TRANSFER OF GENETIC RESISTANCE TO THE RUSSIAN WHEAT APHID FROM RYE TO WHEAT

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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:

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OPSOMMING

'n Oktaploïede triticale is gemaak vanaf die F_1 van 'n kruising tussen 'n Russiese koringluis-weerstandbiedende rog, 'Turkey 77', en die koringkultivar 'Chinese Spring'. Die alloploïed is gekruis met gewone broodkoring en met 'Imperial' rog/'Chinese Spring' disomiese addissielyne. Die F_2 nageslag vanaf hierdie kruisings is getoets vir Russiese koringluis-weerstandbiedendheid en C-bande is ook gedoen. Weerstand is gevind wat geassosieer is met die 1RS chromosoomarm van 'Turkey 77'. Hierdie oorspronklike werk is deur MARAIS (1991) gedoen en uit sy materiaal is 'n monotelosomiese 1RS ('Turkey 77') addissieplant beskikbaar gestel vir die huidige studie. Die F_3 nageslag van hierdie monotelosomiese addissieplant is gebruik om die weerstand teen die Russiese koringluis op chromosoom 1RS te bevestig. Die monotelosomiese addissieplant is ook gekruis met die koringkultivar 'Gamtoos' wat die 1BL.1RS-translokasie dra. Hoewel die 1RS segment van 'Gamtoos' die roesweerstandsgene, *Sr31* en *Lr26* uitdruk, is dit nie die geval met die 'Turkey 77' 1RS telosoom nie. Hierdie gene kon dus as merkergene gebruik word. Vanuit die F_1 is 'n monotelosomiese 1RS addissieplant geselekteer wat ook heterosigoties was vir die 1BL.1RS-translokasie. Hierdie plant is getoetskruis met 'n luisvatbare gewone broodkoring, 'Inia 66'. Meiotiese paring tussen die rogarms het daartoe gelei dat vyf euploïede Russiese koringluis-weerstandbiedende nageslag uit 99 euploïede nageslag geselekteer kon word. Een rekombinant het ook *Sr31* en *Lr26* behou en is toegelaat om self te bestuif. Met behulp van SDS-PAGE profiele is Russiese koringluis-weerstandbiedende 1BL.1RS-translokasie homosigote geïdentifiseer en kon bevestig word dat die weerstandsgeen vir die Russiese koringluis oorgedra is na die 1BL.1RS ('Veery') -translokasie.

Twee strategieë is gevolg om die Russiese koringluislokus of -loci te karteer: (1) 'n Telosomiese analise is gedoen. 'n Plant met 2n = 40 + 1BL.1RS + 1RS is verkry en met 'n luisvatbare koring bestuif. (2) 'n Gerekombineerde,

disomiese plant met Russiese koringluis-weerstandbiedendheid maar sonder die *Lr26* en *Sr31* allele is gekruis met 'Gamtoos' en die F_1 getoetskruis. Die toetskruisouer in beide die strategieë was 'Chinese Spring'. In die eerste eksperiment is die *Sr31*-lokus 10.42 kaarteenhede vanaf die *Lr26*-lokus gelokaliseer. Die roesdata het geïmpliseer dat onbetroubare genetiese kaarteenhede geskat sou word en daarom is die omslagtige Russiese koringluis weerstandsbepalings nie gedoen nie. In die tweede eksperiment is die Russiese koringluis-weerstandsgeen op 14.5 kaarteenhede vanaf die *Lr26*-lokus gelokaliseer. Nie-Mendeliese segregasie van die Russiese koringluis-weerstand in hierdie karteringseksperiment het geïmpliseer dat die berekende kaartafstand onakkuraat mag wees. Dit was ook nie moontlik om op grond van die data die aantal gene betrokke af te lei nie.

SUMMARY

An octoploid triticale was derived from the F_1 of a Russian wheat aphid resistant rye, 'Turkey 77', and 'Chinese Spring' wheat. The alloploid was crossed (a) to common wheat, and (b) to the 'Imperial' rye to 'Chinese Spring' disomic addition lines. F_2 progeny from these crosses were tested for Russian wheat aphid resistance and C-banded. Resistance was found to be associated with chromosome arm 1RS of the 'Turkey 77' rye genome. This initial work was done by MARAIS (1991) who made a RWA resistant, monotelosomic 1RS ('Turkey 77') addition plant available for the study. The F_3 progeny of this monotelosomic addition plant was used to confirm the RWA resistance on chromosome 1RS. The monotelosomic addition plant was then crossed with the wheat cultivar 'Gamtoos', which has the 1BL.1RS 'Veery' translocation. Unlike the 1RS segment in 'Gamtoos', the 'Turkey 77'-derived 1RS telosome did not express the rust resistance genes *Sr31* and *Lr26* which could then be used as markers. From the F_1 a monotelosomic 1RS addition plant that was also heterozygous for the 1BL.1RS translocation, was selected and testcrossed with an aphid susceptible common wheat, 'Inia 66'. Meiotic pairing between the rye arms resulted in the recovery of five euploid, Russian wheat aphid resistant plants out of a progeny of 99 euploids. One recombinant also retained *Sr31* and *Lr26* and was allowed to self pollinate. With the aid of SDS-PAGE profiles, Russian wheat aphid resistant 1BL.1RS translocation homozygotes were identified and it was possible to confirm that the Russian wheat aphid resistance gene was in fact transferred to the 1BL.1RS ('Veery') translocation.

Two attempts were made to map the Russian wheat aphid locus or loci. (1) Telosomic mapping was attempted. For this purpose a plant with 2n = 40 + 1BL.1RS + 1RS was obtained, and testcrossed with a Russian wheat aphid susceptible wheat. (2) A disomic, recombined 1BL.1RS translocation line with Russian wheat aphid resistance but lacking the *Lr26* and *Sr31*

alleles was crossed with 'Gamtoos' and the F_1 testcrossed. The testcross in both strategies were done with 'Chinese Spring'. In the first experiment the *Sr31* locus was located 10.42 map units from the *Lr26* locus. The rust resistance data implied that the genetic distance estimates may be unreliable and therefore the laborious Russian wheat aphid resistance tests were not done. In the second experiment a Russian wheat aphid resistance gene was located 14.5 map units from the *Lr26* locus. In the latter cross non-mendelian segregation of the Russian wheat aphid resistance evidently occurred which implied that the estimated map distance may be inaccurate. It was also not possible to determine the number of genes involved from the data.

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1. LITERATURE REVIEW

1.1 INTRODUCTION

Africa, consisting mainly of third world countries where the food demand greatly exceeds food production, poses a great challenge to plant breeders. Poor soils, pests, diseases and droughtlike environmental conditions complicate the food production problem even more. Worldwide, crop yields have generally been enhanced by applying better cultivation methods and directed breeding. Skillful plant breeders can combine novel genes in one plant that can withstand many adverse environments. However, useful germplasm available to plant breeders remains the driving force in crop improvement. Diverse genes introgressed from related and sometimes distantly related grass species can serve to expand the genetic basis of cultivated wheat and to shape the crop to the demands of Africa and that of the rest of the world.

Wheat is the most extensively produced crop in the world (BRIGGLE and CURTIS 1987) and its widespread cultivation created a need for higher levels of disease and insect resistance, drought and salt tolerance, and tolerance of toxic components from increased pollution. Novel natural variation within the wheat gene pool has largely been depleted. Further, genes beneficial to the improvement of wheat can only be obtained by employing costly and time consuming alternatives such as: (a) introgression from wild relatives through hybridisation and chromosome engineering (POTGIETER 1991), (b) genetic transformation with original or reconstructed genes from distantly related or unrelated organisms and (c) mutation (KONZAK 1987).

The introgression of foreign genes into common wheat from related species became a reality in the 1950's when Ernie Sears first introduced a small piece of an alien chromosome into wheat. Based upon this idea, desirable genetic material has been identified and similarly introduced into wheat by others. Homologous transfer from the progenitors of the A and D genomes of hexaploid wheat is relatively easy. Resistance to powdery mildew (*Erysiphe graminis*) has successfully been transferred from *Triticum*

timopheevi (genome formula = AAGG) to wheat (ALLARD and SHANDS 1954). Also, resistance for eyespot (*Pseudocercosporella herpotrichoides)* has been transferred from *Ae. ventricosum* (genome formula = DDUnUn) (MAIA 1967). However, successful transfers have also been achieved with distantly related species. For example, stem rust resistance has been transferred from *Thinopyrum elongatum* into Canadian wheat (KNOTT 1961).

Rye (*Secale cereale*) was identified as an agronomically useful distant relative of wheat that carries genes for leaf rust, stem rust, yellow rust, powdery mildew and greenbug resistance (McINTOSH 1983). Agronomically, wheat-rye hybrids (triticales) exhibit higher protein levels and out-yield wheat. The useful genes in rye can be incorporated into common wheat following the hybridisation of the two genera. In 1879, Stephen Wilson produced the first wheat-rye hybrid plants (GALE and MILLER 1987). These plants were completely sterile and only a curiosity. Today, synthetic amphiploids of rye with durum or common wheat can be produced readily using the embryo rescue and colchicine chromosome doubling techniques.

The most effective procedures to incorporate genes from rye into wheat is by establishing chromosome substitution and translocation lines. This can only be done when the absence of a wheat chromosome can be compensated for by the presence of a certain rye chromosome. GEORG KATTERMAN (1937) first reported a wheat-rye substitution. A rye chromosome was substituted for a chromosome of wheat. Since then several wheat-rye chromosome substitutions have been reported.

With rye chromosome arms and fragments transferred to wheat, mostly in the form of translocations, researchers today aim to retain only the useful rye chromosome fragments in the wheat genome as the flanking chromosome fragments usually have a negative effect on the quality of wheat products.

1.2 THE RUSSIAN WHEAT APHID

The Russian wheat aphid (RWA) belongs to the superfamily Aphidoidea, the family Aphididea and the genus and species *Diuraphis noxia* (Mordvilko). The RWA is fairly small (1,4 - 2,3 mm), elongated, pale green and dusted with powdery white wax. It can readily be distinguished by its short, under developed antennae (Fig. 1a,b).

1.2.1 Origin and distribution

The RWA is a recent and serious pest in many wheat-producing areas of the world, having been introduced accidentally from countries of the Middle East, Asia Minor and southern Russia. The RWA was first detected in 1900 in the Ukraine in the southern Soviet Union (GROSSHEIM 1914). In 1978 the RWA was identified in South Africa as causing extensive damage to wheat yields. It subsequently appeared in Central and South America (first Mexico, Chile and Argentina) and then in the western United States of America (Arizona, Colorado, Kansas, Nebraska, New Mexico, Oklahoma, South Dakota, Texas and Wyoming). By the end of the 1980's the RWA was present in sixteen western USA states and the Canadian provinces of Alberta, British Columbia and Saskatchewan. The presence of the RWA was also reported in Pakistan, Afghanistan, Iran, Israel, Lebanon, Turkey, Jordania, Syria, Romania, Egypt, Mozambique as well as areas in northwestern China (ROBINSON 1992). Kenya has also reported RWA infestation on barley as well as wheat.

1.2.2 Habitat and mode of infestation

The RWA occur mainly where their host plants are common. The host plant range of the RWA is quite wide. Barley and wheat are the most favoured crops while the RWA also infest non-preferentially oats, rye and triticale. Greenhouse experiments by

Fig. 1a.

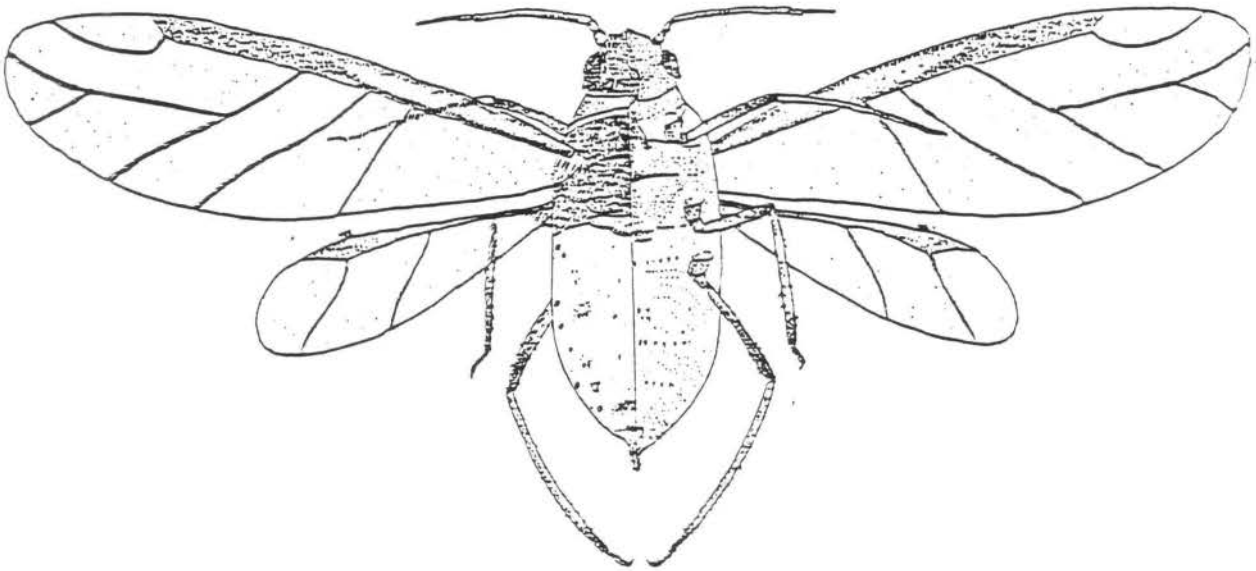

Fig. 1b.

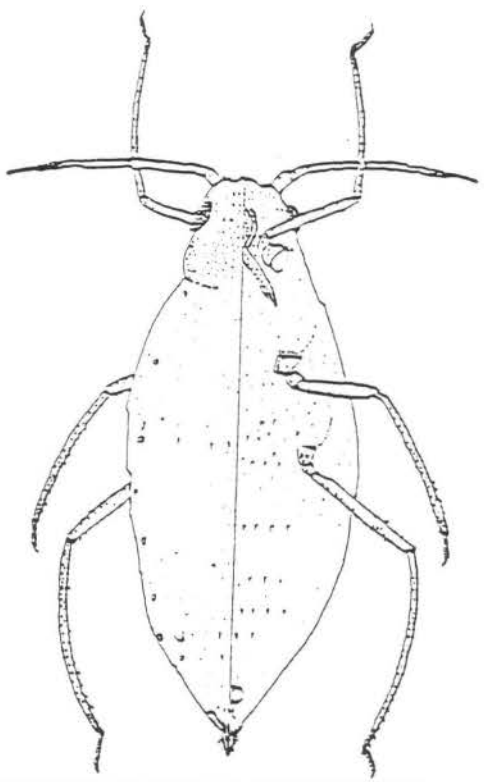

Fig. 1a. Alate viviparous female of *Diuraphis noxia*

Fig. 1b. Apterous viviparous female of *Diuraphis noxia*

KINDLER and SPRINGER (1989) demonstrated that some cool-season and warm-season grasses are suitable alternative hosts. Aphids alternate hosts seasonally (EASTOP 1986). In South Africa *D. noxia* survives on volunteer wheat and *Bromus* grass species during the summer months (FOUCHÉ et al. 1984). The nutritional quality of the host plant determines the relative growth rate of an aphid (DIXON 1987). When the host plant is no longer nutritionally sufficient for the aphids, they migrate to younger plants. Aphids normally infest a crop between the seedling and tillering stages whereafter the population increases slowly. Landing and settling of aphids in the field correlate with plant density and are directed by green colour and sometimes by olfactory cues (ÅHMAN et al. 1985). With the onset of stem elongation an exponential increase in the population occurs. RWA development and reproduction can proceed over a broad range of temperatures. Nymphal production by adult aphids reaches a peak before the population starts to decline shortly after the ears emerge. Other factors that contribute to population decline are predators, parasitoids, fungal diseases, weather, emigration by alates (winged aphids) or some combination of these factors (CHAMBERS et al. 1986). The numbers of aphids can vary from site to site and from year to year depending on environmental conditions. The aphid population usually declines between the summer and autumn migration. The reasons for the decline can be as follows: natural enemies, intraspecific competition in cereals and intraspecific competition in grasslands.

1.2.3 Damage-causing mechanism and damage symptoms

Aphids are devastating pests of crop plants. Their ability to extract the sap from the phloem of a plant together with parthenogenetic reproduction assist in their success as severe plant parasites. Aphids possess enzymes in their watery saliva that can degrade the complex cell wall and middle lamella tissue so that the stylets can penetrate between the mesophyll cells to reach the phloem (McALLEN and ADANS 1961). Apart from depriving the host plant of nutrients, the RWA is classified as a 'phytotoxic' aphid because of the adverse, direct injury it can cause to the plant tissue. Characteristic of RWA infestation is first the appearance of droughtlike symtomps

(RIEDELL 1989) and then the rolled leaves which result in misformed ears. Chlorotic streaks and purple discolorations become very obvious on the leaves. The chlorosis symtoms result from extensive damage to the chloroplasts (FOUCHÉ et al. 1984). Indirect damage can also appear. Ageing of the leaves may be faster due to the honeydew that covers the leaves and viruses can be transmitted to the plant. RWA damage to the host plants has been found to result in low grain protein content, altered dough-mixing characteristics, a reduced winterhardiness, reduced plant height and extensive yield losses (BURD et al. 1993).

1.2.4 Control of the RWA

In South Africa, especially in the Orange Free State, damage caused by the RWA results in winter wheat yield losses of approximately 35-60% when the crop is not protected with insecticides (ROBINSON 1992). Systemic insecticides can be applied with success (90-100% effective) (HILL et al. 1993, PEAIRS et al. 1992), but this is a costly practice. The aphids usually thrive inside rolled leaves which complicate the penetration of contact insecticides. It should also be taken into account that the RWA may in future develop resistance against insecticides that are used routinely. The biological fitness of the RWA seems to be dependent on leaf rolling which also provides protection against natural enemies and therefore if this phenomenom could be limited, it may aid in the control of RWA populations (BURD et al. 1993).

Cultural management is not well developed but a combination of late sowing and application of insecticide is the best option under some circumstances. Delayed planting as well as healthy crop stands may help to minimize infestation by the RWA. In Northeastern and -western Colorado, USA, fields planted later in the autumn (i.e. after the 20th of September) rarely become infested as the young crop thus escapes the major aphid flights. In the following spring, infestations also develop later on the later planted fields (PEAIRS 1990, ROBINSON 1992). Delayed plantings that extend the aphid's critical host support period between harvest and the emergence of the new crop in the autumn can also help to reduce aphid infestation. RIEDELL (1990)

observed a linear correlation between yield loss and the application of nitrogen fertilizer. As more fertilizer was applied the grain yield losses were reduced. The economic and environmental repercussions of increased nitrogen fertilizer applications should, however, be investigated for different environments.

It is difficult to use natural predators and parasitoids for the biological control of the RWA because it is difficult to optimize the natural enemy population size with respect to that of the aphids. The population growth of natural enemies normally lags behind that of the aphid population mainly because of limited initial food supplies and the activities of natural enemies of the predators and parasitoids. A parasite of *Diuraphis holci* in Holland, *Trioxys lambersi*, can penetrate rolled-up leaves (AALBERSBERG et al. 1984) and may be useful in the biological control of *D. noxia*. Recently, resistance to insects in grasses has been linked with the presence of fungal endophytes (SAHA et al. 1987). The fungal endophytes of the genus, *Acremonium*, spend their lifecycle within the host plant (BREEN 1993). BREEN (1993) stated that fungal endophytes are promising in enhancing the control of the RWA, especially in turfgrass and conservation grasses in the USA. VON WECHMAR et al. (1990) investigated the possibility of aphid control by a virus called the aphid lethal paralysis virus (ALPV). Leaves were painted with a solution containing the virus. The aphids died soon after feeding and after analysis of the bodyfluid of the dead aphids, ALPV virus was found in high concentrations. The spraying of solutions containing the virus may serve as a means to prevent RWA infestation, but the following may pose problems: maintaining the virus, duration of the viability of the virus after spraying and the high cost involved.

1.2.5 Breeding for resistance to the RWA

Plant aphids have caused damage to crops over centuries and the value of breeding for resistance was already recognized in 1831 when an aphid resistant apple cultivar was released (LINDLEY 1831). Cultivated common wheat has almost no resistance to the RWA (SOUZA et al. 1991). In those instances where resistance to the RWA has been identified it occurred more often in winter wheats than in spring wheats

(ROBINSON 1992). No resistance has thus far been found among durum wheats, whereas triticale and rye genotypes often exhibit possibly useful levels of resistance. Wild relatives of wheat, i.e. *Triticum monococcum, T. timopheevii, T. dicoccoides* and *T. tauschii* also possess possibly useful levels of RWA resistance (BUTTS and PAKENDORF 1984, DU TOIT and VAN NIEKERK 1985). Resistance to the RWA has often been identified in collections from the aphid's region of origin, i.e. Russia and Iran (SMITH et al. 1991). Biotype and host resistance diversity exist and are highest in the native countries (DU TOIT 1992, PUTERKA et al. 1993). Attempts to find effective resistance in the hexaploid wheats have yielded the dominant genes *Dn1, Dn2, Dn4,* and *Dn5* (DU TOIT 1987, 1988, NKONGOLO et al. 1991a). The resistance found in a *Triticum monococcum* accession (DU TOIT, 1987) proved to lose its expression when transferred to a hexaploid wheat background (POTGIETER et al. 1991). A single recessive gene in *T. tauschii* (accession SQ 24) was designated, *dn3*, by NKONGOLO et al. (1991b). QUICK et al. (1993) reported the introgression of a single dominant gene located on chromosome 4R of *Secale montanum*. Interspecific hybrids obtained by NKONGOLO et al. (1990) showed that RWA resistance genes from wheat relatives are expressed in the presence of wheat genomes and thus are accessible for wheat improvement. RWA resistance also exists in *Hordeum* (barley) species (KINDLER and SPRINGER 1991). COMEAU et al. (1988) demonstrated that intergeneric hybrids can be achieved between *Hordeum* species and common wheat (*Triticum aestivum* L.). A primary objective of geneticists is to incorporate these potentially useful resistance genes into well adapted common wheat cultivars with acceptable quality.

DU TOIT (1989) identified two RWA resistance genes in the wheat lines PI 137739 (*Dn1*) and PI 262660 (*Dn2*). He concluded that these are single dominant genes that inherit independently. DU TOIT (1988) also reported resistance in the common wheat PI 294994. ELSIDAIG and ZWER (1993) reported that RWA resistance in the latter line is conferred by a dominant allele at one locus and a recessive allele at a second locus. MARAIS and DU TOIT (1993) concluded that the RWA resistance of PI 294994 is due to a single dominant gene on chromosome 7D, designated *Dn5*. Single dominant genes can readily be manipulated in breeding to incorporate resistance in new selections. However, *Dn* genes may prove to interact on a gene for gene basis

with aphid virulence genes. Unfortunately, simply inherited, monogenic major gene resistance is often short-lived once the aphid is put under selective pressure in a resistant germplasm environment and it will almost certainly develop new biotypes (the ability of populations within an aphid species to produce genotypes that can differentially damage plant resistance sources during commercial cultivation) (ROBINSON 1992). New aphid variants may arise through : (a) sexual recombination following induction (a genotype X temperature X photophase interaction that induces the development of sexual morphs), (b) mutation - a process that changes the gene or chromosome complement by inducing translocations, gene duplications and point mutations, and (c), selection - a process that is driven by host availability and the genotypic composition of the aphid population during seasonal changes. Selective pressure is increased when the RWA is confronted with resistant germplasm (ROBINSON 1992).

Plants can employ three types of defense mechanisms against insects, namely: (a) avoidance or antixenosis whereby contact with the parasite is avoided - usually by means of thorns, unpleasant smells or tastes, (b) resistance or antibiosis which reduce the growth and development of the parasite, and (c), tolerance - there is no interference with the growth and development of the parasite, but the damage resulting from the parasite's activities is reduced. Avoidance is not commonly employed against insects but more generally against animal parasites and herbivores. Tolerance is very difficult to assess and to manipulate through breeding. Although the mechanism by which plants confer resistance against the RWA is still unclear, RYAN et al. (1990) found significant differences in the pectins of isogenic lines susceptible and resistant to attack by the greenbug (*Schizaphis graminum*). Gene for gene interactions may in this instance be based on the differential abilities of aphid pectinases to degrade wheat pectin (DREYER and CAMPBELL 1987). Host plant resistance may be based on genes encoding alternative pectin compositions and structures.

Due to the accidental introduction of the RWA into South Africa it is possible that the local aphids arose from a single ancestor and hence are genetically very similar. The RWA can reproduce sexually (in unfavourable conditions) or asexually via

parthenogenesis (in favourable conditions). Only asexual reproduction has been observed in South Africa (WALTERS et al. 1980). Since only anholocyclic aphids occur, the South African climate appears to be very favourable for the RWA. In the absence of a sexual phase and an opportunity for recombination, new biotypes may arise primarily through chance mutations. However, the possibility of sexual reproduction occurring in South Africa cannot be ruled out. Also, the large population sizes makes it very likely that spontaneous mutation may be an effective means for the insect by which to produce new biotypes.

The ultimate goal of breeding is for resistance that is durable. The ideal cultivar should confer multibiotypic resistance. Although resistant cultivars will be the most effective buffer against the RWA, both economically and environmentally, insecticides, biological control and resistance gene employment should be integrated to be most effective. Genetic diversity for resistance must be expanded and therefore the search for new and varied RWA resistance sources must be continued. Molecular biologists succeeded in incorporating insecticidal genes into dicotyledons from bacteria such as *Bacillus thuringiensis*. Transgenic plants then produce crystal proteins that are toxic to insects only. However, no success has been obtained in monocotyledons with this technique. On the other hand, this kind of resistance may also be lethal to natural enemies of the RWA. Insect resistance in plants should be compatible with biological control to maximize the combined effect resulting from plant resistance and biological control. Plant breeders still rely on conventional recombination for incorporating alien genes into wheat. The obvious choice as source of alien genes are the wild relatives of wheat. The question arises unequivocally whether it is worth transferring resistance genes from wild species because the useful genes are often associated with undesirable genes. ALLARD (1949) reported that he spent 30 years trying to break deleterious effects associated with genes from *Triticum timopheevii* transferred into common wheat. He succeeded with a few. Some of these genes are still being used in wheat breeding to confer resistance to, for example, stem rust.

The advent of biotechnology about a decade ago can in the future perhaps help to shorten these time consuming efforts. Molecular genetic markers based on the

polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques can possibly be used to identify useful chromosome fragments free of deleterious genes.

1.2.6 Future spread of the RWA

Impact studies in Australia suggest that RWA may pose a threat to their wheat industry (HUGHES 1990). Although the yield loss caused by RWA damage is not expected to be as extensive as in the USA and South Africa, the Australian environment is more favourable for the year around survival of RWA. HUGHES (1990) has also predicted that northeastern China are potentially in danger of RWA infestation.

1.3 STUDIES ON WHEAT-RYE HOMOEOLOGY

Triticum aestivum L., generally called common or bread wheat, has a chromosome complement that comprises 3 genomes containing 7 pairs of chromosomes each (2n = *6x* = 42; genome formula = AABBDD). Each of the genomes originated from a different diploid ancestor and *T. aestivum* L. is therefore an allohexaploid. The A, B and D genomes derived, respectively, from *T. monococcum*, an unknown B genome donor(s) and *T. tauschii*. Chromosomes within the same genome belong to homologous pairs while the relationship of corresponding chromosomes in different genomes is referred to as homoeology.

The chromosome complement of the genus *Secale* (rye) consists of one genome with 14 chromosomes (2n = *2x* = 14; genome formula = RR). The chromosomes of wheat and rye show homoeology. *Secale* is a distant relative of wheat that belongs to the same tribe as wheat, namely the Triticinae. Both cereals probably evolved from a common ancestor.

The chromosomal homoeology between wheat and rye triggered interest in the possibility of transferring desirable agronomic characters from rye to wheat. Well

known genes of this kind were found, for example, on chromosome 1R. Desirable genes on 1R occur as a linked complex that confer resistance to stem rust (*Sr31*), leaf rust (*Lr26*), yellow rust (*Yr9*) and powdery mildew (*Pm8*) (McINTOSH 1983) and have mostly been transferred to wheat in the form of Robertsonian translocations such as the 1BL.1RS translocation. However, linked flanking genes which have detrimental effects on wheat productivity and quality are also transferred and due to the genetic distances involved are difficult to elliminate through the induction of homoeologous pairing and recombination.

1.3.1 Translocations

Spontaneous translocations have been observed involving a rye chromosome and a wheat homoeologue (ZELLER 1973, METTIN et al. 1978, DRISCOLL 1983, SHEPHERD 1973, LAWRENCE and SHEPHERD 1981, SEARS 1973, BIELIG 1970, MAY and APPELS 1978, 1980). Two spontaneous translocations have been observed by ZELLER and KOLLER (1981) which involved segments of wheat chromosome 4A and rye chromosome 7R in one, and in the other, segments of wheat chromosome 7B and rye chromosome 4R. These translocations were, respectively, named translocation line T8 and translocation line T22. The segment of the rye chromosome 7R which is involved in the translocation T8 is homoeologous to chromosome arms 4AS, 4BS and 4DS (HART and LANGSTON 1977). T22, which is a 7BL.4RL translocation, further confirmed the homoeology between chromosome arms 4RL, 7BS and 7DS. Orthologous genes for red coleoptiles were previously shown to be located on these chromosome arms by LAW (1966), ROWLAND and KERBER (1974) and SUTKA (1977).

Translocations are thought to have played an important role in the evolution of the genus *Secale* (STUTZ 1972). Only chromosome arms 1RS, 1RL, 2RL, 3RS and 5RS may have complete homoeology with their wheat counterparts. Relative to wheat, multiple translocations involving the chromosome arms 4RL, 5RL, 6RS and 7RS have been detected as well as a reciprocal translocation between 3RL and 6RL. Evolutionary rearrangements may also be responsible for partial homoeology between

Chromosomes 1R, 2R, 3R, 5R and 6R show homoeology to the wheat chromosome groups 1, 2, 3, 5 and 6, respectively. The homoeology was confirmed in that the rye chromosomes can replace the wheat chromosomes of the corresponding group (ZELLER 1973, METTIN et al. 1978, CHAPMAN and MILLER 1978, BIELIG and DRISCOLL 1970, 1973, JENKINS 1966). No successful substitutions have been achieved with chromosome 7R (MILLER 1984).

1.3.3 Chromosome pairing

Homoeology among chromosomes can also be identified by applying banding techniques to study the pairing of chromosomes at metaphase I of meiosis in allohaploid wheat X rye hybrids (GILL and SEARS 1988, SYBENGA et al. 1985, 1990). Chromosome arm associations are then reflected by chiasmata formation which can result in recombination. Analysis of chromosome associations of metaphase I in wheat X rye F_1 hybrids by NARANJO et al. (1987) suggested that chromosome arms 1AL, 1BL, 1DL and 1RL are homoeologous. Pairing of chromosome arms 5AL, 4BL and 4DL with 5RL was also detected. Nonhomoeologous chromosome associations were observed involving chromosome arms 1BL-4BL and 1AS-4BL. The results of NARANJO et al. (1989) showed that the level of association at metaphase I and the frequency of recombination for the long arms of the group 1 chromosomes were mainly influenced by the wheat genotype. Pairing of homoeologous chromosomes is restricted mainly by the presence of the *Ph1* gene on chromosome 5B (SEARS and OKAMOTO 1958, RILEY and CHAPMAN 1958a). KOEBNER and SHEPHERD (1985) showed that by deleting the *Ph1* gene, homoeologous pairing can be induced among wheat and rye chromosomes. The promotor-suppressor system which regulates chromosome pairing is, however, very complex and involves both weak suppressors on chromosomes 3DS (*Ph2*) and 3AS (DRISCOLL 1973), and promotors on the homoeologous group 3 long arms, 5AL, 5DL, 5AS, 5BS, and 5DS (FELDMAN 1966, 1968, FELDMAN and MELLO-SAMPAYO 1967, RILEY 1966, DVO□ÁK 1976, DRISCOLL 1973). These regulators may even affect homologous pairing. While the absence of *Ph1b* has the strongest effect in promoting homoeologous pairing,

intermediate levels of homoeologous pairing are achieved in the absence of *Ph2* (SEARS 1982, WALL et al. 1971).

NARANJO et al. (1991) concluded that telomeric C-heterochromatin blocks of rye chromosomes hinder wheat-rye pairing and thus homoeologous pairing during metaphase I. Prominent C-bands correlate with the almost total suppression of pairing between chromosomes 1RS, 3RS, 3RL and 4RS and wheat chromosomes (NARANJO et al. 1991). On the contrary, the presence of wheat chromosome arm 5BS raises the level of homoeologous chromosome pairing in wheat X rye hybrids (NARANJO et al. 1988).

Recently, the technique of genomic *in situ* hybridisation (GISH) has also been used to determine the extent of wheat-rye chromosome homoeology (MILLER et al. 1994) in (a) chromosome-5B-deficient wheat-rye hybrids, (b) euploid wheat-rye hybrids and (c) wheat-rye hybrids carrying an extra chromosome 5D. Higher wheat-rye chromosome pairing was observed in (a) than in (b), but significantly less wheat-rye chromosome pairing in (c). Except for the well known pairing suppressor on chromosome 5B that was confirmed, this also indicated a pairing suppressor on chromosome 5D.

The GISH technique provides a more reliable means for determining wheat-rye homoeology than the banding techniques. Hence, the potential level of wheat-alien recombination in interspecific and intergeneric hybrids can be determined.

1.3.4 Genetic maps and markers

A valuable aid to identify harder-to-find characteristics or particular segments of chromatin is the use of easily identifiable characteristics as genetic markers. These markers can also indicate homoeology between chromosomes. Extensive studies with respect to the storage proteins of wheat and rye endosperm have been conducted to determine the composition of the products as well as the chromosomal locations of the

genes (PAYNE et al. 1982, SHEWRY and MIFLIN 1985). Enzyme markers can be used to the same effect in studies of the homoeology between wheat and rye chromosomes. The seven rye chromosomes have been marked by a total of approximately 20 isozyme loci, while wheat chromosomes have been marked with even more (HART 1987).

Genes coding for products with the same properties can be found on rye and corresponding wheat chromosome groups (HART 1975, TANG and HART 1975, LELLEY 1976, MILLER et al. 1983, AINSWORTH et al. 1983, DABROWSKA 1983, NISHIKAWA and NOBUHARA 1971, NISHIKAWA et al. 1976, NISHIKAWA et al. 1981, MAY et al. 1973). Partial homoeology of chromosome 7R to the group 4 and 7 chromosomes of wheat have been demonstrated with orthologous genes. The acid phosphatase genes in wheat were localized on chromosomes 4A, 4B and 4D while chromosome 7R of rye carries the same gene (HART and LANGSTON 1977). The alpha-amylase set of genes on chromosomes 7A, 7B and 7D can also be found on chromosome 7R of rye (NISHIKAWA and NOBUHARA 1971). Linkage data obtained for the genes, *Got-R2, Got-R3* and *Adh-R1* suggested that some homoeology may exist between the 3R, 6R chromosomes of rye and the group 3 and 6 chromosomes of wheat (RILEY and CHAPMAN 1958b, TANG and HART 1975). These biochemical markers aid both in gaining knowledge of homoeologous relationships between wheat and rye chromosomes and in planning the introgression of rye chromatin into wheat.

1.3.5 Molecular investigations

(i) *In situ* hybridisation (ISH)

In situ hybridisation (ISH) was first used by RAYBURN and GILL (1985) in cytogenetic studies of wheat. They detected the presence of wheat-rye translocations by hybridising a highly repeated, biotinilated rye DNA sequence on wheat chromosomes. This application revolutionised the study of homoeology between related species. If appropriate DNA probes are used, the ISH karyotype of individual rye chromosomes

can now be compared with the ISH karyotype of wheat from which similarity and thus homoeology can be deduced. Total genomic DNA from alien species or species specific repeated DNA can be used as probes to detect overall homoeology of genomes. Fluorescence *in situ* hybridisation (FISH) is a modified application of ISH where fluorochromes are used as dyes. Probe chromatin from one species may be labeled to fluoresce as a different colour from another species' chromatin. Even two or more sequences can now be detected in the same cell (multicolour FISH) by using fluorochromes of different colors (LEICHT et al. 1991, LICHTER et al. 1990, REID et al. 1992, MUKAI et al. 1993b). Detailed physical maps of specific chromosome regions can be constructed using multicolour FISH.

In order to construct a physical map it is important to detect smaller low copy number DNA sequences. DONG and QUICK (1995) used such a probe, called *pTtksul26*, that hybridised on two rye chromosomes and several wheat chromosomes. The multiple hybridised sites in wheat are probably the result of the homoeologous nature of the three genomes in wheat. The hybridised sites on the rye chromosomes show that there is homoeology between these two chromosomes and the wheat chromosomes.

The pSc119 and pSc74 probes that were derived from rye can be used to detect rye chromatin. These clones contain highly repeated sequences from 120 and 350 bp repeat families (BEDBROOK et al. 1980, JONES and FLAVELL 1982). The pSc119 clone was subcloned and two new clones were derived: a sequence that hybridises to the entire length of each rye chromosome and a sequence that hybridises to telomeric regions and some interstitial sites of rye and wheat chromosomes (McINTYRE et al. 1990).

Chromosome-specific ISH patterns can be observed in all B genome chromosomes as well as in chromosomes 4A, 2D, 3D and 5D when DNA probes pSc119 and pSc74 are used (MUKAI et al. 1993a). The probe pSc119 produced two interstitial and one telomeric ISH site in the 6RL telosome of rye, while in the translocated chromosome 6BL of wheat a distal and a telomeric ISH site were observed. Probe pSc74 only labeled the 6RL arm of rye at two distally located interstitial sites. Double labeling with

pSc119 and pSc74 produced all five ISH sites on the translocated chromosome 6BL. The translocated chromosome 4BL of wheat shows two interstitial pSc119 sites as well as telomeric pSc119 sites on both arms. A telomeric pSc119 ISH site can also be observed in chromosome 4AL of wheat (MUKAI et al. 1993a). The translocations investigated here confirmed the compensating ability of chromosome arm 6RL for chromosome arm 6BL. A non compensating translocation was also observed but only a very small segment was involved in the interchange.

(ii) Restriction fragment length polymorphisms (RFLPS)

DEVOS et al. (1992) constructed a RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. Twenty two DNA probes and two isozyme markers were used. Homoeology between the group 3 chromosomes of wheat and the 3R chromosome of rye was very clear. Fourteen markers of 3R could also be found on one or more of the homoeologous group 3 chromosomes of wheat. Apparently the loci around the centromere are well conserved for all four chromosomes as all the common RFLP-loci are clustered around the centromere.

DNA probes were used by LIU et al. (1992) to locate similar sequences on chromosomes 4AL, 5BL and 5DL. The same probe, PSR580, located a similar sequence on chromosome 7R. Thus, the respective chromosomes contain homoeologous regions.

1.3.6 Homoeology between 1BS and 1RS

The storage proteins found in endosperm are mostly coded for by genes located on the group 1 chromosomes of both wheat and rye. The locations of the genes and properties of the gene products correlate with the chromosome arms involved. The high molecular weight (HMW) glutelin subunits of wheat are coded by the *Glu-1* locus on chromosome arms 1AL, 1BL and 1DL (LAWRENCE and SHEPHERD 1980, BIETZ et al. 1975, ORTH and BUSHUK 1974) while the HMW glutelin subunits of rye (also

called secalins) are coded by the *Glu-R1* (also designated as *Sec3*) locus on chromosome arm 1RL (LAWRENCE and SHEPHERD 1981).

Homoeology of chromosome arms 1BS and 1RS is also suggested by the evident genetic similarity of genes coding for similar storage proteins. Among these are the *Gli-1* genes (SINGH and SHEPHERD 1988) which are located on the satellites of the short arms of the group 1 wheat chromosomes (PAYNE et al. 1984) and the *Gli-R1* (also designated as *Sec1*) gene which is also distally located and is possibly linked to the telomere of the 1RS chromosome arm. The *Gli-1* loci code for the prolamins (gliadins and secalins) in wheat and rye, respectively (ORELLANA et al. 1993). SHEWRY et al. (1984b) considered omega-secalins and omega-gliadins to be homologous. BENITO et al. (1990) reported two separate loci for the *Sec* site on 1RS namely, *Sec-1a* and *Sec-1b*. The *Sec-1a* locus controls the 40K gamma-secalins while the *Sec-1b* locus controls the omega-secalins. These loci are closely linked (0.33 ± 0.33 cM). PAYNE et al. (1988) mapped the *Gli-3* locus on wheat chromosome arms 1AS and 1BS while CARRILLO et al. (1992) mapped a *Gli-R3* (*Sec4*) locus on chromosome arm 1RS of rye. Comparison of the linkage data and arm locations of biochemical and molecular marker genes among the group 1 chromosomes of wheat and rye indicates the precise homoeology between these chromosomes (PAYNE et al. 1982, SINGH and SHEPHERD 1984, LAWRENCE and SHEPHERD 1981, SHEWRY et al. 1984a, LAWRENCE and APPELS 1986, CARRILLO et al. 1990, BENITO et al. 1990, ORELLANA et al. 1993).

The linkage map established for chromosome 1RS by LAWRENCE and APPELS (1986) and KOEBNER et al. (1986) showed the gene order as follows: centromere - *NOR* (RFLP marker for NOR locus) - *Gpi-R1* (glucose phosphate isomerase) - *Sec-1b* - *Sec-1a* - *5S RNA* - *Lr26/Sr31/Yr9* - telomere (Fig. 2). All the loci are located on the satellite of chromosome 1RS of rye. The location of *Sec1* was determined as follows: by using translocations SYBENGA et al. (1990) placed *Sec1* approximately 30 cM from the *NOR*-locus; GUSTAFSON et al. (1990) physically mapped the *Sec1* locus to the satellite region of 1RS using *in situ* hybridisation and SINGH et al. (1990) estimated recombination between the *Sec1* locus and the disease resistance genes by utilizing a

polymorphism in the terminal region of 1RS. The polymorphism contained the dominant stem rust resistance gene but the leaf and yellow rust resistance genes were reccessive. The *Sec1* locus was located 26.1 ± 4.3 cM from the centromere and the rust resistance genes were located 5.4 ± 1.7 cM from the *Sec1* locus. Very little recombination was observed between the centromere and the *NOR* locus, a segment which represents two-thirds of the 1RS chromosome arm (LAWRENCE and APPELS 1986). However, 22.5% recombination was observed between the *NOR* and *Sec1* that is located more distally.

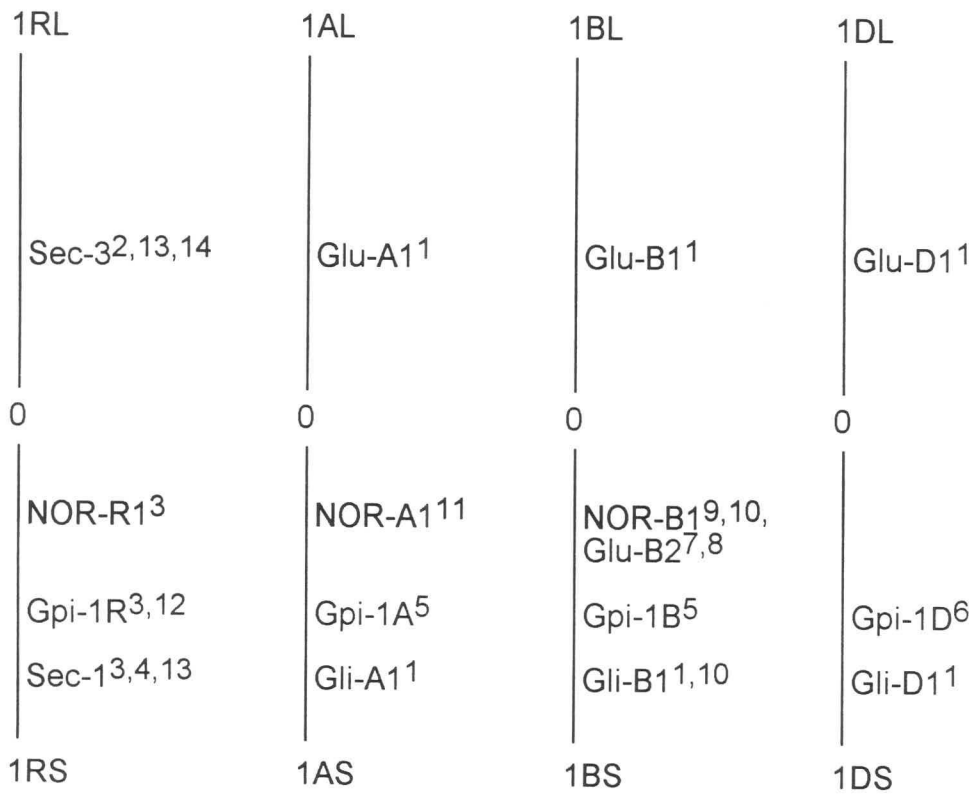

Fig. 2.: Relative map positions of marker loci on the homoeologous group 1S arms of wheat and rye.

1. PAYNE et al. (1982), 2. SINGH and SHEPHERD (1984), 3. LAWRENCE and APPELS (1986), 4. SHEWRY et al. (1984a), 5. HART (1979), 6. CHOCECKI et al. (1983), 7. JACKSON, HOLT and PAYNE, 8. GALLILI and FELDMAN (1984), 9. PAYNE et al. (1983), 10. SNAPE et al. (1985), 11. MILLER, GERLACH and FLAVELL (1980), 12. ORELLANA et al. (1993), 13. CARRILLO et al. (1990)

The glucose phosphate isomerase structural gene (*Gpi-1*) was located on the short arm of chromosome 1D of wheat (CHOJECKI et al. 1983). Allelic variation at this locus allowed mapping of the *Gpi-1* gene between the *Gli-D1* and *Glu-D1* loci of chromosome 1D. The estimated recombination frequency (%) between the centromere and *Gpi-D1* is approximately 26.0 and between *Gli-D1* and *Gpi-D1* about 34.5. The same structural gene coding for the same gene products was found on chromosome 1RS of rye. The *Gpi-R1* locus was also found to be located between the *Sec1* and *Sec3* loci and was mapped approximately 18.9 cM from the *Sec1* locus (BENITO et al. 1990). These corresponding genes on the group 1 wheat chromosomes and the 1R chromosome of rye indicate clear homoeology among these chromosomes. Recombination of these chromosomes should be possible following the induction of homoeologous chromosome pairing. This should allow for combining the advantagous genes of rye with those of wheat.

1.4 THE 1BL.1RS TRANSLOCATION

Natural or deliberate hybridisation of wheat and rye followed by open pollination of the hybrids with wheat pollen, have sometimes given rise to wheat selections that retain all or part of chromosome 1R of rye. Crossbreeding of wheat lines that contained chromosome 1R with normal wheats have often given rise to translocations involving the short arm of 1R and the long arm of one of the wheat homoeologous group 1 chromosomes. Such translocations were mostly associated with an improved agrotype and high levels of disease and pest resistance. 'Amigo', favoured for its greenbug (*Schizaphis graminum*) and powdery mildew resistance, was the first wheat cultivar released containing a 1AL.1RS wheat-rye translocation (SEBESTA and WOOD 1978), the rye chromosome arm having come from 'Insave' rye. This translocation is now present in the North American wheat cultivars 'TAM 107', 'TAM 200' and 'Century' (LOOKHART et al. 1991). Among wheat-rye translocations the 1BL.1RS translocation was also identified. Several European wheat cultivars as well as the CIMMYT 'Veery' releases, the Nebraska variety 'Siouxland' and the local cultivar 'Gamtoos' contain the

1BL.1RS translocation derived from the Soviet wheat lines 'Kavkaz' and 'Aurora'. The rye chromosome segment originally came from the rye cultivar 'Petkus'. The 1BL.1RS translocation lines are favoured over 'normal' wheat cultivars because of the yield advantage associated with the 1RS segment (RAJARAM et al. 1983). Common wheat cultivars containing the 1BL.1RS translocation, were also identified as highly regenerable in the anther culture technique (HENRY et al. 1993). While results up to now were unsatisfatory in the number of wheat plants regenerated via anther culture, the presence of the 1BL.1RS translocation in a cultivar showed a large increase in the number of plants obtained. LANGRIDGE et al. (1991) and HENRY and DE BUYSER (1985) hypothesized that the ability of the embryos to regenerate is greatly dependent on genes located on chromosome 1RS which influence the differentiation of callus. Despite all the advantages of the 1BL.1RS translocation, there is one major defect caused by the presence of the 1BL.1RS translocation in a wheat background. The end-use quality of 1BL.1RS translocation wheats is, however, poor and poses problems to the baking industry.

1.4.1 Identification of 1BL.1RS translocations

1.4.1.1 Cytological techniques

APPELS (1982), GILL and KIMBER (1977), HUTCHINSON et al. (1981) and LAPITAN et al. (1986) demonstrated that rye chromosome fragments can be detected readily in wheat using cytological techniques.

(i) C-banding

The short arm of chromosome 1R of 'Imperial' rye can be identified cytologically by the presence of a satellite (SYBENGA 1983). However, Feulgen stained mitotic chromosomes spreads of the 'Veery' 1BL.1RS translocation lines and hexaploid triticale do not show the satellite (MERKER 1973, 1982). Identification of the 1BL.1RS chromosome can fortunately be accomplished by the technique of Giemsa C-banding.

The telomere, after C-banding, characteristically exhibits two heterochromatic bands. One band occurs distally on the satellite and the other band more proximal (DARVEY and GUSTAFSON 1975; BENNETT and SMITH 1975; MERKER 1982).

In an attempt to simplify the conventional Giemsa C-banding technique, STÖßER et al. (1993) pretreated rye chromosomes with restriction enzymes. After incubation in the presence of the enzymes *AluI*, *DraI, HpaIII* or *MspI*, the slides were washed and stained with a 2.5% Giemsa solution. Similar banding patterns were observed as in conventional Giemsa C-banding. Some differences were observed in the centromeric, interstitial as well as telomeric bands. Differences were also dependent on the restriction enzyme that was used. When the DNA was treated with *DraI*, an area on chromosome 3R showed a clear zone, called a 'negative' band. Although the enzyme mechanism in producing banding patterns cannot be explained, it can be applied for the identification of single rye chromosomes in rye and triticale. Whether the restriction enzyme banding technique can replace conventional C-banding is still to be seen.

(ii) N-banding

The N-banding technique was not successful in staining heterochromatin of rye chromosomes (JEWELL 1978). FUNAKI et al. (1975) observed stained NOR regions by applying N-banding. However, ENDO and GILL (1984) failed to obtain the same results with rye chromosomes. After N-banding of 1BL.1RS translocation lines RAYBURN and CARVER (1988) observed four bands on the 1BL arm while no bands showed on the 1RS arm. The NOR region on chromosome 1RS confirmed the identification of the translocation chromosome. Thus the N-banding technique is not suitable for the identification of 1BL.1RS translocations.

(iii) Meiotic pairing analysis

A study of meiotic pairing in Feulgen stained pollen mother cells can aid in the identification of a wheat-rye translocation.

Root tip somatic cells analysed by ZELLER (1969) showed that the cultivar 'Zorba' contains only two satellited chromosomes while hexaploid wheat normally contains four satellited chromosomes. SASTROSUMARJO and ZELLER (1970) later concluded that wheat chromosome 1B of 'Zorba' was replaced by a rye chromosome. 'Zorba' was then used in many crosses because the rye chromosome fragment conferred good rust resistance. One such cultivar, 'Benno', was derived from crosses of 'Zorba' with hexaploid wheat. ZELLER (1973) determined the chromosome constitution of 'Benno' through meiotic pairing analysis. After crossing 'Benno' the meiotic pairing of the F_1 hybrids was good and 20 ring bivalents and one heteromorphic rod bivalent could be observed implying that the rod bivalent comprised a complete 1B wheat chromosome and a 1B/1R translocation chromosome. Although a 1B/1R translocation appeared to be present in 'Benno', better cytological evidence was needed.

The wheat varieties 'Kavkaz' and 'Aurora' were crossed with 'Chinese Spring' monosomic lines. During meiotic pairing studies of the pollen mother cells of the monosomic F_1's, mostly bivalents and multivalents were observed with univalents at a low frequency. From the results ZELLER (1973) concluded that each of 'Kavkaz' and 'Aurora' possess a 1B/1R interchanged chromosome.

1.4.1.2 Gel electrophoresis

(i) Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Protein electrophoresis provides a rapid and reliable technique by which 1BL.1RS translocations can be detected (GUPTA and SHEPHERD 1992). The *Sec1* gene located on chromosome arm 1RS codes for a prolamin storage protein (secalin) (SHEWRY et al. 1984). When unreduced endosperm protein is extracted from the wheat kernel and electrophoretically analysed on a gel, the 1BL.1RS translocation lines

can be distinguished from lines in which the translocation is absent (GUPTA and SHEPHERD 1992).

Plants containing 1BL.1RS translocations were also identified by KOEBNER and SHEPHERD (1986). The *Gli-B1* locus on chromosome 1BS codes for proteins present as three bands on a SDS-PAGE gel. The *Sec1* locus on chromosome 1RS codes for proteins present as four bands on a SDS-PAGE gel. Plants having a 1BL.1RS translocation could easily be identified by the presence of the *Sec1* bands and absence of the *Gli-B1*. When a 1BL.1RS translocation was not present, the *Sec1* bands were absent and the *Gli-B1* bands present.

(ii) Acid polyacrylamide gel electrophoresis (APAGE)

BITTEL and GUSTAFSON (1992) explored the dosage response of rye storage protein genes in a wheat background with the APAGE method. They could similarly use the *Gli-1* and *Sec1* polymorphisms to distinguish among 1B homozygotes, 1B/1BL.1RS heterozygotes and 1BL.1RS homozygotes.

(iii) Zymograms

Glucose phosphate isomerase zymograms showed that 1DL.1RS translocations can be identified by the absence of *Gpi-D1* encoded bands and the presence of a new band, R1, associated with chromosome arm 1RS. This method should also be applicable for the identification of 1BL.1RS translocations (KOEBNER et al. 1986).

Another technique for the rapid identification of 1BL.1RS translocations was reported by HARTMANN et al. (1994). They used starch gel electrophoresis for two isoenzymes, malate dehydrogenase (MDH) and glucose phosphate isomerase (GPI). Clear diagnostic banding patterns could be observed to distinguish among normal wheat, 1B/1R substitutions and 1BL.1RS translocations. However, gel profiles of both these two isoenzymes need to be done in order to distinguish between the three lines.

The benefit of most electrophoretic procedures are that the procedure can be completed in one day, which is not possible with C- or N-banding.

1.4.1.3 Biochemical techniques

(i) *High-performance liquid chromatography*

Another method for identifying 1BL.1RS translocation wheat lines is the high-performance liquid chromatography (HPLC) method. Extracts from flour samples are analysed by a diode-array detector to detect the eluted components. The data are then stored on a computer in order to plot graphs. Comparative graphs were replotted of rye, 1BL.1RS translocation lines and 'normal' wheat. Three unique peaks were observed which probably reflected the presence of *Sec1* products and were associated with the presence of the 1RS rye chromosome segment. Although various electrophoretic methods are available to identify 1BL.1RS translocation lines, the co-migration of wheat and rye proteins may occur in some genotypes. HPLC can aid in distinguishing translocation carriers in such cases (LOOKHART et al. 1991).

(ii) *Monoclonal antibodies*

The monoclonal antibody method for detecting 1BL.1RS translocation lines is based on the absence of a wheat gene product. The *Gli-B1* locus located on chromosome arm 1BS of wheat codes for gamma-gliadin 45 or the allelic gamma-gliadin 42. These gliadins bind strongly to their monoclonal antibodies. HOWES et al. (1989) argued that 1BL.1RS translocation lines do not produce the gamma-gliadin 45 protein which therefore can not bind to the monoclonal antibodies. HOWES et al. (1989) used an enzyme immunosorbent assay (ELISA) to screen almost ten thousand kernels. He proposed that translocations involving the 1BS arm as well as 1B substitutions, 1B nullisomics and the 1B wheat chromosome in triticale and transgenic plants can be identified in this way. However, LOOKHART et al. (1991) cautioned that the absence

of the gene product may in some instances be caused by a mutation or chromosomal deletion independent of 1BL.1RS.

1.4.1.4 Molecular techniques

The major limitation to most of the above techniques is the failure to obtain markers for both advantageous as well as deleterious chromosome fragments of rye in wheat. The use of molecular markers may overcome this limitation since they cover the entire genome.

(i) *In situ hybridisation (ISH)*

The very useful technique of C-banding can only detect translocated rye chromosome fragments if these contain heterochromatic regions. The recently developed *in situ* hybridisation (ISH) technique also has application in detecting alien chromosome fragments in the wheat genome and has made it possible to accurately identify translocation breakpoints and transferred alien chromosome fragments. Presently, with ISH, genome rearrangements such as intercalary wheat-rye translocations can also be detected (FRIEBE et al. 1991a, 1991b, MUKAI et al. 1993a) mostly due to biotin-labeled probes that can recognise and bind both heterochromatic and euchromatic fragments of rye chromosomes. LAPITAN et al. (1986) were able to locate a small rye chromosome fragment using ISH where it was not possible through C-banding. Novel genes transferred to the wheat genome can be traced in this manner.

The 1BL.1RS translocation can specifically be identified with ISH using the probe pSc119 (MUKAI et al. 1993a). When the whole 1RS chromosome arm of rye is probed, the nucleolus organizer region (NOR) charateristically displays a very distinctive gap in the chromosome arm (MUKAI et al. 1993a).

HESLOP-HARRISON et al. (1990) hybridised the chromosomes of a 1BL.1RS

translocation wheat variety 'Beaver' with labeled genomic DNA of rye. The two rye chromosome arms fluoresced brightly, while the other chromosomes fluoresced very little. The NOR region on the 1RS arm could be observed as a dark gap.

The specificity of probing can be improved upon with the use of blocking DNA. The blocking DNA hybridises with sequences common to both the rye and wheat genome. The block, consisting of 100 - 200 base pairs, hybridises with the probe in solution and also with sequences on the chromosomes. This prevents subsequent binding of the labeled probe and the use of high ratios of block DNA to probe DNA can increase the specificity of hybridisation. However, background signals on chromosome spread preparations may also be enhanced (HESLOP-HARRISON et al. 1990).

Genomic probes hold the potential to detect alien chromosomes or chromosome segments and also to detect changes in the physical size of an alien chromosome segment (HESLOP-HARRISON et al. 1990).

(ii) *RFLPs*

Labeled total genomic rye DNA can be used as a probe to identify the presence of rye chromosomes or a segment of a rye chromosome in wheat (HESLOP-HARRISON et al. 1990). The total genomic DNA was cut with the restriction endonucleases *EcoR1* and *Dra1*. The luminograph after Southern hybridisation showed strong hybridisation to rye chromosome fragments while the wheats without rye chromosome fragments showed very little detectable hybridisation.

The same membrane was then probed with the wheat ribosomal DNA (rDNA) clone, pTA71, that contains spacer and coding sequences. The hexaploid and diploid wheats as well as triticale showed a restriction fragment of approximately 9 kb. The wheat genotypes carrying chromosome 1RS, triticale and rye showed two restriction fragments of approximately 4 and 4.5 kb on the luminograph (HESLOP-HARRISON et al. 1990). APPELS et al. (1986) claim that this clone indicates the presence of the NOR region, but give no information about the size of the translocation.

Total plant DNA of 'Chinese Spring' was extracted and digested with the restriction endonuclease *Taq1*. Three characteristic bands were observed on a Southern blot after hybridisation with the DNA sequence, pScR4-T1. When the rye translocation 1BL.1RS was present, and the same DNA sequence was hybridised to the total plant DNA, four characteristic bands on the Southern blot could be identified. Together with the three 'Chinese Spring' bands an extra band was present assaying the *NOR-R1* locus (KOEBNER et al. 1986). The *5S RNA* locus, located interstitially between *NOR-R1* and the telomere on 1RS (APPELS et al. 1980) can be assayed with the same method by using the DNA sequence pSc 5S-T7. While no hybridisation with the DNA sequence pSc-het1 could be observed when total 'Chinese Spring' DNA was *Taq1* digested, one major band could be seen on a Southern blot when the DNA from a 1BL.1RS translocation plant was used. Thus, the pSc-het1 probe assays rye telomeric heterochromatin (KOEBNER et al. 1986).

(iii) *RAPD-analysis*

IQBAL and RAYBURN (1995) conducted a study to identify marker loci which could be used to detect the 1RS rye fragment in a wheat background by using RAPD-analysis. Near-isolines, where the 1BL.1RS translocation was absent or present, were used. Two out of a total of 120 primers produced rye-specific amplification fragments. The 1BL.1RS and 1AL.1RS introgressions exhibited the two fragments that were missing in the non-translocation lines. These two markers associated with 1RS introgressions were designated *Xim1* and *Xim2*. IQBAL and RAYBURN (1995) found that these two sequences were not homologues and represented different loci. Thus, random primers can be used to target rye translocations in wheat backgrounds.

FRANCIS et al. (1995) converted a RAPD-generated PCR product that contained a unique dispersed repetitive element (AF1/AF4) into a quicker robust assay for the presence of rye chromosome fragments in wheat. This can then be used to screen for the presence of 1RS or any other wheat-rye translocation.

1.4.2 Quality defects associated with the 1BL.1RS translocation

The release of wheat cultivars containing the 1BL.1RS translocation is restricted, also in South Africa, in spite of high yield potential and environmental stability (MORENO-SEVILLA et al. 1991, 1992) because of undesirable dough properties associated with the 1RS chromosome arm of rye (MARTIN and STEWART 1986, DHALIWAL et al. 1987). Characteristic of the dough defects are dough stickiness, reduced strength and intolerance to overmixing. Ideally, only the traits of interest and no flanking material of chromosome arm 1RS should be transferred. The location of the *Sec1* locus, reputed to be the problem area, makes it more difficult to rectify the translocation. In order to get rid of just a small rye chromosome fragment, genetic markers evenly spread along the chromosome are needed. ROGOWSKY et al. (1993) found it impossible to detect possible breakpoint differences in the satellite because the 11 RFLP and three PCR markers they used, are clustered proximal to the *Sec1* locus. A study by PENA et al. (1990) could also not associate the 1RS chromosome arm with dough stickiness.

Considerable variation exists in end-use quality of wheat possessing 1RS and some lines display acceptable quality. The identification of appropriate genetic backgrounds might improve the breadmaking quality of 1RS wheats (LEE et al. 1995). Numerous genetic backgrounds should be examined before the precise causes of the detrimental effects of 1RS are known. By combining 1RS lines with parents possessing high quality glutenin and gliadin, or easily degradable salt-water soluble proteins and non-1RS lines with strong gluten type, the detrimental quality effects may be neutralized (LEE et al. 1995).

1.5 OBJECTIVE OF THIS STUDY

This study reports the outcome of an attempt to transfer genetic resistance to RWA from the rye cultivar 'Turkey 77' to a common wheat that has the 'Veery' translocation. An attempt was also made to map the resistance locus or loci in question on the short arm of chromosome 1R and to determine the number of genes involved.

2. MATERIALS AND METHODS

2.1 Origin of a Russian wheat aphid (RWA) resistant monotelosomic 1RS addition plant (plant A):

A wheat plant (plant A) that had a 1RS addition chromosome carrying a gene for resistance to the RWA was used as the starting material in this study. This addition line was derived by MARAIS (1991) as follows and summarized in Fig.3.

An accession from the World Rye Collection, 'Turkey 77', was found to be resistant to local collections of RWA. 'Chinese Spring' (CS) wheat was crossed as the female parent with 'Turkey 77' in order to derive RWA resistant wheat X rye hybrids. Fifty-eight F_1 wheat X rye hybrid seeds were obtained and RWA resistance tests were done on the resulting seedlings. The RWA feeding symptoms varied considerably among the F_1 plants (1,2,3,4 and 5 readings were obtained) suggesting that more than one resistance gene may have been present in 'Turkey 77'. Twenty-six RWA resistant hybrids were identified, cloned and treated with colchicine to double their chromosome numbers. Thirteen adult plants were raised. A number of C_1 seeds were harvested from these plants. Root tip chromosome counts were done on the C_1 progeny using Feulgen staining. Root tip chromosome counts revealed that only one C_1 plant was octoploid (2n = 56), the chromosome numbers of the remaining C_1 plants ranging from 49 to 63. The octoploid plant was fertile and produced well developed seeds upon selfing. Three plants found with $2n < 56$ probably arose from open pollination (wheat pollen) of egg cells having restitution nuclei. Chromosome numbers > 56 appeared to result from the presence of rye B-chromosomes in the respective F_1 plants which were found to be very infertile.

The RWA resistant amphiploids and partial amphiploids identified from the C_1 progeny were then pollinated with: (i) the 'Imperial' rye to CS disomic addition lines (1R - 7R, 2n =44), and (ii) the hexaploid wheat, 'Inia 66'. The pollinators used in both

strategies were RWA susceptible. By crossing the amphiploids and partial amphiploids with an addition line it was hoped that the transmission of a specific rye chromosome would be stabilized. The use of the different (1R - 7R) disomic additions was expected to result in the retention of a different 'Turkey 77' chromosome in the selfed progeny of each cross, thus facilitating the identification of the critical chromosome(s). The 'Imperial' rye to CS disomic additions were evaluated for resistance to the RWA and proved to be highly susceptible. The 2R and 5R addition plants were poorly developed, male sterile and were not used in the crosses.

For both strategies (i) and (ii) the F_1 embryos had to be rescued. The resulting F_1 plants were allowed to self-pollinate. F_2 plants from the successful crosses were tested for resistance to RWA. The F_2 families obtained following the pollination of RWA resistant amphiploids and partial amphiploids with the disomic additions of 'Imperial' rye to CS, gave results similar to the use of hexaploid wheat pollen. Resistant monosomic addition plants were recovered at similar frequencies from the respective crosses of both strategies. The use of the 'Imperial' to CS addition lines also failed to produce resistance-carrying progeny disomic for a specific rye chromosome. The progeny from crossing strategy (i) containing 44 chromosomes were monosomic for a specific rye chromosome. Eventually it appeared that strategy (ii) i.e. backcrossing to euploid wheat, was a simpler and more effective way to obtain resistant addition plants.

Sixty-eight resistant F_2 plants were recovered from crossing strategies (i) and (ii). Plants were raised in a greenhouse and root tips were cut with the aim of doing chromosome counts and C-banding. The chromosome numbers of these plants varied from 42 - 52 with an average of 46. Twenty-three of the plants with 43 or 44 chromosomes were successfully C-banded, 19 of them had 1R present as the only, or one of the added chromosomes while one (plant A) had an addition of 1RS only. Plant A resulted from crossing strategy (ii). None of the 44-chromosome plants were disomic

additions. The results suggested that chromosome 1R of 'Turkey 77' has a resistance gene on its short arm.

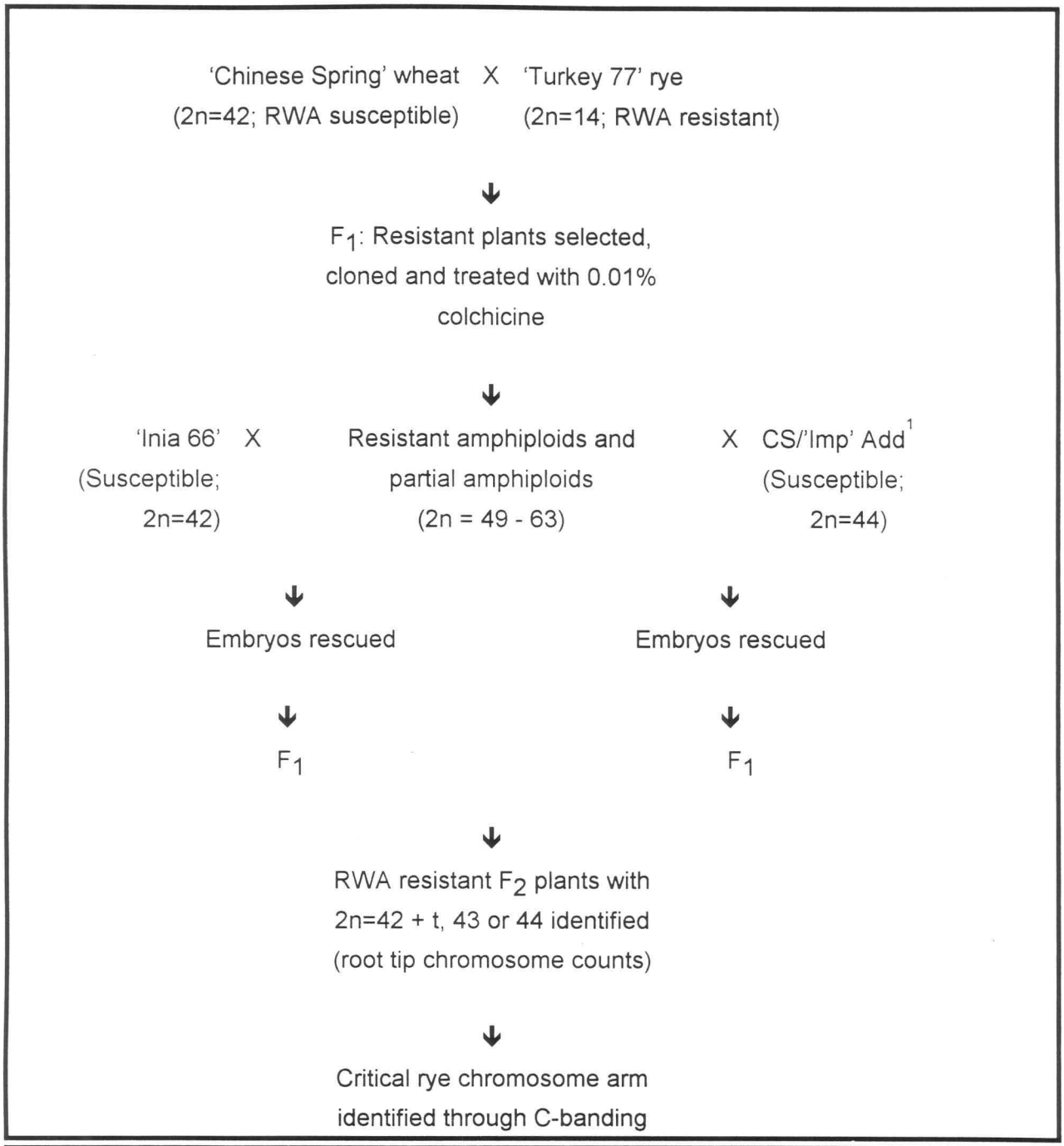

Fig. 3. Development of Russian wheat aphid (RWA) resistant octoploid triticales and determination of the chromosome location of the RWA resistance. [1] Disomic additions of 'Imperial' rye chromosomes 1R-7R to 'Chinese Spring' wheat.

2.2 Confirmation of the location of the RWA resistance gene on chromosome arm 1RS of 'Turkey 77':

In order to confirm the location of a RWA resistance gene on 1RS of 'Turkey 77', root tip chromosome counts and RWA resistance tests were done on 50 F_3 progeny of plant A ($2n = 42 + t^{1RS}$; derived by MARAIS 1991). Seeds were germinated and root tips were cut. The seedlings were then sent by courier to Dr. F. du Toit (PANNAR, P.O. Box 17164, Bainsvlei, 9388, Republic of South Africa) who planted and screened them for RWA resistance. The data were studied to determine whether the resistance cosegregated with the 1RS telosome.

2.3 The transfer of the resistance gene on 1RS to common wheat:

In an attempt to transfer the resistance gene on 1RS of 'Turkey 77' rye to wheat, the common wheat cultivar, 'Gamtoos', was used to pollinate a RWA resistant selection with the chromosome constitution: $2n = 42 + t^{1RS}$ (plant A)(Fig. 4). 'Gamtoos' has the 1BL.1RS ('Veery') translocation. By crossing plant A with 'Gamtoos' it was hoped that the RWA resistance gene could be transferred to the 1RS segment of the 1BL.1RS ('Veery') translocation chromosome through homologous pairing and recombination during meiosis in the F_1. This will make the 1BL.1RS translocation even more useful in wheat breeding since it already carries the rust resistance genes *Lr26*, *Sr31* and *Yr9* and is known to be associated with a yield advantage in many countries (DHALIWAL et al. 1987, LOOKHART et al. 1991, METTIN and BLUTHNER 1984, MORENO-SEVILLA et al. 1991, 1992). Root tips were cut on the F_1 progeny of the cross between plant A and 'Gamtoos'. Following root tip chromosome counts and C-band screening, one plant was selected which had the chromosome constitution: $2n = 40 + 1B + 1BL.1RS + t^{1RS}$ (plant B). Ears were cut from plant B for meiotic studies to determine the frequency of pairing between the 1RS 'Veery' and the 1RS 'Turkey 77' chromosome arms and thus the expected recombination frequency betweem them.

This was expected to indicate the feasability of and strategy for transferring the RWA resistance gene to the 1RS arm of the 1BL.1RS ('Veery') translocation.

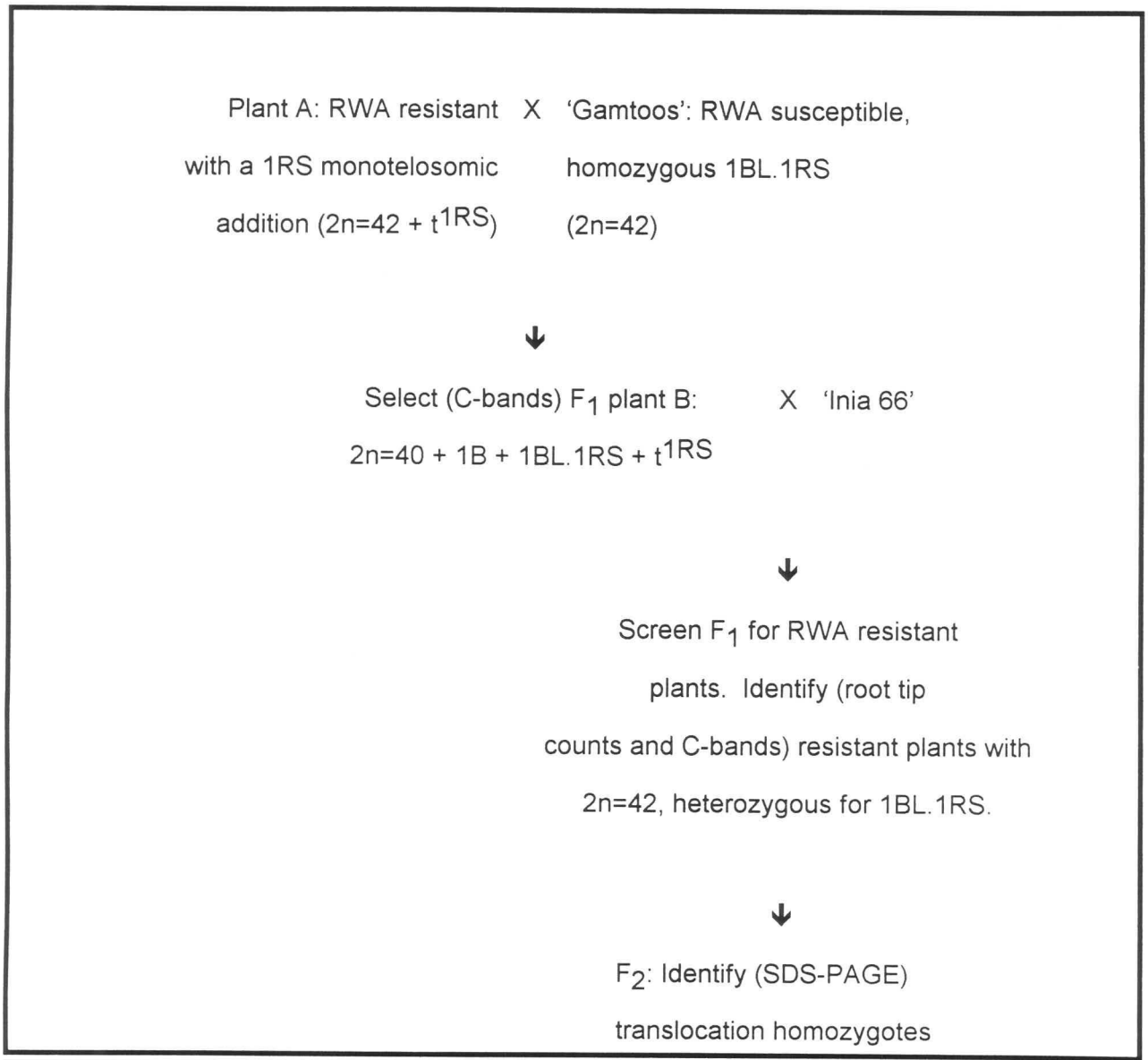

Fig. 4. The transfer of the RWA resistance gene from 1RS of 'Turkey 77' rye to the 1BL.1RS translocation of common wheat.

The remaining ears of plant B were pollinated with a RWA susceptible tester, 'Inia 66', in an attempt to recover resistant disomic translocation heterozygotes (2n = 42). If such plants could be recovered, they would probably have resulted from the transfer through homologous recombination of a 1RS segment of 'Turkey 77' to the 1RS segment of the 1BL.1RS ('Veery') translocation. Root tip chromosome counts and RWA resistance tests were done on 144 F_1 progeny. Root tips from the seven RWA resistant, euploids (2n = 42) that were identified, were C-banded to confirm that they were heterozygous for the 1BL.1RS translocation. Five confirmed 1BL.1RS translocation heterozygotes were inoculated with stem rust pathotype, 2SA4, and the leaf rust pathotype, 3SA132, to test for the presence of the *Lr26* and *Sr31* genes. 3SA132 and 2SA4 are respectively avirulent on *Lr26* and *Sr31* which are carried on the 1BL.1RS ('Veery') translocation in 'Gamtoos'. Both pathotypes are virulent on CS and 'Inia 66'.

2.4 Secalins used as marker genes:

Storage proteins were studied in order to determine (i) whether the 'Turkey 77' and 'Veery' derived alleles of the *Sec1* locus produce polymorphic bands that could be used to map the RWA resistance gene, and (ii) whether the *Sec1* locus could be used to select indirectly for the RWA resistance gene. To be useful in selection, the *Sec1* allele products should be unambiguously distinguishable from the wheat storage proteins.

Storage proteins were studied by extracting total proteins from wheat kernels and separating it by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The procedure was as described by HOLT and PAYNE (1981) except that 12% gels were used.

The bands associated with the wheat storage proteins were determined by studying the SDS-PAGE profiles of CS telosomic and nullisomic tetrasomic stocks (obtained from the late professor E.R. Sears, Department of Agronomy, University of Missouri, Columbia, Missouri, 65211,USA). SDS-PAGE profiles were obtained of the following: CS ditelosomic 1AL (CSDT1AL), CSDT1DL, CS nullisomic 1A tetrasomic 1B (CSN1AT1B), CSN1AT1D, CSN1BT1A, CSN1BT1D, CSN1DT1A, CSN1DT1B.

Kernels obtained following selfing of the monotelosomic addition plants (2n = 42 + t^{1RS}) were cut in half and the endosperm halves were used for SDS-PAGE. The embryo halves of the kernels were germinated and root tip chromosome counts were done on them. The *Sec1* proteins were identified as those that cosegregated with the 1RS telosome.

Since the *Sec1* bands could be distinguished from the 1B encoded protein bands, SDS-PAGE profiles were then used to distinguish between 1BL.1RS translocation homozygotes, 1B/1BL.1RS translocation heterozygotes and 1B homozygotes. Fifty-six F_2 seeds, derived from RWA resistant F_1 translocation heterozygotes, were halved. SDS-PAGE was done with each of the 56 endosperm halves. RWA resistance tests were performed on the embryo halves of the 56 F_2 seeds. The SDS-PAGE profiles were compared with the RWA resistance data to determine if it was possible to identify the RWA resistant F_2 progeny through SDS-PAGE.

2.5 Determining the location of the resistance gene:

The monotelosomic additon of chromosome arm 1RS of 'Turkey 77' rye to common wheat (plant A; 2n = 42 + t^{1RS} - MARAIS et al. 1994) carries a gene for RWA resistance, however, the 1RS telosome was found to lack *Lr26* and *Sr31*. As a result the polymorphic differences between the 1RS telosome derived from 'Turkey 77' rye

and the 1RS segment in the 1BL.1RS 'Veery' translocation, which carries the *Lr26* and *Sr31* resistance genes, could be used to map the RWA resistance gene. In an attempt to determine the number of RWA resistance loci involved and their locations relative to known markers on 1RS, the following crosses were made:

(1) Plant A ($2n = 42 + t^{1RS}$) X 'Gamtoos': 'Gamtoos' is RWA susceptible and carries the rust resistance genes *Lr26* and *Sr31*. From this cross plant B with $2n = 42 + t = 40 + 1B + 1BL.1RS + t^{1RS}$ was selected. Plant B was then used to pollinate 'Inia 66' monosomic 1B. Plant B was used as the pollinator since the transfer of the monosomic condition through the female gametes takes place at a higher frequency than through the male gametes. Monotelodisomic F_1 progeny were obtained and C-banded to identify plants in which 1B was absent while 1BL.1RS and 1RS was present. No such plants were recovered although it was expected that $\pm$ 50% of the progeny would carry the 1BL.1RS translocation. Plants with $2n = 40 + 1B + t^{1RS}$ were inoculated with stem and leaf rust to confirm no resistance to *Sr31* and *Lr26* and therefore no 1BL.1RS translocation chromosome. These plants were then crossed with 'Gamtoos'. 'Gamtoos' was used as the pollinator to ensure frequent transfer of 1RS through the egg cells. Chromosome counts and C-banding were used to identify a plant with the required chromosome constitution ($2n = 40 + 1BL.1RS + 1RS$). One such plant was recovered from the cross (indicated as plant C). Meiotic studies were done with plant C to determine the frequency of pairing between the 1BL.1RS 'Veery' translocation and the 1RS 'Turkey 77' telosome. Plant C was also testcrossed with a RWA susceptible tester, CS, in order to do telocentric mapping of the RWA resistance gene. The testcross F_1 seed were germinated and root tips were collected. Chromosome counts and stem rust (2SA4) resistance tests were done. The rust resistance data obtained implied that very abnormal segregation occurred and that the data would not give reliable estimates of genetic distances. Due to the laborious nature of the RWA screening tests, it was decided no to go ahead with these tests.

(2) In a second attempt at mapping the RWA resistance gene, a plant, disomic for a recombined 1BL.1RS translocation that carries the RWA resistance but not *Lr26* and *Sr31*, was crossed with 'Gamtoos'. With this strategy the location of the RWA resistance gene can only be determined relative to the rust resistance genes. The F_1 progeny from this cross were C-banded to confirm that they were translocation homozygotes. The F_1 was then testcrossed with CS. The testcross F_1 seeds were grown in a greenhouse and the seedlings were left to self pollinate. Three samples of 15 - 20 TF_2 seeds per TF_1-derived family were then grown and tested for the segregation of leaf rust (3SA132) resistance, stem rust resistance (2SA4) and RWA resistance. The data were used to derive linkage distances and to deduce the number of RWA resistance genes segregating.

2.6 Leaf and stem rust seedling tests:

2.6.1 Inoculation

Seedlings at the 2-3 leaf stage were inoculated by thorough spraying with a suspension of rust spores in distilled water to which a few drops of a wetting agent, Triton, were added. Plastic bags were wetted on the inside and the seedlings were covered with the bags. The seedlings were put in a growth chamber where the temperature ranged from 18 - 20°C . After 24h of incubation the plastic bags were removed and the seedlings placed on a bench under lights where the temperature ranged from 22 - 25°C and a day/night cycle of 16h/8h was maintained.

2.6.2 Disease assessment

Leaf rust infections were scored 7 - 10 days after inoculation, while stem rust infections were evaluated 14 - 16 days after inoculation. Infections were scored according to

STAKMAN et al. (1962) on a 0 - 4 scale. Zero to two indicated resistance, while 3 - 4 indicated susceptibility.

2.7 RWA resistance tests:

All RWA resistance tests were done by Dr. F. du Toit (PANNAR, P.O. Box 17164, Bainsvlei, 9388, Republic of South Africa). Plant damage caused by the RWA was scored as described by DU TOIT (1987).

2.8 C-banding:

The C-banding procedure described by GIRALDEZ et al. (1979) was used.

2.9 Root tip chromosome counts

Root tips were cut and chromosomes counted following Feulgen staining.

3. RESULTS AND DISCUSSION

3.1 Confirmation of the location of the RWA resistance gene on chromosome arm 1RS of 'Turkey 77':

In order to confirm that a RWA resistance gene is associated with the 1RS telosome, root tip chromosome counts and RWA resistance tests were done on 50 F_3 progeny from a F_2 plant with the chomosome constitution 42 + t^{1RS} (plant A). Seventeen plants had 42 + t or 42 + 2t chromosomes and were resistant to the RWA, whereas 33 plants had 42 chromosomes and were susceptible. Thus, chromosome arm 1RS of 'Turkey 77' carries a RWA resistance gene. The reason for the larger euploid progeny is that unpaired chromosomes, like in this instance the 1RS telosome, are prone to be lost during meiosis and therefore a larger number of selfed progeny without the 1RS telosome results.

3.2 The transfer of the resistance gene on 1RS to common wheat:

The RWA resistant plant with 2n = 42 + t^{1RS} (plant A) was crossed with the common wheat cultivar 'Gamtoos' (which has the 1BL.1RS 'Veery' translocation) in an attempt to transfer the resistance gene(s) on 1RS of 'Turkey77' to the 1RS segment of the 1BL.1RS ('Veery') translocation. Root tip chromosome counts on the F_1 led to the identification of one plant with 2n = 42 + t chromosomes. The inferred chromosome constitution of this plant (plant B; 2n = 40 + 1B + 1BL.1RS + t^{1RS}) was confirmed by means of C-banding.

The 1BL.1RS translocation chromosome and the 1RS telosome can be readily identified by their C-banding patterns. The 1RS segment shows a large terminal and a subterminal C-band which are characteristic of the short arm of chromosome 1R of rye.

The 1BL.1RS translocation can similarly be distinguished from chromosome 1B by its 1RS arm fused with the 1BL chromosome arm of wheat that characteristically displays one terminal, two proximal and one centromeric C-bands (FRIEBE et al. 1991a) (Fig. 5.).

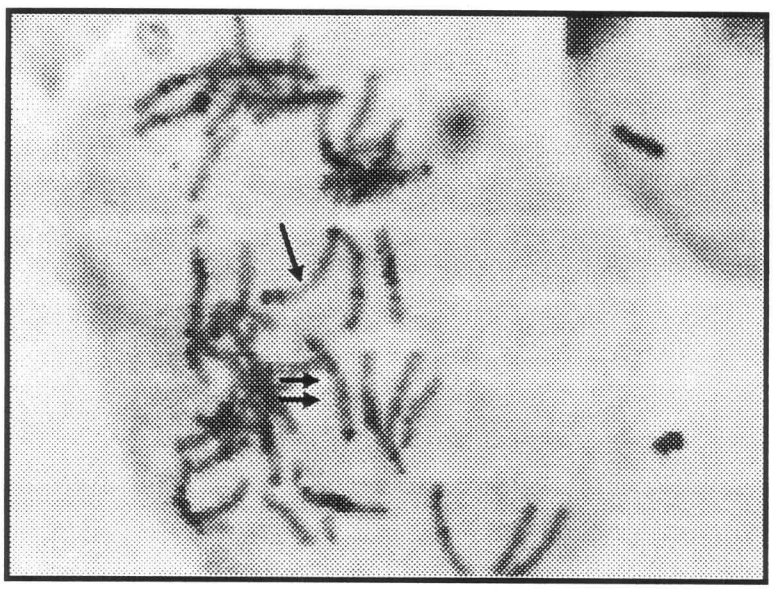

Fig. 5. C- banded chromosomes of a translocation heterozygote. The translocated 1B/1BL.1RS chromosome (arrow) and the normal chromosome 1B (two arrows) are indicated.

Meiotic studies that were done on plant B showed that the telosome paired with the rye segment of the 1BL.1RS translocation in 32% of the pollen mother cells (PMC's) (Fig. 6.). The 22 PMC's studied had the following average configurations: 20.05 II + 0.14 II(H) + 1.23 I + 0.68 t + 0.18 III(H) + 0.05 IV. The relatively high frequency of univalents appears to relate to the observation of PIENAAR (personal communication) that the

'Imia 66' and CS genomes may differ with respect to at least two translocations. The results imply that it should be possible to transfer the resistance to the 1BL.1RS translocation through crossing over.

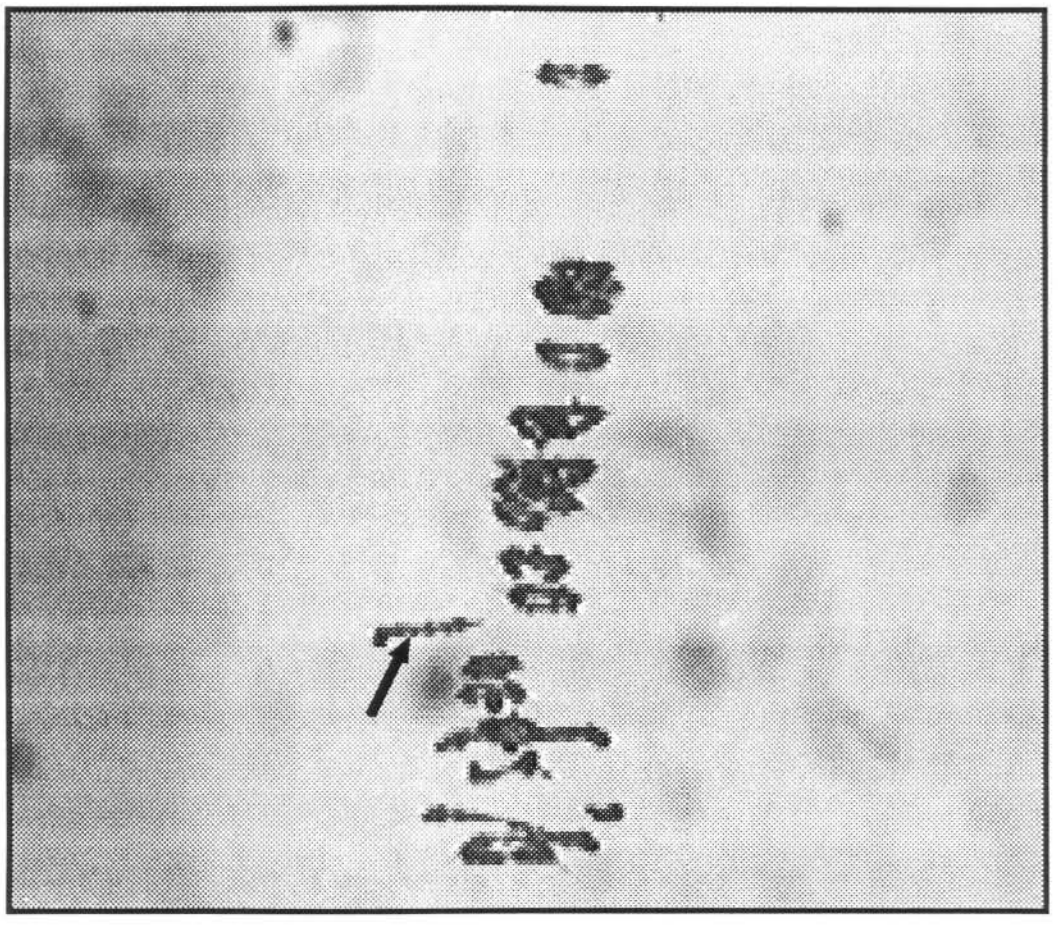

Fig. 6. A PMC of plant B containing a heteromorphic bivalent that is the result of pairing between the 1RS 'Turkey 77' telosome and the 1RS arm of the 1BL.1RS 'Veery' translocation.

Root-tip chromosome counts and RWA resistance tests of 144 TF_1 progeny obtained following the pollination of plant B (2n = 40 + 1B + 1BL.1RS + t^{1RS}) with a RWA susceptible tester ('Inia 66') (Table 1), revealed that seven of the 99 euploid (2n =42) progeny were resistant . The seven euploid, resistant plants were then C-banded and a 1BL.1RS translocation was observed in five of the plants. One of the seven apparently disomic, RWA resistant testcross F_1 plants had an isochromosome (1RS.1RS). Another plant died and could not be tested.

Table 1. Chromosome numbers and RWA resistance of F_1 plants obtained following the pollination of plant B (2n = 40 + 1B + 1BL.1RS ('Veery') + t^{1RS}('Turkey 77') with 'Inia 66' (RWA susceptible)

Chromosome number	Number of plants		
	Resistant	Susceptible	Total
43 + t	1	0	1
42 + t	27	11	38
42	7	92	99
41 + t	1	1	2
41	0	4	4
Total	36	108	144

The results of seedling rust resistance tests performed on the five recombined plants and the parental controls are given in Table 2. A disomic 1RS ('Turkey 77') addition was also tested for resistance to leaf rust (3SA132) and stem rust (2SA4) and proved to be susceptible to both pathotypes.

Table 2. Seedling leaf and stem rust resistance data of 5 recombined lines and their parental controls.

	Seedling infection type: Leaf rust (3SA132)	Stem rust (2SA4)
Disomic addition of 1RS('Turkey77')	4	4
'Gamtoos'	;	;1-
'Chinese Spring'	4	4
'Inia 66'	4	3-4
93M45-5[1]	3-4	4
93M45-14[1]	;	;
93M45-66[1]	3-4	4
93M45-67[1]	4	not tested
93M45-83[1]	3-4	4

[1]RWA resistant selections, heterozygous for recombined 1BL.1RS translocation chromosomes.

This means that the 'Turkey 77'-derived 1RS segment does not have *Lr26* and *Sr31*. However, the 'Veery' translocation is resistant to both pathotypes. One plant, RWA resistant and resistant to both rust pathotypes, was obtained among the five recombined plants. The results suggest that in at least one instance recombination took place between the linked rust resistance genes (*Sr31* and *Lr26*) and the RWA resistance gene. This is potentially the most useful recombinant chromosome, since it combines all the resistance genes (leaf rust, stem rust and RWA). The latter selection will subsequently be referred to as plant D.

Twelve plants were also recovered that had the 1RS telosome but were RWA susceptible. A total of 18 plants therefore appeared to be recombinant types (13.2% recombination). Since the 1BL.1RS translocation and 1RS appeared to pair in approximately 30% of the PMC's, the RWA resistance gene is probably loosely linked or not linked to the 1RS centromere. The five plants with recombinant 1BL.1RS translocations were allowed to self pollinate in order to derive translocation homozygotes.

3.3 Secalins used as marker genes:

SDS-PAGE profiles, of the CS telosomic and nullisomic tetrasomic stocks (nulli-tetras) that were used to identify the 1B encoded wheat gliadin bands, showed two bands that were associated with chromosome 1B. These two bands were present in all the nulli-tetras except CSN1BT1A and CSN1BT1D. They were also absent in 1BL.1RS homozygotes which would suggest that they occur on chromosome arm 1BS.

In an attempt to confirm the storage protein bands produced by the 1RS 'Turkey 77' telosome and the 1RS rye arm of the 1BL.1RS 'Veery' translocation, SDS-PAGE

profiles were obtained of 73 progeny of plants that had a monotelosomic, 1RS, addition chromosome. 'Gamtoos' was included as a control. Based on its correlation with the presence of the 1RS telosome, an omega-secalin band (*Sec1* locus) was identified . Unfortunately this band coincided with the omega-secalin produced by the 1BL.1RS 'Veery' translocation and could not serve as a genetic marker to differentiate between the 'Turkey 77' 1RS and the 'Gamtoos' 1RS ('Veery') segments (Fig. 7.).

The *Sec1* locus was then used as a genetic marker for the presence of the 1BL.1RS translocation and an attempt was made to confirm that the RWA resistance is associated with the recombinant translocation chromosome in some of the recombinants.

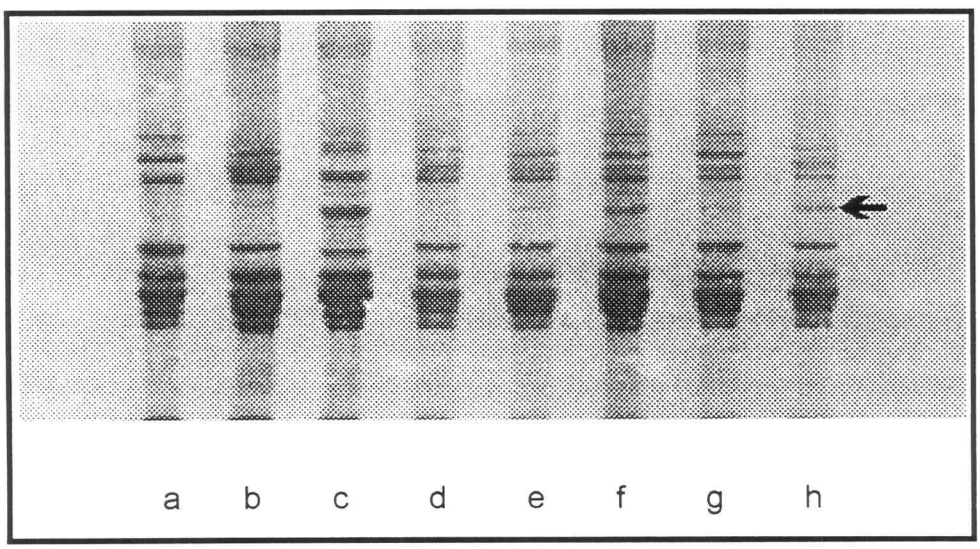

Fig. 7. SDS-PAGE profiles of endosperm storage proteins of: **a** CS; **b** 'Inia 66'; **c** 'Gamtoos'; **d** 1B homozygote, 2n = 42; **e - h** 1B homozygote with monotelosomic addition chromosomes, 2n = 42 + t. The position of the omega-secalin band (←) is indicated.

A total of 56 F_2 seeds derived from two plants, each having a normal chromosome 1B and a recombined 1BL.1RS translocated chromosome, were halved. The parental plants were both RWA resistant, one (plant D) also carried *Sr31* and *Lr26*. SDS-PAGE profiles were obtained from the endosperm halves and the presence of the secalin and/or two 1BS encoded gliadin bands were used to classify progeny as 1BL.1RS homozygotes, 1BL.1RS heterozygotes or 1B homozygotes. The embyo halves were planted for RWA resistance tests. In 1BL.1RS translocation homozygotes the *Sec1* associated band was more intense and no 1BS gliadins were present. When the 1BL.1RS translocation was absent, gliadin bands associated with the 1BS arm of the wheat genome were present and no *Sec1* band could be observed. In 1BL.1RS translocation heterozygotes the 1BS gliadin bands as well as the *Sec1* band could be seen (Fig. 8).

The RWA seedling tests performed with the embryo halves showed that eight plants were RWA resistant homozygotes for 1BL.1RS and 34 plants were RWA resistant heterozygotes for 1BL.1RS. The 14 plants that were homozygous for wheat chromosome 1B were susceptible to RWA. Three plants expressed the secalin band, yet were identified as being susceptible to RWA. However, the halved seeds gave rise to weak seedlings and it is very likely that in these instances misclassification occurred as a result. The data therefore confirm that the RWA resistance gene(s) was transferred successfully onto the 1BL.1RS translocation. It was also possible to retain the genes *Sr31* and *Lr26* on the translocated segment of plant D.

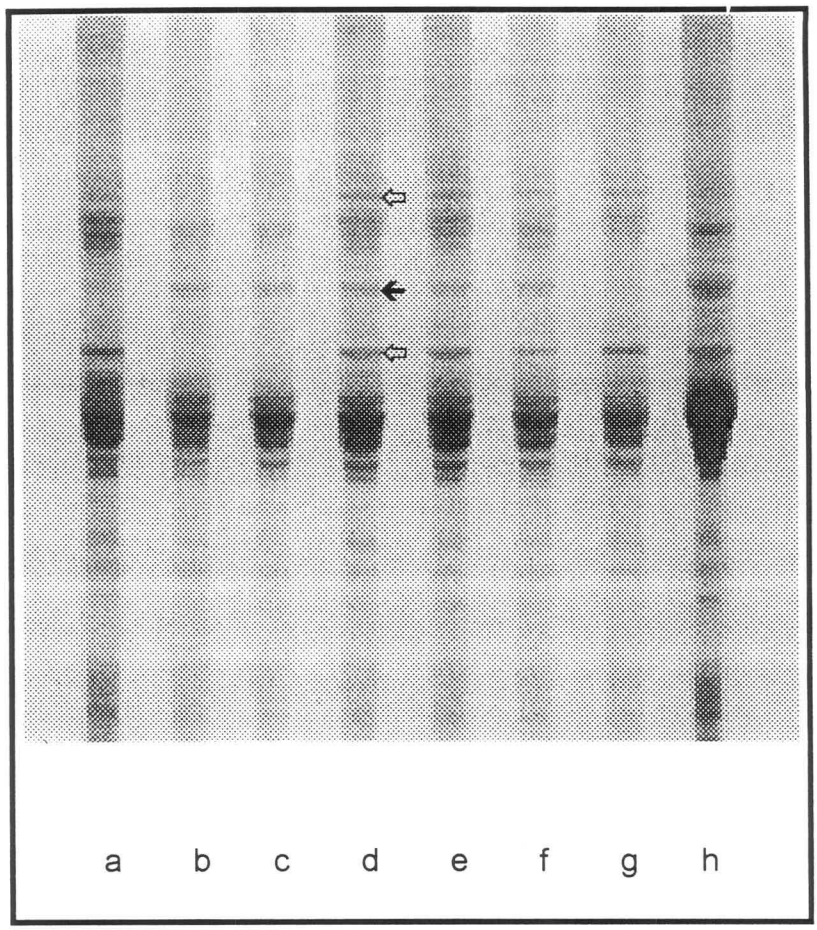

Fig. 8. SDS-PAGE profiles of endosperm storage proteins: **a** 'Inia 66'; **b-c** 1BL.1RS homozygotes; **d-f** 1BL.1RS heterozygotes; **g** 1B homozygote and **h** 'Gamtoos'. The positions of the 1BS encoded gliadins (⇦) and *Sec1* (🡐) products are indicated

3.4 Determining the location of the resistance gene:

(1) Genes of allopolyploid higher plants can be assigned to chromosome arms and their relative distance from the centromere determined by the use of telocentric chromosomes (RéDEI, 1983). 'Inia' monosomic 1B was pollinated with plant B (2n =

40 +1B +1BL.1RS ('Veery') + t^{1RS}) in order to obtain progeny with a chromosome constitution : 2n = 40 + 1BL.1RS ('Veery') + 1RS, which were required for the telocentric mapping of the resistance gene. Chromosome counts were done on 47 F_1 progeny from this cross. Only three plants had a chromosome constitution of 2n = 41 + t. They were C-banded and no progeny with the required chromosome complement (2n = 40 + 1BL.1RS + 1RS) could be found. All the plants were monotelodisomic or disomic, implying that the 1RS telosome and the 1BL.1RS translocation were seldomly transmitted together through the pollen. The three monotelodisomic progeny, 2n = 40 + 1B + t^{1RS} , (labeled as 93M46-2, 93M46-5, 93M46-7) were then inoculated with stem (2SA4) and leaf rust (3SA132) to confirm that no rust resistance genes (*Sr31* and *Lr26*) occurred in them. The results of the rust resistance tests are shown in Table 3.

Table 3. Seedling leaf and stem rust resistance of plants with a chromosome constitution of 2n = 40 + 1B + t^{1RS}

	Leaf rust (3SA132)	Stem rust (2SA4)
93M46-2[1]	4	3-4
93M46-5[1]	4	4
93M46-7[1]	4	4

[1]RWA resistant , monotelodisomic plants.

The selected monotelodisomic F_1 plants, susceptible to stem and leaf rust, were then crossed with 'Gamtoos' which has the 1BL.1RS translocation chromosome and thus the rust resistance genes *Sr31* and *Lr26*. One F_1 plant with 2n = 40 +1BL.1RS + 1RS (plant C) was selected by doing chromosome counts and C-banding. Meiosis was studied in thirty six PMC's of plant C. Five cells had univalents. In 31 PMC's all the chromosomes were paired and in four of these cells the heteromorphic bivalent could be distinguished. Thus, the frequency of pairing between the rye segment of the 1BL.1RS ('Veery') translocation chromosome and the 1RS ('Turkey 77') telosome was very high and the mapping data derived from this plant was expected to be accurate. The remaining ears on this plant (plant C) were pollinated with a RWA susceptible tester, CS, in order to map the RWA resistance gene(s) on chromosome 1RS relative to the centromere. Root tip chromosome counts were done on the F_1 seedlings and they were then inoculated with stem rust (2SA4). The results are summarized in Table 4.

It can be seen from these results that the parental telosome type progeny is a small percentage of the total progeny (9.2%). Thus, the original 'Turkey 77' telosome was transmitted at a relatively low frequency through the female gametes. LAWRENCE and APPELS (1986) calculated that the *Sr31*-locus is located at $\pm$31,4 cM from the centromere.

However, no linkage was detected in the present experiment by the standard chi-square test (P = 0.3588; Table 4). The segregation ratios of the two markers involved deviate significantly from the expected ratios as can been seen from the P values in Table 4.

Table 4. Results of chromosome counts and stem rust seedling resistance tests of the F_1 progeny from the testcross between plant C (2n = 40 + 1BL.1RS + 1RS) and 'Inia 66'.

Root tip chromosome counts	*Sr31*		
	present	absent	Total
41 + t	19[2]	7[1]	26
42	27[1]	23[2]	50
Total	46	30	76

[1]Parental types. [2]Recombinant types.

Chi-square test:

	df	χ^2	P
Sr31 Segregation	1	3.3684	0.066
Telo Segregation	1	7.7589	0.006
Linkage	1	0.8421	0.3588
Total	3	11.7895	0.008

(2) A second strategy to map the RWA resistance gene relative to the rust resistance genes (*Sr31* and *Lr26*) entailed the following. Translocation homozygotes were obtained by crossing a recombinant 1BL.1RS translocation line with RWA resistance but without stem and leaf rust resistance (line 93M54-83, Table 2), with 'Gamtoos'. F_1 translocation homozygotes were testcrossed with CS. The TF_1 progenies were selfed to derive large TF_2 families. The TF_2 seeds were used to test for the presence of *Sr31*, *Lr26* and RWA resistance. For each determination a sample of 15 - 20 TF_2 seeds were employed. The results obtained with *Sr31* and *Lr 26* are summarized in Table 5 where significant linkage is evident but also severe deviation from expected ratios for the two loci. However, the TF_1 plants were not isolated during selfing and it is possible that a degree of cross pollination occurred which subsequently resulted in misclassification of some of the families.The estimate of linkage is 10.42 map units suggesting very close linkage. SINGH et al. (1990) have previously reported 'unbroken linkage' between the three rust resistance genes implying that no recombinants were found in their material.

The RWA and leaf rust resistance data obtained for TF_2 families are summarized in Table 6. Sixty-one TF_1 plants were RWA susceptible while only 22 were resistant. The proportion of RWA resistant parental type progeny were similarly low. It appears that the 1BL.1RS translocation carrying the RWA resistance (that is the recombined translocation chromosome) was transmitted at a lower rate compared to the normal 'Veery' translocation. Thus, the transmission of the recombined 1BL.1RS chromosome appeared to be impaired and the data could not be used to estimate the number of RWA resistance genes involved. The reason for the lower transmission of the RWA resistance carrying chromosome is not clear.

The chi-square test for linkage in the results of Table 6 is highly significant ($P < 0.001$) and the estimate of linkage is 14.5 map units. However, it should be noted that highly significant deviations from expected segregation ratios are also present.

Table 5. Data obtained in an experiment to calculate the recombination frequency between the *Sr31* and *Lr26* loci.

Lr26	*Sr31*		
	present	absent	Total
present	34[1]	2[2]	36
absent	3[2]	9[1]	12
Total	37	11	48

[1]Parental types. [2]Recombinant types.

Chi-square test:

	df	χ^2	P
*Sr31*Segregation	1	14.083	<0.001
Lr26 Segregation	1	12.00	<0.001
Linkage	1	8.333	= 0.004
Total	3	34.417	<0.001

Table 6. Data obtained in an experiment to calculate the recombination frequency between the *Lr26* and RWA resistance gene(s).

	RWA resistance		
Lr26	present	absent	Total
present	4[2]	53[1]	57
absent	18[1]	8[2]	26
Total	22	61	83

[1]Parental types. [2]Recombinant types

Chi-square test:

	df	χ^2	P
RWA Segregation	1	18.3253	<0.001
Lr26 Segregation	1	11.5783	=0.001
Linkage	1	41.9398	<0.001
Total	3	71.8434	<0.001

4. CONCLUSION

Secale cereale (cultivated rye) is a distant relative of wheat that has proved to be an accessible source of genes that can be incorporated into wheat. Rye genes have been introduced into wheat as whole genomes, individual chromosomes or arms of chromosomes (GALE and MILLER 1987). Useful translocations such as the 1BL.1RS 'Veery' (leaf-, stem-, yellow rust and mildew resistance) (McINTOSH 1988) and the 1AL.1RS 'Amigo' translocation (mildew and greenbug resistance) (HEUN et al. 1990, HOLLENHORST et al. 1983, LOWRY et al. 1984) were derived in this manner. Using wheat-rye translocation lines as the starting material, and making use of the *ph*-mutants in wheat, rare recombination between wheat and rye chromosomes occured leading to rye segments smaller than the chromosome arms present in wheat (ROGOWSKY et al. 1992). NKONGOLO et al. (1990) detected RWA resistance in 'Imperial' rye and concluded that it results from the additive interaction of genes located on chromosomes 1R, 3R, 4R and 7R. However, 'Imperial' rye and its disomic additions to CS proved to be highly susceptible to collections of the aphid made in South Africa. Other reports of RWA resistance in rye have also been made (BUTTS and PAKENDORF 1984, NKONGOLO et al. 1989).

The 'Veery' translocation is associated with a yield advantage in many environments (MERKER 1982) but is generally ill-suited to the production of leavened bread products (ZELLER et al. 1982), primarily because secalins produced by the *Sec1* gene on 1RS cause dough stickiness during processing. However, indications are that the deleterious effects of *Sec1* may be masked in the presence of wheat genes coding for high quality glutenin and gliadin subunits (BARNES 1990). Furthermore, results obtained by KOEBNER et al. (1986) demonstrated that it may be difficult, yet not impossible, to replace the deleterious chromosome fragments of 1RS with homoeologous wheat chromosome fragments. Apart from its potential use in breeding bread wheats with acceptable quality, the 'Veery' translocation is also a valuable source of resistance for incorporation into feed wheats.

In this study a RWA resistance gene was successfully incorporated through homologous recombination in the 1BL.1RS translocation of wheat while the leaf and stem rust resistance genes, *Lr26* and *Sr31*, were retained. While this should improve the usefulness of the 1BL.1RS translocation, it is not clear whether the rye alleles coding for a yield advantage in 'Veery' selections were also retained. To determine this, isogenic lines having the recombined and 'Veery' translocations, respectively, should be constructed and compared in yield trials. The location of the RWA resistance locus on the 1RS arm of the 1BL.1RS translocation also means that its selection may in future be based on the more readily detectable linked 1RS loci, *GPi*, *Sec1*, *Sr31*, and *Lr26* or on C-bands rather than biotests.

The *Sec1* locus was physically mapped to the satellite region of 1RS (GUSTAFSON et al. 1990). Recombinationally it is located close (26.1 cM) to the centromere while the tightly linked rust resistance loci (*Sr31*, *Lr26* and *Yr9*) are situated 5.4 cM distally from *Sec1* on 1RS (SINGH et al. 1990). Present indications are that the RWA resistance locus is located distally from the rust resistance loci. Its location is therefore reconcilable with attempts to improve the genetic utility of 1BL.1RS translocation by removing the *Sec1* locus through homoeologous recombination.

Due to the distorted segregation ratios obtained in the mapping attempts of this study, it could not be determined with certainty that the RWA resistance locus is situated distally from the rust resistance loci (*Sr31, Lr26).* Distorted segregation ratios of the RWA resistance prevented the determination of the number of genes involved. However, the RWA resistance gene was located closely to the *Lr26* locus (14.5 map units) in this study.

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6. ADDENDUM

Part of this study has been published in Plant Breeding. The reference is as follows:

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