3 Study of a Russian wheat aphid resistance gene that may have been derived from 'Turkey 77'

3.3.1 Monosomic analysis

The F_2 results of the monosomic analysis of a RWA resistance gene present in 91M37-51 are given in Table 13. As the total data for non-critical families conform to a 3:1 segregation ratio (1611 resistant: 494 susceptible $\chi^2_{3:1} = 2.64$, $P = 0.104$), it is concluded that 91M37-51 carries a single dominant RWA resistance gene. Two susceptible plants out of a total of 107 plants were encountered in the monosomic chromosome 7D F_2 population. Chromosome 7D is therefore identified as carrying the resistance gene. These two plants were probably nullisomic for chromosome 7D and comprise 1.87% of the total number of plants.

3.3.2 Molecular study

The probe pSc119.1 produced distinct bands when hybridized to HindIII digested genomic DNA (Figure 9). No differences were observed between the resistant and susceptible material tested. McIntyre *et al.* (1990) reported that pSc119.1 hybridizes to the entire length of all the rye chromosomes, except some telomeres and the nucleolar organiser region. It also hybridizes specifically to rye-chromatin and cross-hybridizes only weakly to the centromeres of a few wheat chromosomes. This probe could therefore be useful for the detection of transferred rye chromosome fragments in wheat.

Probe pSc119.2 gave very faint bands when hybridized to HindIII digested genomic DNA. Much background was observed, despite the precautions taken. The membranes were washed more stringently using a 0.5x SSC, 0.1% SDS solution for 20 min at 65°C. The bands subsequently observed did not show any differences between the resistant and susceptible material tested (Figure 10). McIntyre *et al.* (1990) reported that pSc119.2 hybridizes predominantly to the telomeric regions of rye chromosomes and it also assays repetitive sequences in wheat chromosomes. Cuadrado and Jouve (1995) showed that the FISH hybridization sites of pSc119.2 are similar to the C-banding pattern of *S. montanum* chromosomes and that the FISH karyotypes

Table 13. Results of the monosomic analysis of the Russian wheat aphid resistance gene present in 91M37-51.

Monosomic chromosome	Number of F_2 plants		χ^2 (P) [a]
	Resistant	Susceptible	
1A	84	25	0.2477 (0.619)
2A	77	26	0.0032 (0.955)
3A	77	30	0.5868 (0.444)
4A	55	50	28.6508 (<0.001)
5A	73	33	1.5977 (0.206)
6A	80	29	0.8349 (0.361)
7A	101	8	18.1315 (<0.001)
1B	75	32	1.3738 (0.241)
2B	60	18	0.1538 (0.695)
3B	85	22	1.1246 (0.289)
4B	86	16	4.7190 (0.030)
5B	83	23	0.6164 (0.432)
6B	79	31	0.5939 (0.441)
7B	93	17	5.3455 (0.021)
1D	81	21	1.3612 (0.243)
2D	80	25	0.0794 (0.778)
3D	100	9	16.2966 (<0.001)
4D	81	21	1.0588 (0.303)
5D	77	32	1.1040 (0.293)
6D	84	26	0.1091 (0.741)
7D	105	2	30.5327 (<0.001)
Total	1164	494	2.635 (0.104)

[a] Probability that the observed data conform to a 3 : 1 segregation ratio.

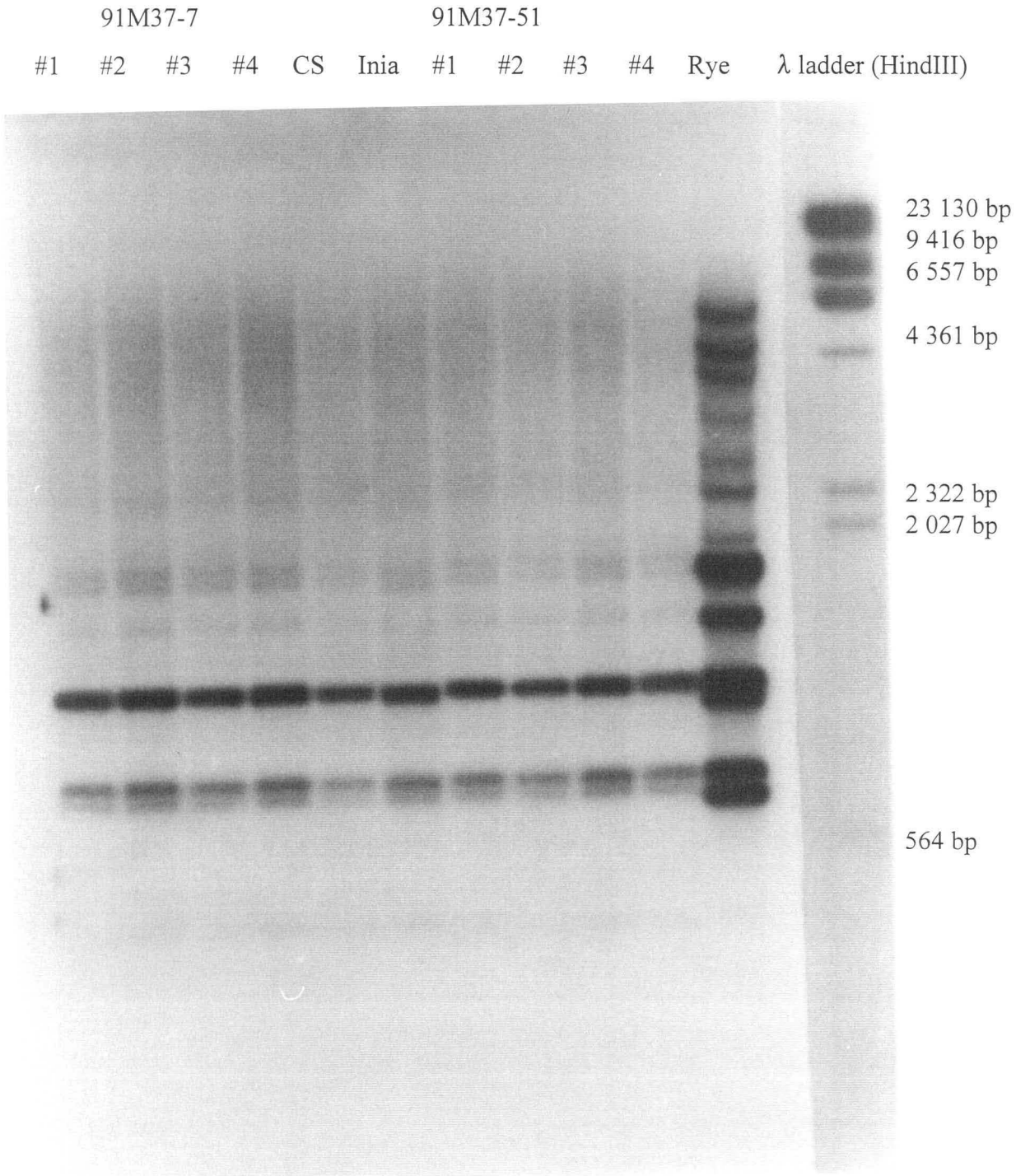

Figure 9. Autoradiogram of HindIII digested genomic DNA hybridized with radio-labelled pSc119.1.

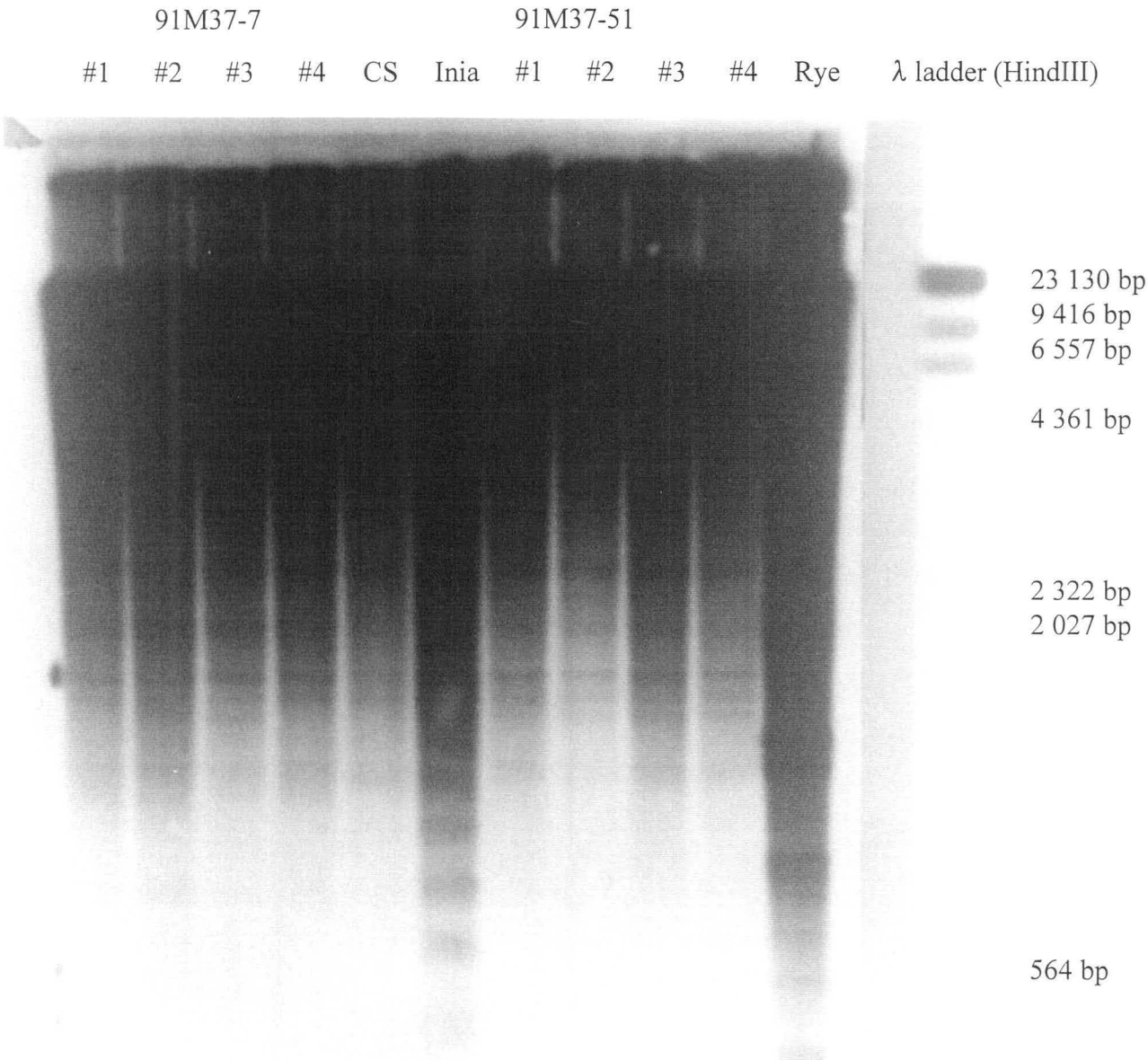

Figure 10. Autoradiogram of HindIII digested genomic DNA hybridized with radio-labelled pSc119.2.

of *S. cereale* and *S. montanum* are similar. pSc119.2 will therefore only reveal rye chromosome fragments in wheat that coincide with rye C-band staining regions. pSc119.2 may therefore be less suited for the purpose of the study and the lack of polymorphisms is not unexpected as C-banding did not reveal the presence of rye chromosome fragments in 91M37-51 (Marais *et al.* 1994a).

No differences between the RWA resistant and susceptible material were observed when pAW173 was hybridized to HindIII digested genomic DNA (Figure 11). Probe pAW173 hybridized very strongly to the rye genomic DNA illustrating the high specificity of this probe to rye DNA. The probe pAW173 is a member of the R173 family and Guidet *et al.* (1991) reported that approximately 15 000 copies of this R173 repeated sequence are present per rye genome, explaining the strong signals observed when pAW173 hybridized to rye DNA.

pAW173 was hybridized to HindIII digested genomic DNA of 'Gamtoos', WRT238-5 and 90M128-10-BL. Despite the fact that the transfer of the digested DNA from the gel to the membrane was not good and that 'Inia 66', the negative control, was not digested at all, hybridization with pAW173 did occur. It seems, however, as if no polymorphisms occured between the different rye translocation material and negative control (Figure 12). Ten μg of digested genomic DNA was loaded per gel slot. The use of less genomic DNA and longer running times for the gel will probably be better to see if polymorphisms did occur. This shows that probe pAW173 probably does not identify the presence of sizeable blocks of rye-chromatin transferred to wheat. Small rye chromosome fragments transferred to wheat will therefore also be difficult to detect by RFLP analyses.

The monosomic analysis of the RWA resistance gene present in 91M37-51 revealed chromosome 7D as the location of a single dominant gene. The molecular study with rye-dispersed probes did not detect the presence of rye chromatin in the RWA resistant lines 91M37-7 or 91M37-51. Schroeder-Teeter *et al.* (1990, 1991) mapped the RWA resistance gene, *Dn1*, on chromosome 7D of common wheat, while Marais and Du Toit (1993) mapped the RWA resistance gene, *Dn5*, also on chromosome 7D. The possibility therefore exists that the RWA resistance gene in 91M37-7

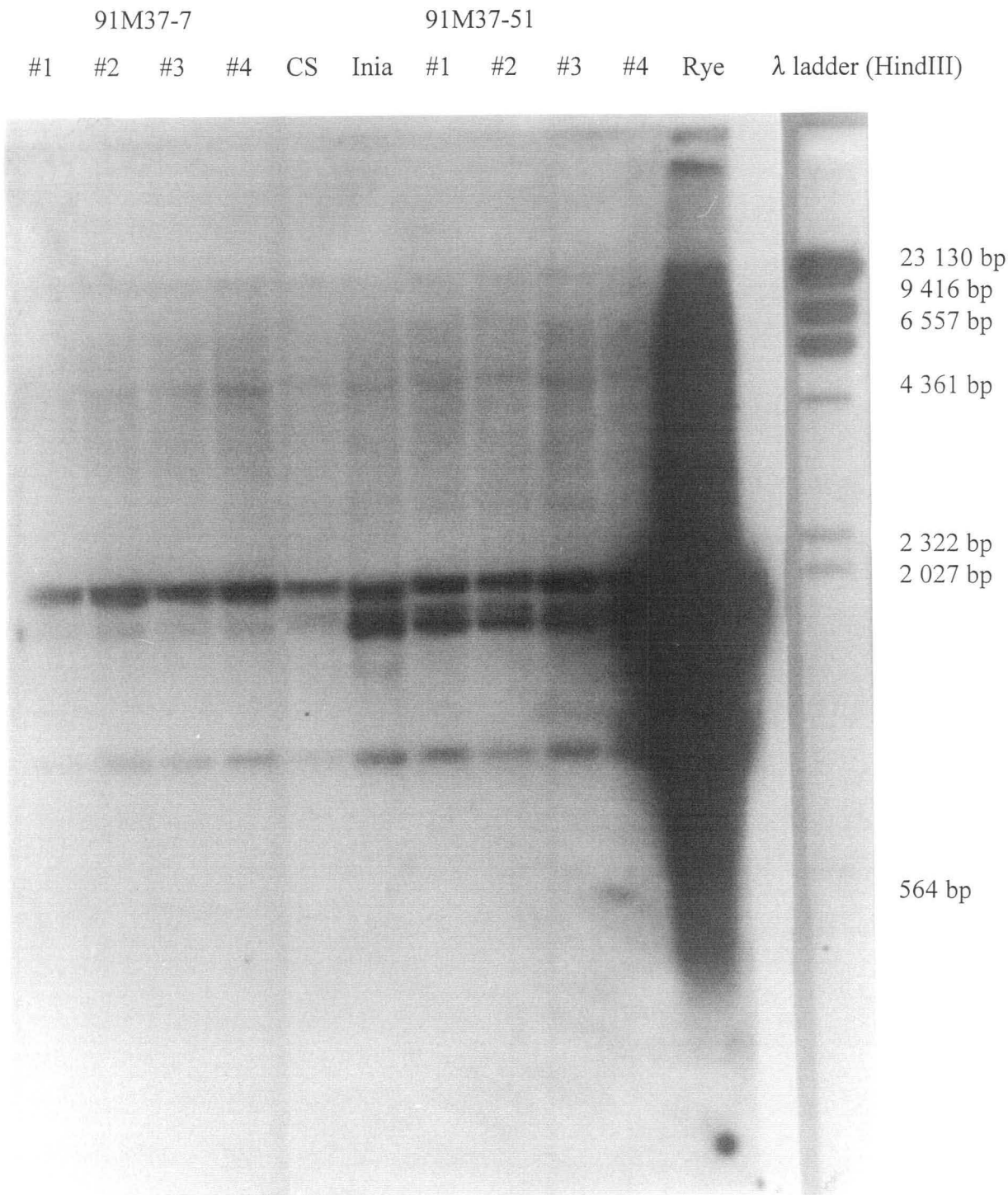

Figure 11. Autoradiogram of HindIII digested genomic DNA hybridized with radio-labelled pAW173.

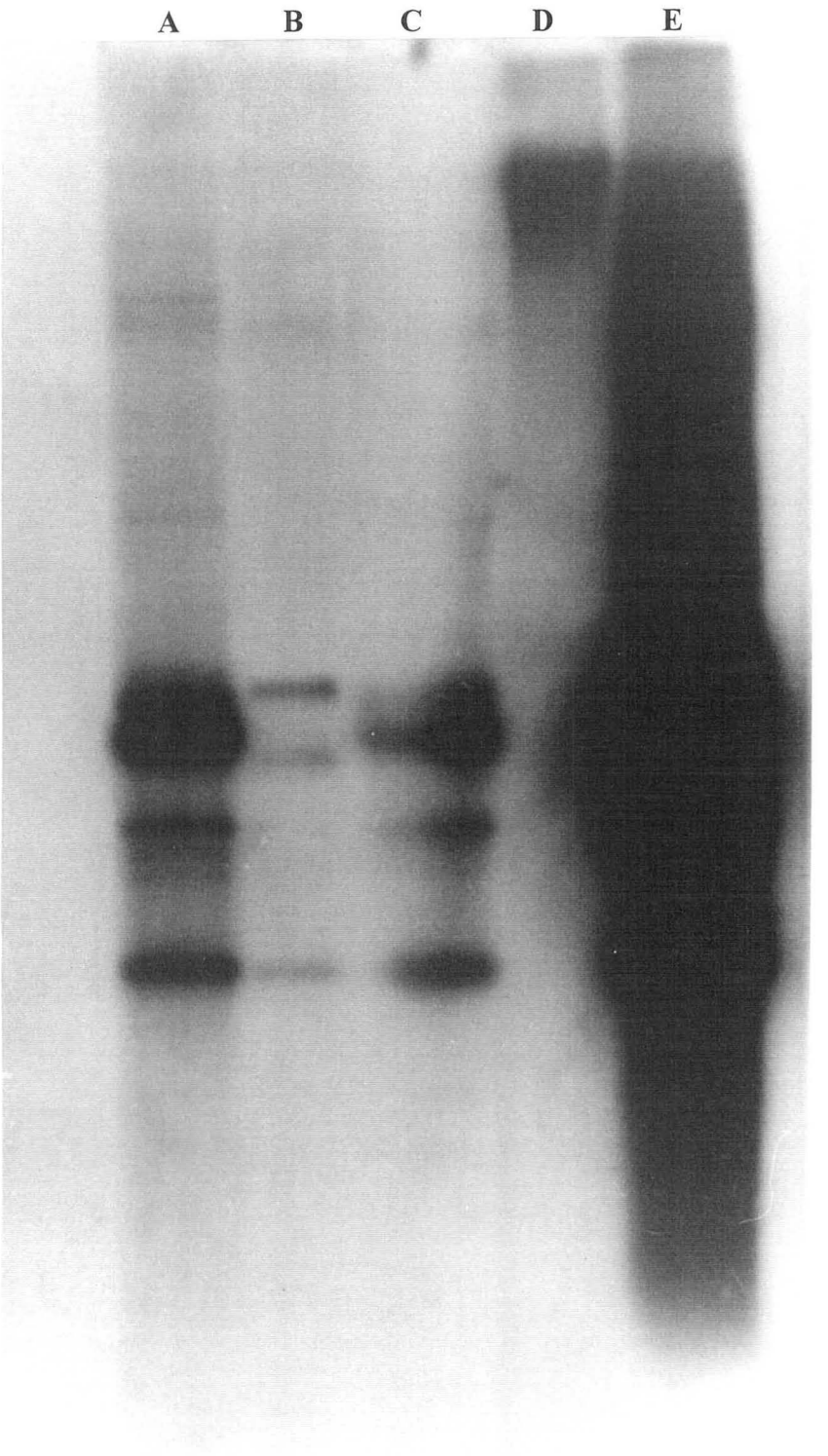

Figure 12. Autoradiogram of HindIII digested genomic DNA of **A**: 'Gamtoos'; **B**: 'WRT238-5'; **C**: '90M128-10-BL'; **D**: 'Inia 66'; **E**: 'Rye' hybridized with radio-labelled pAW173.

and 91M37-51 could have resulted from cross pollination of secondary amphiploids during an attempt to transfer a RWA resistance gene on 1RS of 'Turkey 77' rye to wheat (Marais *et al.* 1994a). The pollen could have been contributed by wheat plants having either *Dn1* or *Dn5* that were used in a monosomic study (Marais, personal communication). In order to investigate this possibility, crosses will have to be made between the RWA resistant lines 91M37-7, 91M37-51 and lines having either *Dn1* or *Dn5*.

4. SYNTHESIS OF MAJOR CONCLUSIONS

- A telosomic analysis has shown that the stem rust resistance gene, *Srtau*, is located on chromosome arm 1DS. *Srtau* is linked to the centromere with a recombination frequency of 21 ± 3.40%.

- The occurrence of glume blotch and a heavy mildew infection in 1996 made it impossible to distinguish plants in the TF_2:W70/87M66-2-1//Inia 66 populations that had *Rg2*, the gene conferring brown glume colour at maturity, from those without *Rg2*. Linkage between *Lr21* and *Rg2* on chromosome arm 1DS could therefore not be determined.

- *Sr33* and *Srtau* give similar reactions to the stem rust pathotype UVPgt50. The distance between these two genes was estimated as being 9 ± 1.9 map units and *Sr33* and *Srtau* are therefore not allelic. The linkage estimate was based on the presence of susceptible TF_1:W72/87M66-2-1//Inia 66 seedlings when screened with the stem rust pathotype UVPgt50.

- The occurrence of TF_2:W70/W72//Inia 66 populations consisting of susceptible seedlings only (when screened against the stem rust pathotype UVPgt50) suggested that recombination did occur between *SrX* and *Sr33*. The estimated recombination frequency between these two genes was calculated as being ± 6 map units.

- The estimated recombination frequency between *SrX* and *Srtau* is ± 10 map units. This estimate is based on the presence of only susceptible seedlings in 7 TF_2:W70/87M66-2-1//Inia 66 populations when these TF_2 populations were screened for resistance to the stem rust pathotype UVPgt50.

- The linkage data of this study suggest that *SrX*, *Sr33* and *Srtau* are closely linked on chromosome arm 1DS. The most likely order of the genes, taking existing map data into consideration, is : centromere — *Srtau* — *Sr33* — *SrX* (Figure 5).

- A telosomic analysis has shown that the Russian wheat aphid resistance gene, *Dn5*, is located on chromosome arm 7DL. A recombination frequency of 60 ± 4.53% between *Dn5* and the centromere suggested the absence of linkage.

- The RWA resistant line 'SA 463' produced 2 endopeptidase polymorphisms that differ from the alleles described in the gene catalogue (Figure 6). F_2 monosomic analyses of bands 8 and 9 encountered in 'SA 463' were performed (Table 10). Band 8 co-segregates with band 10 (*Ep-D1a*) and the designation *Ep-D1e* is suggested. Band 9 of 'SA 463' on the other hand appears to be an *Ep-A1* allele and the designation *Ep-A1d* is suggested.

- Linkage between *Ep-D1b* and *cn-D1* could not be calculated as the endopeptidase bands *Ep-D1b*, encountered in 'VPM-1', and *Ep-B1a*, encountered in 'W311', could not be distinguished because of their similar isoelectric points.

- Recombination occurred between *Dn5* and *Ep-D1* in the TF_2:93M168/Inia 66 and TF_2:93M168/W84-17 families and it was estimated that these 2 genes are linked with a recombination frequency of 32 ± 4.97%.

- *Dn5* and *cn-D1* are linked with a recombination frequency of 37 ± 6.30%.

- Recombination frequencies encountered in this study were integrated in the existing map data for chromosome arm 7DL (Figure 8).

- A monosomic analysis of the RWA resistance gene present in 91M37-51 showed that a single dominant RWA resistance gene occurs on chromosome 7D.

- A RFLP study with rye specific dispersed probes was conducted in order to verify the presence of rye-chromatin in the RWA resistant lines 91M37-7 and 91M37-51. Neither of the rye specific dispersed probes used in this study showed polymorphisms between the negative controls and the RWA resistant lines 91M37-7 and 91M37-51. It was concluded that the RWA resistance gene in 91M37-7 and 91M37-51 could have resulted

from cross pollination by wheat plants having either *Dn1* or *Dn5*, known to be located on chromosome arm 7DL.

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