TRANSFER OF RESISTANCE TO LEAF RUST AND STEM RUST FROM *Triticum tauschii* TO HEXAPLOID WHEAT, CYTOGENETIC MAPPING OF THE GENES INVOLVED, AND RESTRICTION MAPPING OF RIBOSOMAL DNA IN *T. tauschii*.

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by

Robert L. Innes

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ABSTRACT

Genetic diversity was examined in a group of 12 accessions of *Triticum tauschii* (2n=14, DD) with regard to variability for resistance to leaf rust and stem rust, and variability in the ribosomal DNA (rDNA) repeat unit. Intercrosses among the 12 accessions indicated the presence of four distinct genes for seedling leaf rust resistance and one gene conferring adult-plant stem rust resistance. In synthetic hexaploids (2n=42, AABBDD) produced from crosses between three accessions of *T. tauschii* and TetraCanthatch (2n=28, AABB), three adult-plant leaf rust resistance genes were detected. Additionally, one seedling gene for stem rust resistance was detected.

The nine rust resistance (seven leaf rust and two stem rust) genes identified in these accessions were transferred to common wheat (2n=42, AABBDD) by direct introgression, by the production of amphiploids, and by the recovery of restitution gametes from triploid hybrids (3x=21, ABD) between TetraCanthatch and the *T. tauschii* accessions RL5688 and RL5778. In the direct introgression method the genes from *T. tauschii* were backcrossed directly into the common wheat cultivars "Chinese Spring" and "Marquis". Amphiploids were produced by colchicine-doubling of triploid hybrids (2n=21, ABD) between TetraCanthatch and *T. tauschii*. Spikes in

undoubled sectors of these hybrids were pollinated by "Marquis" to take advantage of restitution gametes. The three methods were compared with regard to the time and effort required to obtain meiotically stable hexaploids homozygous for the transferred gene(s). Two of the seedling leaf rust resistance genes were not expressed at the hexaploid level. The other two, designated *LrA* and *LrB*, were mapped to chromosome 2DS more than 50 cM from the centromere and to chromosome 5D, respectively. The seedling stem rust resistance gene was mapped to chromosome 1DS. The adult-plant resistance genes were not mapped, although preliminary results indicate that the stem rust resistance gene is on one of chromosomes 1D, 4D, or 7D.

Variability among the *T. tauschii* accessions was noted in the rDNA repeat unit for both repeat unit length and for the presence of two Bam HI restriction sites in the intergenic region (IGR). The rDNA repeat units in the accessions showed three distinct restriction maps. Bam HI sites in the IGR occurred only in three accessions of *T. tauschii* ssp. *strangulata*. Such sites have not previously been reported in *Triticum* species.

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CHAPTER I

Introduction

Leaf rust, caused by *Puccinia recondita* Rob. ex Desm., and stem rust, caused by *P. graminis* Pers. f.sp. *tritici* Eriks. and Henn., are the two most important diseases of wheat in western Canada (Martens *et al.* 1984). The most effective and economical measure for the control of these two diseases is genetic resistance (Martens *et al.* 1984). In this region, therefore, wheat cultivars must be resistant to all prevalent races of the two pathogens. Due to selection pressure exerted by resistant cultivars, new races of the rust fungi evolve periodically which are virulent against the existing resistance genes in the crop. To minimize the genetic vulnerability of the crop, the trend is to broaden the genetic basis of resistance among cultivars with additional sources of resistance (Dyck and Kerber, 1985). As common wheat (*Triticum aestivum* L.) has been extensively studied, it is unlikely that many new genes will be found in this species. The most promising sources of new resistance genes are, therefore, the wild relatives of wheat. *Triticum tauschii* (Coss) Schmal. (syn. *Aegilops squarossa* L.) is a diploid species (2n=2x=14, DD) with the D genome, homologous to the D genome in common wheat (2n=6x=42, AABBDD). Recombination occurs freely between the D genome of *T. tauschii* and the D genome of *T. aestivum* when the two

are combined in hybrids, enabling gene transfer between the two species. Many genes of agronomic importance, including rust resistance, have been detected in *T. tauschii*. In this study, 12 accessions of *T. tauschii* which had previously been shown to exhibit a high degree of resistance to a broad spectrum of leaf rust races (E.R. Kerber, unpublished data) were used as source material for new resistance genes. One accession also had seedling resistance to stem rust, and three had adult-plant resistance to stem rust. The resistance in each of these accessions had been determined to be different from genes previously transferred from *T. tauschii* to *T. aestivum*. These accessions were intercrossed to determine the number of genes involved in the rust resistance exhibited and their inheritance.

Several methods for transferring genes from wild relatives to common wheat have been described in the literature. In this investigation two methods were chosen and compared for effectiveness and efficiency of gene transfer. The methods used were direct introgression, in which the target gene is backcrossed directly into the recurrent hexaploid parent, and the production of synthetic hexaploids in a manner reminiscent of the evolution of hexaploid wheat. These methods were compared for efficiency of gene transfer.

The expression of resistance genes transferred to hexaploid wheat

may be altered or suppressed, so that not all genes are successfully transferred and expressed. In this study, the inheritance of resistance transferred to the hexaploid level was determined, and each of the genes involved was mapped to a specific chromosome or chromosome arm.

Some controversy exists in the literature regarding the taxonomy of the wild wheats. In this thesis the system of Morris and Sears (1967) will be used. To gain some insight into the differentiation of the botanical varieties of *T. tauschii*, variability at the DNA sequence level was investigated by restriction mapping of the ribosomal DNA (rDNA) repeat unit of several different botanical varieties. DNA sequence similarity is a measure of relatedness among taxa (Appels *et al.* 1980).

CHAPTER II

Literature Review

2.1 Leaf rust and stem rust of wheat.

Leaf rust, caused by *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Eriks. & Henn., and stem rust, caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn., are the two most damaging diseases of wheat in western Canada (Martens *et al.*, 1984) and the great plains area of the United States (Loegering *et al.*, 1967). The epidemic of stem rust in 1954 and other epidemics have resulted in over 50% yield loss of the spring wheat crop and 65% to 75% of the durum wheat crop in the United States (Loegering *et al.*, 1967). The epidemic of 1954, caused by race 15B, resulted in the greatest losses in Canadian history (Martens *et al.* 1984). Optimum stem rust development occurs at a daytime maximum temperature of 30°C, which coincides with the reproductive period of spring wheat, resulting in substantial losses in yield (Roelfs, 1985). Both stem rust and leaf rust result in increased transpiration and, therefore, water requirement due to the rupture of the epidermis by the rust pustules. Although leaf rust has a less devastating effect, it results in the killing of leaves and the subsequent loss of photosynthetic area. It also causes floret and spikelet abortion and fewer, smaller kernels, and reduced protein in the grain (Loegering *et al.*,

1967). Leaf rust generally occurs earlier in the growing season than stem rust and causes heaviest losses (up to 40% yield reduction) if the disease becomes severe prior to flowering (Samborski, 1985). Clearly, to reduce losses in yield and quality, control of these two diseases is required, and genetic resistance is the most economic and effective means of control (Loegering *et al.*, 1967; Jones and Clifford, 1978; Johnson and Lupton, 1987).

Genetic resistance may be broadly divided into two categories - race-specific or vertical resistance, and non race-specific or horizontal resistance (Dyck and Kerber, 1985). Resistance of the vertical type is generally simply inherited and confers a high level of resistance to a limited number of pathogen isolates. Horizontal resistance is generally under polygenic control and gives a lower degree of resistance to a wider range of isolates, which may be more durable (Parlevliet, 1985). In this study, only vertical resistance was considered.

Both the leaf rust and stem rust pathogens are biotrophs which require living host tissue to support their growth, rendering a hypersensitive or necrotic host response an effective defence (Jones and Clifford, 1978). In a number of instances host plants and their biotrophic pathogens have co-evolved and have developed a gene-for-gene relationship

(Flor, 1955, 1956) with regard to resistance in the host and virulence in the pathogen (Person *et al.*, 1962; Dyck and Kerber, 1985). This co-evolution results in a dynamic balance between the host and pathogen populations characterized by a great deal of genetic variability for host resistance genes and pathogen virulence genes (Mundt and Browning, 1985), and allows the pathogen to circumvent host resistance by selecting for pathotypes virulent on hosts with specific resistance genes (Person *et al.*, 1962; Watson, 1981; Dyck and Kerber, 1985). The gene-for-gene concept implies specificity at the genotype level, and also that a study of the genetics of the system in either the host or the pathogen requires the presence of the other component (Person *et al.*, 1962), as it is the interaction phenotype which produces disease.

Due to the high reproductive rate and genetic diversity in the rust pathogens, another consequence of the gene-for-gene concept is that genetic resistance cannot be expected to protect widely grown cultivars over long periods of time when based on only one or two genes for specific resistance (Loegering *et al.*. 1967). Samborski (1985) points out that selection for resistance in wheat breeding programs is based on rows or small plots, thus imposing no selection pressure on the pathogen population, but once released, resistant cultivars are often widely grown, resulting in a high selection pressure on the pathogen population. Experience from both

Australia (Watson, 1981) and North America (Dyck and Kerber, 1985) has shown that resistance based on single genes is ephemeral, but when several genes are combined into a cultivar, the resistance provided is more durable as the pathogen must acquire and combine virulence genes corresponding to all resistance loci in the host. There is a continued need, therefore, to develop new sources of resistance, and to deploy resistance genes in a diverse manner (Dyck and Kerber, 1985; Mundt and Browning, 1985).

Many rust resistance genes are currently available, although not all are effective against the presently predominant races of the rusts in western Canada. McIntosh and Cusick (1987) have catalogued over 30 genes for resistance to each of leaf rust and stem rust, with some loci having several alleles. In addition to genes found in common wheat, resistance genes have been transferred from related species such as *Aegilops speltoides* Tausch, *Ae. squarrosa* L., *Ae. umbellulata* Zhuk., *Agropyron elongatum* Gaertn., *A. intermedium* Gaertn., *Secale cereale* L., *Triticum timopheevi* Zhuk., and *T. turgidum* L. (Jones and Clifford, 1978). Most of these genes are race specific, and most are expressed from the seedling stage of growth onward (Dyck and Kerber, 1985).

There are several different host plant responses conditioned by resistance genes. The most common is the hypersensitive response

[infection type (IT) 0 or ; (Stakman *et al.*, 1962)] in which the host cells in the immediate vicinity of the infection site die, depriving the pathogen of its living substrate. Examples of genes giving this type of response are *Lr1,* the *Lr2* alleles, *Lr3, Lr10, Sr5,* and *Sr6* (Dyck and Kerber, 1985). Other genes such as *Lr11, Lr16, Lr17, Lr18, Lr30, Sr8,* the *Sr9* alleles, *Sr22, Sr24,* and *Sr33* produce a moderate type of resistance characterized by an IT of 1 to 2 (Dyck and Kerber, 1985). The infection type and inheritance of both the hypersensitive and moderate resistance genes can vary according to the rust culture used and the background genotype of the host (Dyck and Samborski, 1974; Rohringer *et al.*, 1979; Dyck and Kerber, 1985). The expression of certain resistance genes is delayed, becoming apparent after the third leaf stage of growth, or as late as the flag leaf stage. Examples of genes of this adult-plant type of resistance are *Lr12, Lr13,* the *Lr22* alleles, and *Sr2* (Dyck and Kerber, 1985).

While resistance genes generally act independently (Dyck and Kerber, 1985), some studies have shown an additive or synergistic effect (eg. Samborski and Dyck, 1982). The expression of resistance genes may also be influenced by the modifying effect of the genetic background in which they function (Dyck and Samborski, 1974; Rohringer *et al.*, 1979). The background genotype may affect the expression of resistance quantitatively by diminishing (Dyck and Kerber, 1985) or completely suppressing (Kerber

and Green, 1980) the effect of the resistance gene. In the case of certain genes in certain genetic backgrounds, dominance reversal has also been reported (Dyck and Samborski, 1974).

There are thus several types of resistance genes, and many sources of novel resistance genes. Durability of resistance in the future will depend on diversity both in the numbers and types of resistance genes employed, and also in their deployment (Dyck and Kerber, 1985; Mundt and Browning, 1985). Rust epidemics are rare in the center of co-evolution of this host-pathogen system due to the great diversity in both host and pathogen genotypes, such that no single pathogen race is virulent on all plants in the host population (Mundt and Browning, 1985). Deployment of resistance genes in such a diverse manner requires an arsenal of many genes from which to select. The search for new resistance genes within *Triticum aestivum* is not likely to be successful as most are known and used (Dyck and Kerber, 1985), and should thus concentrate on ancestral or related species.

2.2 Genetic variation in *Triticum tauschii*.

Due to the relative ease of transfer of characters from the D genome of related species to hexaploid wheat, *T. tauschii* (Coss.) Schmal. (syn. *Ae.*

squarrosa L.) has been used as a source of desirable characters. This species has been extensively surveyed (Kihara *et al.*, 1965; Kerber and Dyck, 1978; Marshall and Brown, 1981) and found to contain desirable genetic variation for cold hardiness (Limin and Fowler, 1981) and resistance to insects and diseases (Kerber and Dyck, 1969, 1973, 1978; Dyck and Kerber, 1970; Pasquini, 1980; Merkle and Starks, 1985; Gill *et al.*, 1986; Gill and Raupp, 1987; Raupp *et al.*, 1989; Wilson *et al.*, 1989; Appels and Lagudah, 1990). *Triticum tauschii* contains more variability for disease resistance, isozymes, and seed storage proteins than does the D genome of *T. aestivum* (Lagudah and Halloran, 1988; Lubbers *et al.*, 1991). This is a reasonable finding, since evidence indicates that only a small number of *T. tauschii* genotypes contributed to the D genome component of *T. aestivum* (Lagudah *et al.*, 1991). Several investigators have implicated *T. tauschii* ssp. *strangulata* as the donor of the D genome to *T. aestivum* (Lagudah *et al.* 1991; Lagudah and Halloran, 1988; Jaaska, 1981). This subspecies occurs only around the Caspian Sea, which is the primary geographic center of the species (Jaaska, 1981; Nakai, 1978) and the center of diversity (Lagudah *et al.*, 1991; Lubbers *et al.*, 1991). Lubbers *et al.* (1991) found that great genetic variability exists in *T. tauschii* with many rare alleles, and suggested that a search for new genes of agronomic importance should begin in the area around the Caspian Sea, and concentrate on ssp.

eusquarrosa as this subspecies is most likely to contain genetic material not found in the D genome of hexaploid wheat.

Since *T. tauschii* is a species with great diversity (Kimber and Feldman, 1987), it would be useful to determine the genotypic diversity based on DNA markers as DNA sequence similarity is a measure of relatedness (Appels *et al.* 1980, Flavell 1982, Molnar *et al.* 1989). Due to their high copy number, ribosomal DNA (rDNA) repeat units have been used as chromosomal markers (Rayburn and Gill, 1986, 1987) and for identification and phylogenetic studies of plant species (Lapitan *et al.* 1987, Roger and Bendich 1987). Each rDNA repeat unit is composed of a highly conserved region coding for 18S, 5.8S, and 26S rRNA, and a more variable intergenic region (IGR) (Flavell 1982, Long and Dawid 1980, Rogers and Bendich 1987). Molnar *et al.* (1989) found both interspecific and intraspecific variation in 25 *Hordeum* species for repeat unit length and BamHI restriction sites. Appels and Dvorak (1982) reported that the main source of variation in rDNA repeat unit length is in the number of repetitive subunits in the IGR. Appels *et al.* (1980) found a single BamHI site in each of the 18S and 26S gene regions in wheat and rye, and two additional sites in the IGR of some barley genotypes. Kim (1990) found polymorphism for repeat unit length among four accessions of *T. tauschii*, and that approximately 20% of the 26S BamHI sites were not restricted.

Appels *et al.* (1980) and Molnar *et al.* (1989) had similar results and suggested that methylation of the BamHI site in the 26S region could prevent cleavage.

All investigated species in the Triticeae have a single EcoRI site in the 26S region, 0.9 kb downstream from the BamHI site (Appels *et al.* 1980, Flavell 1982, Molnar *et al.* 1989). Restriction at this site is therefore useful in determining repeat unit length.

2.3 Gene transfer from other species into hexaploid wheat.

The potential gene pool available for wheat improvement is extensive. Intergeneric hybrids have been successfully produced between *Triticum* and most other genera in the tribe Triticeae (Miller, 1987), although problems may occur in obtaining such hybrids due to zygotic abortion, embryo/endosperm incompatibility, and hybrid lethality.

Difficulties in obtaining hybrid seed from wide crosses may be overcome by several methods. A wheat cultivar recessive at the *Kr1* and *Kr2* loci, such as Chinese Spring, may be used as the seed parent in an intergeneric cross as the dominant alleles at these loci reduce crossability of

wheat with rye, barley, and probably other species (Riley and Chapman, 1967; Gale and Miller, 1987). Post-pollination treatment of developing embryos with plant hormones such as gibberellic acid may reduce seed abortion (Larter and Evans, 1960). Embryos may also be excised and cultured prior to abortion (Lapitan *et al.*, 1984). With these techniques it is possible to obtain hybrids from many wide crosses and thus expand the available gene pool.

Intergeneric hybrids between parents of different ploidy levels are often highly sterile due to irregular meiosis (Riley and Chapman, 1960; Delibes *et al.*, 1977; Fedak, 1977; Merkle and Starks, 1985; Gill and Raupp, 1987; Kerber and Dyck, 1990) and therefore cannot be used directly in a common wheat improvement program. However, un-reduced and therefore functional gametes may be produced at a low frequency (Alonso and Kimber, 1984). Such hybrids will therefore often produce viable seed after many repeated pollinations with pollen from hexaploid wheat and the backcross progeny is partially fertile (Merkle and Starks, 1985; Gill and Raupp, 1987). In order to minimize difficulties with hybrid sterility, bridging crosses may be made between the alien species and a suitable tetraploid wheat such as *T. turgidum* ssp. *durum* (Delibes *et al.*, 1977; Dosba and Doussinault, 1977; Doussinault et al., 1983) or the tetraploid (AABB) *T. aestivum* derivative stock TetraCanthatch (Kerber, 1964). The

resulting sterile triploid (2n=21) hybrid is pollinated by hexaploid wheat to obtain fertile progeny. Alternatively, chromosome doubling in the sterile hybrid may also occur spontaneously in a manner reminiscent of the evolution of tetraploid and hexaploid wheats (Miller, 1987). Spontaneous amphiploids from hybrids between *Aegilops ovata* and *T. dicoccoides* were reported by Kihara and Katayama (1931). The sterile hybrid may also be induced to undergo chromosome doubling to produce an amphiploid (Gale and Miller, 1987). Amphiploids were induced by Dorsey (1936) by heat treatment of zygotes from hybrids between *T. aestivum* and *Secale cereale*. Dvorak (1977) treated crossed heads with N_2O to produce amphiploids from a *Ae. speltoides* X *T. aestivum* cross. Treatment of hybrid seedlings with the alkaloid colchicine is now the most efficient and the most common method of producing amphiploids (Kaltsikes, 1974), although Tsuchiya and Larter (1968) synthesized Triticale by doubling the chromosome number of the parents before hybridizing. Production of amphiploids has become a commonly used method of transferring alien genetic material into wheat (Delibes *et al.*, 1977; Kerber and Dyck, 1990), and one amphiploid, hexaploid triticale (X *Triticosecale* Wittmack) has become a commercial crop in its own right (Larter *et al.*, 1968). Production of synthetic hexaploids of wheat by crossing a tetraploid wheat with *T. tauschii* followed by colchicine-doubling of chromosome number in the hybrid is an efficient method of transferring

genes from *T. tauschii* to hexaploid wheat (Dyck and Kerber 1970, Kerber and Dyck 1969, 1978, 1978; Rowland and Kerber 1974).

2.3.1 Homoeologous transfers.

Most species in the Triticeae have genomes which are homoeologous to the A, B, and D genomes of hexaploid wheat. Due to the diploidizing effect of the *Ph1* gene on chromosome 5BL, these homoeologous genomes will not pair with any of the wheat genomes at prophase I of meiosis in hybrids, making recombination an unlikely event (Riley *et al.*, 1959). More recently, other genes involved in the control of chromosome pairing have been discovered, although these have relatively minor effects (Miller and Reader, 1985). Several methods are available for overcoming the *Ph1* effect. Alien addition or substitution lines may be crossed as pollen parents with a wheat monosomic for chromosome 5B (Joshi and Singh, 1979), or as seed parents with a nullisomic 5B/tetrasomic 5D (Sears, 1972), a mutated or deleted Ph1 (Sears, 1975, 1977b), or certain genotypes of either *Ae. speltoides* or *Ae. mutica* which suppress the *Ph1* effect (Riley *et al.*, 1968; Dvorak and Knott, 1980). Backcrossing to hexaploid wheat with selection for restored pairing control and the alien character will produce recombinant lines carrying a segment of alien chromatin conferring the desired character. However, such lines will often carry loci linked to the target gene which may have a detrimental effect on the recipient wheat

cultivar in terms of quality or agronomic performance. For example, Loegering and Sears (1963) found a pollen killer gene linked to *Sr11*. A segment of alien chromatin may pair infrequently with its wheat homoeologue and therefore usually will be maintained intact (Kerber and Dyck, 1990), so the goal of the cytogeneticist is to ensure that the minimum amount of alien chromatin is transferred (Sears, 1981).

Translocations in alien addition or substitution lines may also be induced by ionizing radiation (Sears, 1956; Knott, 1971; Aung *et al.*, 1977). The amount of alien chromatin introduced by this method may be minimized by intercrossing several different translocation lines and selecting the appropriate recombinants (Sears, 1977b, 1981). Somaclonal rearrangements in callus cultures derived from hybrid embryos may also result in the transfer of alien characters to wheat chromosomes (Lapitan *et al.*, 1984).

2.3.2 Homologous transfers

Transfers to hexaploid wheat from species containing homologous genomes (i.e. A or D) are not subject to difficulties in recombination. Although the A genome in hexaploid wheat has diverged from that in the diploid donor to a greater extent than has the D genome (Riley and Chapman, 1960; Kimber *et al.*, 1981; Kimber and Zhao, 1983), both the A

and D genomes in hexaploid wheat recombine freely with their counterparts in other species (Kimber *et al.*, 1981). Genes from species with these genomes may be transferred with relative ease into hexaploid wheat by backcrossing with selection for fertility and the desired character (Gale and Miller, 1987). Such transfers to common wheat have been made from tetraploid species such as *T. timopheevi* (2n=28=AAGG) as well as from diploids with the A genome (Kerber and Dyck, 1973; The, 1973, 1976; The and Baker, 1975) and the D genome (Kerber and Dyck, 1969, 1978; Dyck and Kerber, 1970; Gill and Raupp, 1987).

2.3.3 Additional problems encountered with the use of alien genetic material.

In addition to the previously discussed problems associated with crossability - linkage of the desired trait to deleterious characters, ploidy differences resulting in reduced embryo viability, and hybrid lethality - genes transferred from diploid progenitors into hexaploid wheat are often expressed to lesser degrees in the hexaploid wheat, and may even be completely suppressed. Quinones *et al.* (1972) reported an example of leaf rust resistance on the R genome not being expressed in hexaploid triticale (AABBRR). Kerber (1983) reported that leaf rust resistance was not expressed in a synthetic hexaploid derived from TetraCanthatch / *T.*

tauschii RL5495, the *T. tauschii* parent being a highly resistant accession. Similarly, an amphiploid between the durum cultivar Stewart 63 and *T. tauschii* RL5261 (an accession susceptible to leaf rust) failed to express the leaf rust resistance known to be in Stewart 63. Kerber and Green (1980) showed that Canthatch plants lacking chromosome 7DL were resistant to several races of stem rust whereas euploid plants were susceptible, demonstrating the presence of a suppressor of stem rust resistance on that arm. Intergenomic suppressors of this nature could be responsible for the relatively common lack of expression of disease resistance genes in amphiploids (Kerber, 1983), although such suppressors affect only certain genes (Kerber and Green, 1980).

In addition to being completely suppressed, the expression of disease resistance genes may be diluted upon transfer to a higher ploidy level. Dyck and Kerber (1970) found that the level of resistance from an amphiploid of TetraCanthatch / *T. tauschii* RL5271 was diminished during successive backcrosses to Thatcher. Similarly, Kerber and Dyck (1973) found that stem rust resistance derived from *T. monococcum* (;1 infection type) was diluted to 1+ at the tetraploid level and 2 at the hexaploid level. These authors noted that such dilution was more pronounced with genes on the A genome than the D genome.

Knott (1978) found that the expression of genes transferred to hexaploid wheat from the A or D genomes was more likely to be decreased or suppressed than genes transferred from homoeologous genomes. However, with genes from homoeologous genomes, linkage of the target gene to undesirable characters is a more serious problem due to the lack of recombination between the alien segment and the native wheat chromatin.

2.4 Cytogenetic mapping of genes transferred to hexaploid wheat.

Mapping of alien genes is often restricted to mapping the boundaries of alien and wheat chromatin as crossing-over between the alien segment and the corresponding wheat chromatin is unlikely (McIntosh, 1987). This problem is evident with homoeologous but not with homologous transfers. Location of genes to specific chromosomes may be accomplished using various aneuploids. Recombination mapping can then establish linkage relationships with the centromere and other known loci (McIntosh, 1987).

2.4.3 Mapping functions.

Recombination frequencies may only be related directly to map distances in centimorgans (cM) over short distances (less than 5 cM) due to the occurrence of double crossovers (McIntosh, 1987). Such double crossovers may obscure recombination products and result in the underestimation of map distances. Several mapping functions have been

proposed to correct for this difficulty, but the most widely accepted is that of Kosambi (McIntosh, 1987) :

$$x = 25 \log_n [(1+2y)/(1-2y)] \quad [1]$$

where x is the map distance in cM and y is the recombination frequency.

CHAPTER III

Materials and Methods

3.1 Plant Material

3.1.1 *Triticum tauschii*

This project used 12 accessions of *T. tauschii*, six each of ssp. *eusquarrosa* and ssp. *strangulata* (see Table 1.) The resistance of all accessions had previously been determined to be different from resistance previously transferred from *T. tauschii* to *T. aestivum* by crossing these accessions to stocks containing the previously transferred genes (E.R. Kerber, unpublished data).

3.1.2 *Triticum aestivum*

The hexaploid wheat cultivars used as recipients of genes transferred from *T. tauschii* in this study were "Chinese Spring" and "Marquis". "Chinese Spring" was chosen as it is homozygous recessive at both the *Kr1* and *Kr2* loci for interspecific crossability and the aneuploid series used for

Table 1. Accessions of *Triticum tauschii* used in this study

Taxon	Accession number	Seedling leaf rust[1] infection type[2]	Stem rust[3] reaction	Geographical origin
T. tauschii				
ssp. *eusquarrosa*	RL5662	1	Susceptible	-----
	RL5663	2+	Susceptible	-----
	RL5764	;1+	Adult resistance	Iran
	RL5766	11+	Adult resistance	Iran
	RL5767	;1+	Adult resistance	Iran
ssp. *strangulata*	RL5683	0;	Susceptible	Turkmenia, USSR
	RL5686	;1+	Susceptible	Azerbaijan, USSR
	RL5688	;1	Susceptible	Turkmenia, USSR
	RL5689	;1	Susceptible	Armenia, USSR
	RL5778	0;	Seedling resistance	-----
	RL5781-1	2	Susceptible	Iran
	RL5782-1	1+2	Susceptible	Iran

[1] Seedlings were inoculated with leaf rust race 1.
[2] Infection type was determined as described by Stakman *et al.* 1962).
[3] Seedling resistance was tested with race C10, and adult-plant resistance with a mixture of the prevalent races.

mapping (see below, section 3.6) was developed in this cultivar. "Marquis" was selected as the other hexaploid recipient because the Winnipeg Research Station breeding program utilizes single-gene resistance lines in a "Marquis" background. Both "Chinese Spring" and "Marquis" are susceptible to leaf rust and stem rust at the seedling stage, although "Chinese Spring" has adult-plant leaf rust resistance. Both the "Chinese Spring" and "Marquis" lines used in this study were derived from a single plant of each cultivar. The "Chinese Spring" line had been self-pollinated

for eight generations and the "Marquis" line for 16 generations to ensure genetic homozygosity and homogeneity.

For the production of synthetic hexaploids, the cytogenetic stock TetraCanthatch was used. This stock consists of the AABB component of the hexaploid cultivar "Canthatch" (Kerber, 1964) and is susceptible to leaf rust. Although TetraCanthatch itself is resistant to stem rust, synthetic hexaploids derived from crosses between this stock and susceptible *T. tauschii* accessions are expected to be susceptible to stem rust due to the suppressor of stem rust resistance on chromosome 7DL of *T. tauschii* (Kerber and Green, 1980).

3.2 Characterization of the *T. tauschii* accessions.

3.2.1 Leaf rust resistance.

Since all 12 of the original accessions of *T. tauschii* were resistant in the seedling stage to leaf rust, it was possible that some accessions had resistance genes in common. In order to determine the number of genes for leaf rust resistance in these accessions, F_1 hybrids from previously completed intercrosses between the original 12 genotypes were grown and F_2 seed was collected from each of two F_2 plants per cross. Fifty F_2 seedlings from each F_1 plant were tested for leaf rust reaction as described below, and the results analysed to determine the number of segregating

genes (if any) in the F_2 population. If no segregation was observed, it was concluded that the parents shared a common gene or genes for leaf rust resistance.

3.2.2 Stem rust resistance.

The three accessions with adult-plant resistance to stem rust were also intercrossed and the F_2 progeny tested in the field nursery for adult plant reaction to stem rust. If no segregation was observed, it was again concluded that the parents shared a common gene or genes for adult-plant resistance to stem rust. Since only one of the 12 accessions had seedling resistance to stem rust, this plant was crossed only with stoks containing other stem rust resistance genes transferred from *T. tauschii* to *T. aestivum* (E.R. Kerber, unpublished data).

3.3 Evaluation of resistance to leaf rust and stem rust.

3.3.1 Seedling leaf rust resistance.

Seedling reaction to leaf rust was evaluated in the greenhouse. Seed was planted in a 3:1 mixture of topsoil : peat moss. Susceptible and resistant control seedlings were planted in 10 cm pots with 10 seeds/pot. After emergence the seedlings were fertilized with 20-20-20 soluble fertilizer. The seedlings were inoculated before the second leaf had fully

emerged. Prior to inoculation, the pots, flats, and the greenhouse bench were soaked with water to provide a humid environment. The seedlings were misted with water containing a few drops of Tween-20, a surfactant, to ensure complete wetting of the leaves. Urediospores of leaf rust race 1 (Samborski 1963) were puffed onto the leaves with an atomizer using talc as a carrier. If only a very few plants were to be inoculated, spores were applied directly to the leaves with wet fingers. After inoculation the greenhouse bench was covered with a 6 mil polyethylene sheet and humidity was maintained by an ultrasonic humidifier for 18-20 hours. Natural sunlight was supplemented by fluorescent lamps 30 - 40 cm above the canopy.

After approximately 12 days, when the susceptible control showed full pustule development, the rust infection was evaluated. The infection type (IT) was scored according to Stakman *et al.* (1962) for each plant. An IT of 3 or higher was considered a susceptible reaction.

3.3.2 Seedling stem rust resistance.

Seedlings were evaluated for stem rust resistance in a manner similar to that of leaf rust resistance, except for the method of spore application. Stem rust spores of race C10 (Green, 1981), isolate F909, in a

mineral oil carrier, were sprayed onto the leaves with compressed air. The oil was allowed to evaporate prior to the bench being covered and the humidifier installed. In all other respects, inoculation and evaluation of stem rust resistance was identical to the procedure outlined above for leaf rust resistance.

3.3.3 Adult plant resistance to leaf rust and stem rust.

To evaluate rust resistance in mature plants of hybrid populations, F_3 families were seeded in 1.5m rows at a rate of 40 seeds/row in the Agriculture Canada rust nursery at Glenlea, Man. Every tenth row was seeded to the susceptible cultivar "Marquis", and the entire evaluation block was surrounded by spreader rows consisting of susceptible cultivars of wheat and barley. To supplement the naturally occuring inoculum, the spreader rows were inoculated with a spore mixture representative of the leaf rust and stem rust pathogen populations as determined by the previous year's rust pathogen survey. Inoculation was accomplished by dusting rust spores in a talc carrier onto the spreader rows the evening prior to an expected heavy dew. Plants were scored for per-cent infection and pustule type, and lines were classified as homozygous resistant, segregating, or homozygous susceptible.

F_2 populations were also evaluated in the rust nursery. F_2 plants were space-planted at 40 plants in a 3m row and inoculated as above. F_2 plants were evaluated on a single-plant basis.

3.4 Interspecific gene transfer from *T. tauschii* to *T. aestivum*.

Genes conferring resistance to leaf rust and stem rust were transferred from *T. tauschii* to *T. aestivum* by several methods: direct introgression, the production of synthetic hexaploids (amphiploids), and the production of derived hexaploids, a modification of the synthetic hexaploid method.

3.4.1 Gene transfer by direct introgression.

In this method of gene transfer the recipient hexaploid cultivars "Chinese Spring" and "Marquis" were pollinated by all accessions of *T. tauschii* and the tetraploid (ABDD) embryos were cultured as described below (section 3.4.1.1). Meiosis was observed in the ABDD hybrids (see below), and the fertility of the hybrids was evaluated. Since the hybrids were completely male sterile, they were backcrossed as seed parents to the appropriate hexaploid cultivar. The partially fertile BC_1 progeny were evaluated for rust resistance and chromosome number, and the resistant plants with closest to the euploid chromosome number (2n=42) were again backcrossed to the appropriate hexaploid cultivar, this time as pollen

parents as it was expected that certation would favor euploid (n=21) or near euploid pollen. The BC_2 plants were again evaluated for rust resistance and chromosome number. Euploid resistant plants were self-pollinated to obtain stable hexaploids homozygous for resistance in the BC_2F_2 generation. Homozygous plants were identified by progeny testing and were examined for meiotic pairing and fertility.

3.4.1.1 Embryo rescue.

In the case of the original inter-ploidy crosses (i.e. 6x/2x and 4x/2x), collapse of the endosperm and abortion of the developing hybrid embryos was observed at 20 to 22 days after pollination (DAP). Crossed spikes were therefore harvested at 18 - 20 DAP. The developing seeds were removed and surface sterilized with 95% ethanol in a laminar flow hood. Embryos were aseptically excised and cultured on 0.7% Difco Bacto Orchid Agar medium. When the first leaf of the seedling was approximately 4-5cm long the seedlings were transplanted to 10cm pots in a growth cabinet under a 16 hour photoperiod with a 16°C/12°C day/night temperature. The pots were covered with a lantern glass to maintain high humidity until the seedlings were well established.

3.4.2 Gene transfer by amphiploid production.

In this method of gene transfer TetraCanthatch was used as the seed parent. Crossing was accomplished and the embryos cultured as described above. When the hybrid (2n=21, ABD) plants were well established and had begun to tiller, they were removed from the pots and the soil was washed from the roots. The roots and crown area of the plants were immersed in 0.01% aqueous colchicine solution for 4 hours. The plants were then thoroughly washed in distilled water, repotted in 18cm pots, and grown in a growth cabinet at a cool temperature (16° days/12° nights) to encourage profuse tillering. Spikes with sectors indicative of doubling of the chromosomes were fertile and could be recognized by plump, well developed anthers. These spikes were bagged to ensure self-pollination, and the homozygous S_1 progeny were evaluated for rust reaction, meiotic regularity, and fertility.

In a modification of this method, undoubled spikes (recognized by their shrivelled anthers) were pollinated by "Marquis" pollen to take advantage of any restitution gametes (n=21) which might occur to produce hexaploid progeny heterozygous for resistance. These progenies were self-pollinated, and homozygous F_2 plants were identified by progeny testing. As previously, all derived hexaploids were examined for meiotic irregularities and fertility.

3.5 Cytogenetic observations.

3.5.1 Determination of chromosome number.

The chromosome number of plants was determined by examining mitotic cells in root tip squashes. Root tips were collected from seeds germinated on silica sand (20-40 grade) in glass petri dishes or from potted plants at the tillering stage of growth. Root tips were harvested into glass vials containing well aerated distilled water and pretreated in ice-water for 18-20 hours, fixed in Farmer's solution (3:1, 95% ethanol : glacial acetic acid) at room temperature for 48 hours, and stored at 4°C until examination. Immediately prior to examination, root tips were hydrolysed for 8 min. at 60°C in 1N HCl, stained in Feulgen's solution for 45 min. at room temperature, and then cooled in the refrigerator. The root apical meristem was dissected onto a microscope slide in a drop of 45% acetic acid, heated and squashed. The chromosome number was determined from confirmed counts of at least 3 cells per root observed at 400X with a Leitz Ortholux microscope.

3.5.2 Meiotic analysis and fertility.

Meiotic pairing was recorded to determine the extent of homology between the D genome of the donor *T. tauschii* genotypes and the recipient *T. aestivum* cultivars in the direct introgression portion of the project. Meiosis of hexaploids derived from all methods was examined for

irregularities which could affect fertility. Immature spikes were collected from tillers at the boot stage of growth, immediately fixed in Carnoy's solution (6:3:1 95% ethanol : chloroform : glacial acetic acid) for a minimum of 48 hours at room temperature and stored in the refrigerator at 4°C until examined. Anthers at metaphase I were macerated in aceto-carmine, covered with a cover slip, heated and squashed. Pairing was analysed in a minimum of 50 microsporocytes per anther, and the number of closed bivalents, open bivalents, univalents and multivalents was recorded for each microsporocyte.

Fertility was determined by examining the primary and secondary florets of all but the uppermost and lowermost spikelets on five spikes for each plant. The per-cent fertility was calculated as the ratio of fertile florets to total florets times 100.

3.6 Cytogenetic Mapping

3.6.1 Gene mapping using monotelosomics.

The and McIntosh (1975) describe a variation of the monosomic method of gene mapping (Morris and Sears, 1967) using monotelosomics (2n = 40 + t). This method has two advantages. First, univalent shift (Person, 1956) is readily detected. Additionally, the initial cross will yield both

monosomic (2n = 41) and monotelodisomic (2n = 41 + t) progeny. The critical chromosome may be identified by examination of the F_2 populations derived from monosomic F_1 hybrids as previously described. Subsequent analysis of F_2 progeny from the appropriate monotelodisomic allows the association of a gene with a specific arm of the critical chromosome (The and McIntosh, 1975). If the gene is on the chromosome arm not present in the original monotelosomic, the F_2 progeny from the monotelodisomic will segregate in the same manner as the F_2 progeny from the monosomic. Alternatively, if the locus of interest is located on the arm present in the original monotelosomic, the F_2 progeny from the monotelodisomic will segregate in the same manner as the disomic control cross. However, the centromere of the telosome may be used as a phenotypic marker, allowing the recombination frequency between the gene and the centromere to be determined.

If the gene of interest is located on the unpaired chromosome arm in the monotelodisomic F_1, no recombinants will occur and crossing with the opposite ditelosomic is required for mapping. If the gene is on the paired arm, recombination will occur and the F_2 progeny will have two rust reaction phenotypes for each of the three possible chromosome configurations, resulting in the six phenotypic classes in Table 2 (The and McIntosh, 1975; McIntosh, 1987).

Table 2. Expected proportion and observed frequencies of F_2 individuals in the progeny of an *Aa* monotelodisomic individual with *A* present in the entire homologue and *a* present in the telosome (p = linkage of *A*/*a* with centromere. x = male transmission of the telosome. i = relative ammount of information yielded by an observastion). (From McIntosh, 1987)

Chromosome number	Phenotype	Expected proportion	Observed number	i
42	A	$1/2(1-x)(1-p^2)$	a	$\frac{2(1-x)p^2}{1-p^2}$
	a	$1/2(1-x)p^2$	b	$2(1-x)$
41 + t	A	$1/2(1-p+p^2)$	c	$\frac{(1-2p)^2}{2(1-p+p^2)}$
	a	$1/2(p-p^2)$	d	$\frac{(1-2p)^2}{2x(1-p)^2}$
40 + 2t	A	$1/2(xp)(2-p)$	e	$\frac{2(p-p^2)}{2p-p^2}$
	a	$1/2(x)(1-2p+p^2)$	f	$2x$
		Total	N	Σi

The recombination frequency may be calculated using the maximum likelihood equation derived from the phenotypic frequencies in Table 2 :

$$a(2p/1-p^2) + b(2/p) - c(1-2p/1-p+p^2) + d(1-2p/p-p^2) + e(2-2p/2p-p^2) + f(2/1-p) = 0 \quad [2]$$

with standard error :

$$s_p = (1/N\sum i)^{1/2} \quad [3]$$

The Kosambi mapping function may then be applied to the calculated recombination frequency to obtain a map distance in cM.

If the gene is too close to the centromere for recombination to be observed with either chromosome arm, the progeny of plants monosomic for the critical chromosome and carrying the gene may be examined for a mis-division product (i.e. a telocentric chromosome) carrying the gene of interest. McIntosh (1980) observed no recombinants between *Sr14* and the centromere of chromosome 1BL, but found a 1BL monotelocentric with *Sr14* in the progeny of a monosomic 1B plant, confirming the presence of *Sr14* on 1BL near the centromere.

If the gene is greater than 50 cM from the centromere it will segregate independently of the centromere, and mapping the gene with reference to other genetic markers in the critical arm is required. Confirmation of the presence of the gene in the critical arm may be obtained by the lack of recombinants in crosses to a plant ditelosomic for the opposite arm (McIntosh, 1987).

Cytological examination of the F_2 progeny from monotelodisomic F_1 plants will also permit the calculation of the pollen transmission rate of the telosome. Maternal transmission of the telosome is expected to be unbiased (The and McIntosh, 1975). If this is the case, the ratio 2n=42 + 2n=42+2t : 2n=41+t will equal unity, and the pollen transmission frequency of the telosome may be calculated from the frequencies in Table 2 as:

$$x = e+f / a+b+e+f \qquad [4]$$

with a standard deviation of:

$$s_x = (2x(1-x)/N)^{1/2} \qquad [5]$$

(McIntosh, 1987).

Although the F_2 method of telosomic mapping outlined above involves a larger standard error for the estimates of gene-centromere distance and pollen transmission of the telosome than would a backcross to the recessive phenotype, it has the advantages that it may be initiated prior to the specific arm location being known and requires fewer manipulations and cytological observations (Driscoll, 1966).

3.6 Restriction mapping of the rDNA repeat unit of *T. tauschii.*

3.6.1 DNA Extraction

DNA was extracted from seedlings derived from a single self pollinated plant of each of the accessions listed in Table 1. Ten seeds of each accession were planted in a 12 cm. clay pot and approximately 5 gm. of leaf material was collected at the 3-4 leaf stage. Leaf material was harvested into liquid nitrogen, ground in dry ice, lyophilized, and stored at -20°C. Genomic DNA was extracted by the CTAB procedure described by Kim *et al.* (1990).

3.6.2 Restriction and hybridization

The yield of genomic DNA was estimated spectrophotometrically (Maniatis *et al.*, 1982), and 1µg was digested to completion overnight at

37°C with 15U of the appropriate restriction endonucleases in the buffer recommended by the manufacturer (Bethesda Research Laboratories, Bethesda Md.). Each sample was digested with the endonucleases BamHI, EcoRI. Samples were also digested with both enzymes simultaneously. The restriction fragments were separated by electrophoresis in a 0.8% agarose gel in Tris-borate (TBE) buffer (89mM Tris-borate, 89mM boric acid, 2mM EDTA) for 500 Volt-hours and transferred to a nylon membrane (Zeta-Probe, BioRad Corp., Mississauga Ont.) as recommended by the manufacturer. The membranes were probed with ^{32}P-labelled pMF2, a plasmid containing the ribosomal RNA genes from *Neurospora crassa* (Free *et al.*, 1979), or pHbR26a, a plasmid containing part of the intergenic region (IGR) from *Hordeum bulbosum* (Procunier and Kasha, 1990) by the method recommended for the Zeta-Probe membrane by the manufacturer. Detection of the label was accomplished by autoradiography using Kodak XOmat XAR5 X-ray film with Hi-Plus intensifying screens.

CHAPTER IV

Results and Discussion

4.1 Variability in *T. tauschii*

4.1.1 Variability for seedling resistance to leaf rust and stem rust

Surveys of *T. tauschii* have found many accessions resistant to leaf rust, some of which have been demonstrated to contain common genes (Kerber and Dyck 1979). Among the 12 accessions examined in this study, reactions to race 1 of leaf rust varied from 0; to 2+ (Table 1.). It was therefore likely that these accessions differed for genes conferring resistance to leaf rust, and intercrosses among these accessions were made to investigate the genetic diversity for leaf rust resistance. The segregation of F_2 progeny from these intercrosses is shown in Table 3. Expected ratios for two-gene segregation (15R : 1S) were tested for all crosses. Crosses involving RL5688 were also tested for the segregation of three genes as two genes were detected in derived hexaploids produced from this accession (see below, section 4.2.1.2). The F_2 populations were classified as segregating or non-segregating, and the parents of a non-segregating cross were concluded to have one or more leaf rust resistance genes in common. Although the cross RL5686/RL5782-1 did not segregate, suggesting that the parental

accessions share a common gene for leaf rust resistance, the gene from RL5782-1 was not expressed at the hexaploid level (see below, section 4.2.1.1). The gene from RL5686, however, was expressed at the hexaploid level, indicating that these accessions do not share a common gene for leaf rust resistance. This cross should therefore have segregated, and it is most likely due to chance (χ^2=4.933, 0.01<p<0.05) and small population that no segregation was observed. It is also possible that the susceptible genotypes escaped infection.

From the results of the crosses in Table 3, the 12 accessions were sorted into four groups. No segregation was observed in crosses within a group, indicating that the individuals within a group share a common gene for leaf rust resistance. Segregation was observed in crosses between members of different groups, indicating that different groups have different genes for leaf rust resistance. The temporary gene designations assigned to the four groups are given in Table 4. Since only RL5778 was resistant to stem rust at the seedling stage of growth, the stem rust resistance gene in this accession will be designated *SrA*.

Table 3. Segregation for resistance to leasf rust race 1 of F_2 populations derived from intercrosses between accessions of *T. tauschii* with seedling resistance to leaf rust

Cross	Segregation Resistant : Susceptible	$P_{15:1}$	$P_{63:1}$[1]	Classification (S/NS)[2]
RL5662/RL5686	84 : 4	0.50 - 0.75		S
RL5781-1	44 : 0	0.05 - 0.10		NS[3]
RL5782-1	77 : 4	0.10 - 0.25		S
RL5683/RL5663	78 : 3	0.50 - 0.75		S
RL5686	83 : 0	0.01 - 0.05		NS
RL5688	92 : 0	0.01 - 0.05		NS
RL5689	88 : 0	0.01 - 0.05		NS
RL5766	73 : 2	0.10 - 0.25		S
RL5778	87 : 0	0.01 - 0.05		NS
RL5686/RL5663	79 : 4	0.50 - 0.75		S
RL5688	71 : 0	0.01 - 0.05		NS
RL5767	63 : 9	0.10 - 0.25		S
RL5781-1	54 : 2	0.25 - 0.50		S
RL5782-1	74 : 0	0.01 - 0.05		NS
RL5688/RL5663	67 : 0	0.01 - 0.05		NS
RL5766	59 : 3	0.50 - 0.75	0.10 - 0.25	S
RL5767	76 : 3	0.25 - 0.50	0.10 - 0.25	S
RL5782-1	81 : 2	0.10 - 0.25	0.50 - 0.75	S
RL5689/RL5686	84 : 0	0.01 - 0.05		NS
RL5688	89 : 0	0.01 - 0.05		NS
RL5766	88 : 6	0.75 - 0.90		S
RL5778	92 : 0	0.01 - 0.05		NS
RL5764/RL5766	87 : 0	0.01 - 0.05		NS
RL5766/RL5686	86 : 1	0.05 - 0.10		S
RL5767	86 : 0	0.01 - 0.05		NS
RL5767/RL5683	63 : 2	0.25 - 0.50		S
RL5689	55 : 1	0.10 - 0.25		S
RL5764	80 : 0	0.01 - 0.05		NS
RL5778	77 : 2	0.10 - 0.25		S
RL5778/RL5688	96 : 0	0.01 - 0.05		NS
RL5766	87 : 2	0.10 - 0.25		S
RL5781-1	90 : 3	0.10 - 0.25		S
RL5781-1/RL5766	93 : 0	0.01 - 0.05		NS

[1] Crosses at the hexaploid level indicate two genes for leaf rust resistance in RL5688. In crosses with accessions not sharing one of these genes, a 63 : 1 ratio is expected.

[2] S - Segregating, NS - Not segregating. Crosses not segregating share a common gene.

[3] Although the χ^2 for a 15:1 ratio is not significant, this cross is classified as NS due to the lack of susceptible plants.

Table 4. Temporary designations assigned to genes involved in this study

Gene	Accessions of *Triticum tauschii* containing gene
LrA	RL5683, RL5686, RL5688, RL5689, RL5778.
LrB	RL5663, RL5688.
LrC	RL5662, RL5764, RL5766, RL5767, RL5781-1.
LrD	RL5782-1.
SrA	RL5778.

Previous surveys of *T. tauschii* for resistance to leaf rust have identified many accessions (usually approximately 50% of the accessions tested) with leaf rust resistance (Kerber and Dyck 1978, Gill and Raupp 1987). However, these authors did not determine the number of different genes contributing to the resistance in their source populations. Among the 12 accessions examined in this investigation, only four different genes for seedling leaf rust resistance were detected. Of these four genes, *LrD* was found in only one accession. The other genes were duplicated in the population, suggesting that these genes may be quite common in this species. Since several genes have been transferred from *T. tauschii* to hexaploid wheat (Rowland and Kerber 1974, Kerber and Dyck, 1979, Kerber 1987, Gill and Raupp 1987, Raupp *et al.* 1989, Wilson *et al.* 1989), and several genes appear to be common in this species, crosses at the diploid level may allow the identification of new resistance genes prior to their

transfer to hexaploid wheat, and thus prevent duplication of previous work. Raupp *et al.* (1989) transferred a gene conferring resistance to leaf rust from *T. tauschii* to hexaploid wheat and mapped this gene to chromosome 2D. The gene designated *LrA* (Table 4) has also been mapped to chromosome 2D (see below, section 4.3.2). Crosses should be made between lines containing these two genes to determine if they are different. Similarly, *Sr33* has been mapped to chromosome arm 1DS (Jones *et al.* 1991). The gene in this study designated *SrA* was also mapped to chromosome arm 1DS (see below, section 4.3.3), although crosses at the diploid level indicate that these are two distinct genes (E.R. Kerber, unpublished data).

4.1.2 Variability for adult-plant stem rust resistance

Three of the original 12 *T. tauschii* accessions (RL5764, RL5766, and RL5767) were resistant to stem rust at the adult stage of growth. F_2 seedlings from intercrosses among these accesssions were vernalized for ten weeks and planted in the field rust nursery in 1990. No segregation for stem rust resistance was observed (Table 5), indicating that these accessions share a common gene(s) for adult-plant resistance to stem rust. These three accessions also share *LrC* (Table 4), and all were collected from the same location in Iran (Table 1).

Table 5. Segregation for adult-plant resistance to stem rust[1] in F_2 populations derived from intercrosses among *Triticum tauschii* accessions

Cross	Segregation		Classification
	R : S	$P_{(15:1)}$	S/NS[2]
RL5764 X RL5766	89 : 0	0.01 - 0.05	NS
RL5766 X RL5767	92 : 0	0.01 - 0.05	NS

[1] The field rust nursery was inoculated with a mixture of races in addition to naturally occuring inoculum (see materials and methods).
[2] S - segregating, NS - not segregating

During the process of mapping *SrA* (see below, section 4.3.3), two ditelosomic plants were observed in which the first two leaves were susceptible to stem rust race C10 but the third leaf was resistant. These plants did not contain *SrA*, and may therefore contain another adult-plant stem rust resistance gene.

4.2 Transfer of rust resistance genes from *T. tauschii* to *T. aestivum*

The rust resistance genes identified at the diploid level (section 4.1.1) were transferred to hexaploid wheat by two methods. Synthetic hexaploids were produced by crossing the *T.tauschii* donor as a pollen parent to TetraCanthatch and doubling the chromosomes of the resulting triploid hybrid to produce an amphiploid homozygous for the rust resistance gene(s) contributed by the diploid parent. Undoubled spikes were pollinated with "Marquis" pollen, taking advantage of the rare formation of restitution gametes to produce derived hexaploids heterozygous for the rust resistance gene(s). The *T. tauschii* accessions were also crossed as pollen parents to the common wheat cultivars "Chinese Spring" (CS) and "Marquis" (Mq) to introgress the rust resistance directly into these cultivars. The crosses made and the resulting numbers of embryos cultured and surviving seedlings are shown in Table 6.

Table 6. Crosses completed between accessions of *Triticum tauschii* and various recipient genotypes (florets pollinated / embryos cultured / surviving seedlings)

***T. tauschii* Parent**	**Seed Parent**		
	Chinese Spring	Marquis	TetraCanthatch
RL5662	244 / 1 / 0	93 / 0 / 0	211 / 4 / 2
RL5663	139 / 13 / 6	58 / 6 / 4	196 / 2 / 2
RL5683	222 / 6 / 2	28 / 0 / 0	216 / 1 / 0
RL5686	168 / 7 / 6	92 / 0 / 0	170 / 0 / 0
RL5688	193 / 6 / 6	139 / 0 / 0	217 / 4 / 2
RL5689	159 / 24 / 16	58 / 8 / 4	214 / 2 / 2
RL5764	214 / 10 / 9	86 / 0 / 0	257 / 2 / 2
RL5766	210 / 16 / 7	28 / 3 / 2	159 / 2 / 1
RL5767	129 / 7 / 2	88 / 3 / 0	116 / 1 / 1
RL5778	128 / 5 / 4	61 / 3 / 3	132 / 1 / 0
RL5781-1	136 / 3 / 3	218 / 13 / 8	96 / 4 / 0
RL5782-1	106 / 3 / 1	303 / 8 / 3	113 / 4 / 2
Mean	171 / 22 / 5	104 / 4 / 2	175 / 2 / 1
% Success (Seedlings / florets)	3.0	2.3	0.7

In all cases the parent with the higher ploidy was used as the seed parent in these crosses. In contrast, Gill and Raupp (1987) used the diploid as the seed parent and reported that while the initial seed set was higher than in the reciprocal cross, embryo abortion occured earlier and was more severe, resulting in a low overall success.

These authors (Gill and Raupp, 1987) obtained no viable progeny from 114 hybrid embryos cultured from crosses between 17 accessions of *T.*

tauschii and hexaploid wheat. However, from their crosses between another 14 accessions of *T. tauschii* (seed parent) and hexaploid wheat cultivars (pollen parent) 105 embryos were cultured, resulting in 24 hybrid plants, 12 of which produced seed after repeated pollinations with pollen from the recurrent hexaploid parent. Thus some diploid genotypes were capable of forming hybrids with the recipient hexaploids and some were not. This illustrates the effect of the genotype of both parents which is also evident in Table 6.

4.2.1 Production of synthetic hexaploids

The seedlings obtained from the crosses between TetraCanthatch and the accessions of *T. tauschii* were handled as previously described (section 3.4.2). The seed obtained from doubled spikes and from spikes pollinated with "Marquis" is given in Table 7. Amphiploids were obtained from RL5662, RL5764, RL5766, RL5767, and RL5782-1. Heterozygous derived hexaploids were obtained from RL5662, RL5688, RL5764, RL5767, RL5778, and RL5782-1.

Meiotic chromosome pairing and fertility of the amphiploids obtained from the doubled sipkes was determined (Table 8). Data is also shown for the two heterozygous synthetic hexaploids derived from accessions from

which no doubled seed was obtained, and for the hexaploid cultivars "Chinese Spring" and "Marquis".

Table 7. Hexaploid seed obtained from doubled[1] and undoubled[2] sectors of colchicine-treated F_1 hybrids from crosses between TetraCanthatch and accessions of *Triticum tauschii*

T. tauschii parent	Seed from fertile spikes in sectors in which chromosome doubling had occured.	Seed from sterile spikes (pollinated by "Marquis")
RL5662	7	1
RL5663	0	0
RL5688	0	4
RL5689	0	0
RL5764	3	1
RL5766	4	0
RL5767	7	4
RL5778	0	4
RL5782-1	283	19

[1] Spikes in doubled sectors were self-pollinated.
[2] Spikes in undoubled sectors were pollinated with "Marquis".

The chromosome pairing at metaphase I (MI) in the amphiploids is essentially normal, with 21 bivalents observed in most cells. Some cells were observed with 20 bivalents and 2 univalents, and rarely 19 bivalents

and 4 univalents. These results are consistent with those observed by Joppa *et al.* (1980) in an amphiploid between *T. turgidum/T. tauschii.* Pairing in the amphiploids and the derived hexaploids was similar, and was comparable to the two hexaploid cultivars. The similarity in the frequency of open and closed bivalents between the homozygous amphiploids and cultivars and the heterozygous derived hexaploids indicates very little chromosome differentiation (as described by Dvorak and McGuire, 1981) between the D genome of the two *T. tauschii* accessions, RL5688 and RL5778, and the D genome of "Marquis".

Table 8. Metaphase I pairing and fertility in plants grown from amphiploid and derived hexaploid seed

Pedigree	Number of microsporocytes recorded	Average bivalents per cell			Average univalents per cell	Fertility(%)[1]
		Closed	Open	Total		
TC/RL5662	125	17.0	3.9	20.9	0.1	92.7
TC/RL5764[2]	96	18.1	1.1	19.2	0.8	46.2
TC/RL5766	119	18.5	2.3	20.8	0.2	87.5
TC/RL5767	114	19.2	1.6	20.8	0.2	91.7
TC/RL5782-1	102	19.7	2.2	19.9	1.1	84.1
TC/RL5688/Mq	175	19.2	1.5	20.7	0.3	93.9
TC/RL5778/Mq	73	17.4	3.1	20.5	0.5	88.4
CS	125	19.5	1.4	20.9	0.1	96.8
Mq	105	19.8	1.1	20.9	0.1	98.4

[1] Evaluated on five open pollinated spikes.
[2] This plant was nullisomic for chromosome 2D.

Triploid hybrids between TetraCanthatch and five accessions of *T. tauschii* (RL5662, RL5764, RL5766, RL5767, and RL5782-1) were successfully doubled to produce amphiploids (Table 7). These five accessions represent two of the identified leaf rust resistance genes, *LrC* in RL5662, RL5764, RL5766, and RL5767, and *LrD* in RL5782-1 (Table 4). While both genes confer a high to moderate level of resistance (IT ;1 to 1+2) in the diploid parents (Table 1), in the amphiploids neither gene is detectable at the seedling stage of growth. Three amphiploids containing *LrC*, however, exhibit varying degrees of resistance to leaf rust at the adult stage of growth (Table 9). Segregation of F_2 populations derived from intercrosses among these amphiploids indicate that the adult-plant leaf rust resistance in each is genetically distinct (Table 10).

Table 9. Adult-plant reactions of amphiploids to a bulk of races of leaf rust and stem rust

Amphiploid	Pedigree	*Lr* Genotype	Adult-plant rust reaction[1] Leaf rust	Stem rust
RL5865	TC/RL5766	*LrC*	10R	15MR
RL5866	TC/RL5767	*LrC*	15R	10R
RL5867	TC/RL5662	*LrC*	2R	40S
RL5868	TC/RL5782-1	*LrD*	40S	50S
RL5869	TC/RL5764	*LrC*	40S	15MR
Marquis[2]			70S	60S
TetraCanthatch[2]			60S	10R

[1] Reactions recorded in the field rust nursery. R = resistant; MR = moderately resistant; S = susceptible. Numbers indicate percentage of infection.
[2] Included as a susceptible control.

Table 10. Segregation for adult-plant resistance to leaf rust of F_2 populations derived from intercrosses of amphiploids containing *LrC*

Cross	Segregation[1] R : S	$P_{(15:1)}$
RL5866 X RL5865	79 : 4	0.50-0.75
RL5867 X RL5865	59 : 8	0.05-0.10
RL5867 X RL5866	70 : 8	0.10-0.20

[1] Tested in greenhouse.

The most probable explanation for the lack of seedling leaf rust resistance in these amphiploids is the suppression of *LrC* and *LrD* by loci on the A or B genomes. Such suppressors are not rare. Quinones *et al.*(1972) reported an example of leaf rust resistance known to be on the R genome in rye not being expressed in hexaploid triticale, suggesting the action of a suppressor of leaf rust resistance on the A or B genome. Similarly, Kerber and Green (1980) found that a gene on chromosome 7D suppressed stem rust resistance carried by the AB component of "Canthatch". The suppression in this case of *LrC* would allow the detection of the expressed adult-plant leaf rust resistance gene(s), previously masked in the diploid by the seedling resistance gene *LrC*.

4.2.2 Production of derived hexaploids

In cases where chromosome doubling is not successful, the triploid hybrids between TetraCanthatch and *T. tauschii* may form the occasional restitution gamete with 21 chromosomes (as per Alonso and Kimber, 1984). To take advantage of these rare events, sterile spikes on colchicine-treated plants were pollinated by "Marquis". The progeny of such pollination is a derived hexaploid that would be heterozygous for the rust resistance gene(s) contributed by the *T. tauschii* donor. In this manner derived hexaploids were produced from two additional accessions, RL5688 and RL5778 (Table 11). These heterozygous hexaploids expressed the resistance to both leaf rust and stem rust as effectively as the diploids from which they were derived (cf. Table 1), indicating that the genes in these lines are not subject to the modifying influences of the A and B genomes. The heterozygous hexaploids were self-

Table 11. Seedling reaction of derived hexaploid lines to leaf rust race 1 and stem rust race C10

Pedigree	Genotype	Seedling infection type	
		Leaf rust (race 1)	Stem rust (race C10)
TC/RL5688/Mq	*LrA LrB*	;1	3+4
TC/RL5778/Mq	*LrA SrA*	;1	;1

pollinated and plants homozygous for each of the three genes *LrA, LrB* , and *SrA* (Table 4) were selected in the progeny and confirmed by progeny testing.

The F_2 progeny from the heterozygous derived hexaploids containing *SrA* segregated for stem rust resistance in a 1:2:1 ratio where the three classes were defined by IT ;1 (R): IT 1+2 (MR): IT 33+ (S) (Table 12). Progeny testing of selected plants with IT ;1 confirmed the homozygosity of these plants. It should be noted that in not all environments is the heterozygote (IT1+2 in this case) distinguishable from the homozygote for this gene.

Table 12. Rust reaction of self-pollinated progeny of heterozygous derived hexaploids containing *LrA* and *SrA*

Pedigree	Pathogen	Segregation IT= ;1 1+2 33+	Ratio tested	P
TC/RL5688/Mq(S_1)	Leaf rust (Race 1)	20 : 9 : 8	12 : 3 : 1	<0.01
TC/RL5778/Mq(S_1)	Stem rust (Race C10)	24 : 41 : 20	1 : 2 : 1	0.75-0.90

In the F_2 progeny from the triploid (ABD) hybrid derived from RL5688 pollinated by "Marquis" the resistant plants also fell into two distinct classes. Insufficient progeny were obtained to determine a segregation ratio (Table 12), but progeny testing identified plants homozygous for each of two genes.

Homozygous plants containing the two genes (*LrA* and *LrB*) extracted from RL5688 were crossed with each other and with "Marquis" to confirm that two independent genes had been isolated (Table 13). When plants homozygous for each of the genes were crossed with "Marquis", a single-gene ratio of three resistant plants to one susceptible was observed in the F_2 generation. When the plants containing the two genes were intercrossed, a modified dihybrid ratio of 12 highly resistant : 3 moderately resistant : 1 susceptible was observed, confirming that two genes had been extracted from RL5688 in accordance with the results obtained from crosses at the diploid level.

Table 13. F_2 segregation for reaction to leaf rust race 1 of crosses involving the two genes transferred from RL5688

Cross	Segregation R : S	Ratio tested	P
Mq X *LrA*	70 : 18	3 : 1	0.25-0.50
Mq X *LrB*	69 : 26	3 : 1	0.50-0.75
	R : MR : S		
LrA X *LrB*	69 : 24 : 1	12 : 3 : 1	0.75-0.90

4.2.3 Direct introgression of rust resistance genes

Since amphiploids had been successfully produced from the accessions RL5764, RL5766, RL5767, and RL5782-1 in which the leaf rust resistance of these accessions was not expressed, work was discontinued with these accessions in the direct introgression part of this study.

4.2.3.1 Seedling resistance to leaf rust and stem rust

The F_1 progenies from the crosses of "Chinese Spring" and "Marquis" with the *T. tauschii* accessions (Table 6) were examined for fertility and chromosome pairing at metaphase I in microsporocytes (Table 14). The meiotic pairing in most plants indicated predominantly seven bivalents and 14 univalents in most cells (Fig. 1), although some cells had fewer than seven bivalents. Riley and Chapman (1960) found an average of 6.54 bivalents in a *T. tauschii* X "Chinese Spring" hybrid. This is consistent with the pairing of the D genome chromosomes only, and

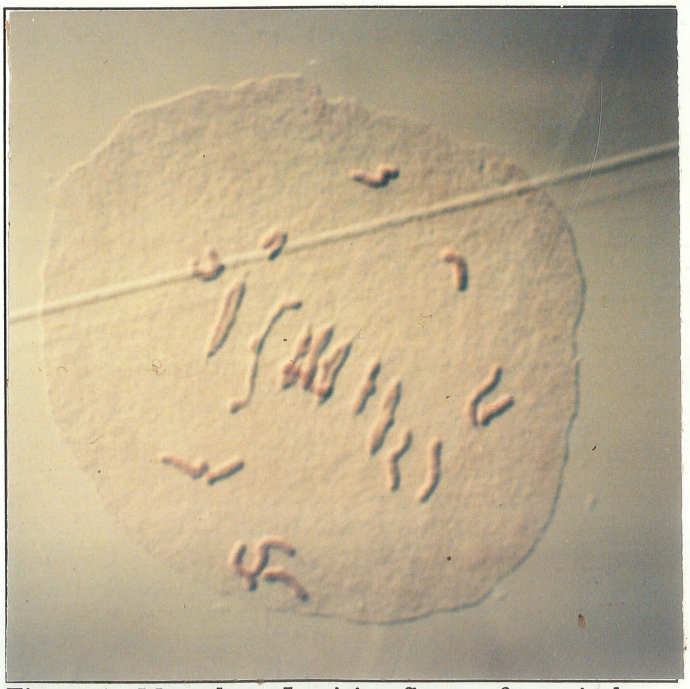

Figure 1. Metaphase I pairing figure of a typical ABDD hybrid.

indicates that the D genome of *T. tauschii* is fully homologous with the D genome of *T. aestivum*. No more than seven bivalents were observed in any cell, and no multivalents were observed, indicating that associations between the two copies of the D genome account for all the observed pairing. This is in agreement with the results of Riley and Chapman (1960) and Gill and Raupp (1987). The unpaired chromosomes of the A and B genomes contribute to the low fertility of these plants.

The F_1 (ABDD) plants were backcrossed as seed parents to the appropriate hexaploid cultivar. The crosses made and the progeny obtained are given in Table 15. The low fertility of the F_1 plants required a large number of pollinations in order to recover any BC_1 seed.

Table 14. Average chromosome pairing at metaphase I and fertility in *Triticum aestivum* X *T. tauschii* F_1 hybrids

Pollen parent	Seed parent											
	Chinese Spring						Marquis					
	No. cells	Bivalents			Univalents	Fertility[1](%)	No. Cells	Bivalents			Univalents	Fertility[1](%)
		Closed	Open	Total				Closed	Open	Total		
RL5663	104	5.6	1.4	7.0	14.0	0.0	125	6.1	0.9	7.0	14.0	0.0
RL5683	96	5.9	1.1	7.0	14.0	0.0	0					
RL5686	170	5.2	1.8	7.0	14.0	0.0	0					
RL5688	110	5.5	1.4	6.8	14.2	0.0	0					
RL5689	99	4.3	2.6	6.8	14.2	0.0						
RL5764	106	5.4	1.5	6.8	14.2	0.0	0					
RL5766	112	5.0	1.9	6.8	14.2	0.0						
RL5778	100	6.3	0.7	6.9	14.1	0.0	103	5.0	2.0	7.0	14.0	0.0
RL5781-1	113	5.4	1.6	7.0	14.0	0.0	105	5.2	1.8	7.0	14.0	0.0
RL5782-1	100	6.0	0.9	6.9	14.1	0.0	102	5.4	1.6	7.0	14.0	0.0

[1] Average open pollinated seed set on five spikes.

Table 15. Backcrosses between tetraploid (ABDD) F_1 hybrids and "Chinese Spring" (CS) or "Marquis" (Mq). (Florets pollinated / seed obtained)

	Pollen parent	
Seed parent	CS	Mq
CS/RL5663	1016 / 6	
Mq/RL5663		1164 / 10
CS/RL5683	304 / 1	
CS/RL5686	1672 / 24	
CS/RL5688	1696 / 13	
CS/RL5689	1308 / 8	
Mq/RL5689		232 / 2
CS/RL5764	708 / 1	
CS/RL5766	1604 / 9	
Mq/RL5766		352 / 0
CS/RL5767	644 / 4	
CS/RL5778	1504 / 6	
CS/RL5781-1	412 / 0	
Mq/RL5781-1		1516 / 11
CS/RL5782-1	244 / 11	
Mq/RL5782-1		504 / 7
Success rate (seed/floret)	0.7%	0.8%

Since the F_1 (ABDD) plants were heterozygous for the rust resistance gene(s) contributed by the *T. tauschii* parent, the BC_1 progeny were evaluated for leaf rust reaction as well as chromosome number (Table 16). Leaf rust resistance was not observed in progeny from the RL5663 lines. This could be due to the low number of progeny obtained (N=7, χ^2=7.00,

$p<0.01$) or to the combination of the parental background genotypes diluting the effect of this gene in this background. The gene *LrB* had already been transferred from the RL5688 lines, and was expressed in that background (RL5688/TC/Mq) with an infection type of 22+.

Table 16. Chromosome number and leaf rust reaction of BC_1 progeny from ABDD F_1 hybrids / "Chinese Spring" (CS) or "Marquis" (Mq)

Backcross	Seed / Seedlings	Seedling chromosome numbers	Rust reaction R	S
CS/RL5663//CS	6 / 5	47, 48, 49(3)	0	5
Mq/RL5663//Mq	10 / 2	46, 47	0	2
CS/RL5683//CS	1 / 1	47	1	0
CS/RL5686//CS	24 / 14	38, 40, 44, 46(2), 47(2), 48(2), 49(5)	12	2
CS/RL5689//CS	8 / 7	39, 46(2), 48, 49(3)	5	2
Mq/RL5689//Mq	2 / 1	36	0	1
CS/RL5778//CS	6 / 2	42, 49	0	2
Mq/RL5781-1//Mq	7 / 6	39(2), 40, 41, 42, 49	0	6

Leaf rust resistance was not observed in progeny from the RL5778 line. As this resistance was well expressed in the synthetic hexaploid derived from this accession (IT ;1), its absence is most likely due to the low number of progeny (N=2, $0.10<p<0.25$). Both plants of this progeny were resistant to stem rust, and that with 2n=42 was selected and self-pollinated

to produce plants homozygous for the stem rust resistance gene *SrA*. The homozygous BC_1F_1 plants were identified by progeny testing. Leaf rust resistance also was not observed in progeny from the RL5781-1 line. As this accession carries the same gene (*LrC*) as found in the synthetic hexaploids derived from RL5662, RL5764, RL5766, and RL5767, and the resistance is not expressed in these synthetic hexaploids, it is not unexpected that the resistance in the BC_1 plants is not expressed.

Gill and Raupp (1987) reported that approximately 50% of their BC_1 (2x/6x/6x) progeny (16 plants in total) had chromosome numbers of 2n = 40 to 43 and approximately 50% had 2n = 49 to 50. This would be consistent with the F_1 plant producing gametes of either near-euploid or euploid chromosome number n=21 (ABD) or n=28 (ABDD). Similarly, Alonso and Kimber (1984) found chromosome numbers of 2n = 41, 42, 49 and 50 in four BC_1 plants. In this study a preponderance of higher chromosome numbers was observed. Five BC_1 plants had 2n<40, five had 2n = 40 to 43, 15 had 2n = 44 to 48, and 13 had 2n = 49 to 50. Of the 38 BC_1 plants obtained, 22 had more than 47 chromosomes, indicating that these progeny resulted predominantly from the formation of restitution gametes (Alonso and Kimber, 1984) with near-euploid or euploid chromosome number n=28 (ABDD).

Gill and Raupp (1987) found that the BC_1 plants with chromosome numbers of approximately 42 or 49 were the most fertile when compared with plants of other chromosome numbers. The data in Table 17 indicates great variability for fertility, although plants with fewer than 40 chromosomes were always the least fertile. Plants with 2n = 42 or 2n = 47 to 49 were generally the most fertile.

Plants were selected from the BC_1 progeny on the basis of resistance to leaf rust. Within the resistant group, plants were selected for chromosome numbers as close to euploidy (2n=42) as possible. These selected BC_1 plants were partially fertile (Table 17), and were crossed as pollen parents to "Marquis" so that certation would favor euploid (n=21) or near-euploid gametes. This should result in near-euploid (2n=42) progeny (Gill and Raupp, 1987), although Cox and Harrell (1989) found that high-chromosome-number plants (2n>47) produced a small proportion of euploid pollen, reducing the competitive advantage of euploid pollen. Since the BC_1 plants were aneuploids, euploid gametes were expected to be relatively rare, and therefore a large number of pollinations were required to obtain resistant BC_2 progeny. The crosses made and progeny obtained are shown in Table 18.

Table 17. Fertility and chromosome number of BC_1 plants

Pedigree	Chromosome number	Average fertility[1]
CS/RL5663//CS	48	60.3
	49	33.7
Mq/RL5663//Mq	46	15.0
	47	81.2
CS/RL5683//CS	40	50.0
	44	5.0
	46	25.0
	47	36.4
	48	83.3
	49	90.3
CS/RL5686//CS	38	8.3
	49	47.9
CS/RL5689//CS	48	94.6
	49	50.5
Mq/RL5689//Mq	36	22.7
CS/RL5778//CS	38	50.0
	39	43.7
	40	22.9
	42	59.4
	47	10.0
Mq/RL5781-1//Mq	39	29.5
	40	60.0
	42	90.9
	49	83.3

[1] Average open pollinated seed set on unpollinated heads of all plants with the given chromosome number.

Table 18. Progeny and selections from crosses of BC_1 plants to "Marquis" (Mq)

Pollen parent	Progeny[1]	Chromosome number(s)	Segregation for leaf rust reaction R : S	Selected progeny[2]
CS/RL5683//CS	214/3/1	42	1 : 0	1(42,1+)
CS/RL5686//CS	602/13/5	42,44,45(2),47	1 : 4	1(42,;1)
CS/RL5689//CS	378/21/21	42, 44, 45(3),47(2)[3]	7 : 14	1(42,;1)
Mq/RL5689//Mq	62/3/0			
Average success rate	2.1%			

[1] Florets pollinated/seeds/seedlings
[2] Number of plants selected (2n, IT)
[3] Only resistant progeny were evaluated for chromosome number

The selected BC_2 plants were self-pollinated, and BC_2S_1 plants homozygous for the leaf rust resistance gene *LrA* were identified by progeny testing. In this manner *LrA* was transferred by direct introgression.

4.2.4 Summary of genes transferred.

Of the five amphiploids produced containing either of the seedling leaf rust resistance genes *LrC* or *LrD*, none expressed these genes. However, three of these amphiploids (RL5865, RL5866, and RL5867) expressed adult-plant

Table 19. Genes transferred from *Triticum tauschii* to *T. aestivum*

Gene	Source accessions
LrA	RL5683, RL5686, RL5688, RL5689, RL5778.
LrB	RL5663, RL5688.
ALrA	RL5766.
ALrB	RL5767.
ALrC	RL5662.
SrA	RL5778.
ASrA	RL5764, RL5766, RL5767.

resistance to leaf rust. This leaf rust resistance was shown to be genetically different in each amphiploid, and the three genes involved will be temporarily designated *ALrA*, *ALrB*, and *ALrC*, respectively. There were also three amphiploids (RL5865, RL5866, and RL5869) which were resistant to stem rust in the adult stage of growth. The resistance in these amphiploids was shown to be genetically identical, and the gene will be temporarily designated *ASrA*.

Three genes (*LrA*, *LrB*, and *SrA*) were transferred by the derived hexaploid method. All three were expressed at the seedling stage of growth in the hexaploids. *LrA* and *SrA* were also transferred by the direct introgression method. The genes were expressed at the seedling growth stage, although the stem rust resistance gene *SrA* conferred a higher level of resistance in the direct introgression line than in the derived hexaploid line (Figure 2). This is likely due to the opportunity to select for maximum expression of resistance in the direct introgression method, and the lack of such an opportunity for

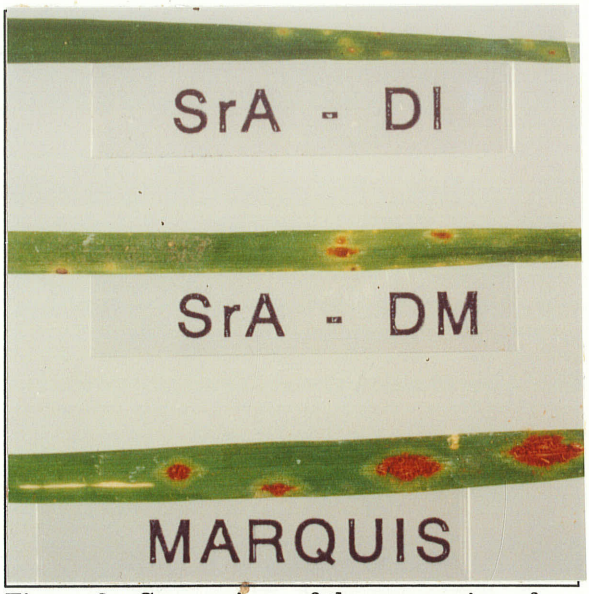

Figure 2. Comparison of the expression of *SrA* in the derived hexaploid (*SrA*-DM) and direct introgression (*SrA*-DI) lines.

selection in the background genotype in the derived hexaploid or amphiploid methods. The leaf rust gene *LrA* did not show improved expression in the direct introgression lines, suggesting that the modifying effect of the background genotype is not as pronounced with this gene. The differential effects of background genotypes on different genes has been previously reported (Samborski and Dyck 1982).

In total, two seedling-effective leaf rust resistance genes, three adult-plant leaf rust resistance genes, one seedling stem rust resistance gene, and one adult stem rust resistance gene were transferred from *T. tauschii* to *T. aestivum* (Table 19).

4.2.5 Comparison of methods of gene transfer.

Genes were successfully transferred from *T. tauschii* to *T. aestivum* by three methods: direct introgression, amphiploid production, and derived hexaploid production. The number of manipulations and the number of generations involved in these three methods are compared in Table 20.

The production of amphiploids involves one cross and colchicine treatment of the hybrids to produce a hexaploid homozygous for the target gene. Obtaining hybrids may require many pollinations of the tetraploid parent by the diploid, and the success of the cross is limited by the

genotypes of both parents. Otherwise, this method is rapid, straightforward, and does not require selection for the target gene. This method is also flexible in that it allows the use of the derived hexaploid method in the event that chromosome doubling is not successful. This method is therefore recommended as the first method of choice for gene transfer from *T. tauschii* to *T. aestivum*.

Table 20. Comparison of amphiploid production, derived hexaploid production, and direct introgression as methods of gene transfer from *Triticum tauschii* to *T. aestivum*

	Amphiploid	Derived hexaploid	Direct introgression
Number of crosses	1	2	3
Cycles of selection for resistance	0	0	2
Cycles of selection for chromosome number	0	0	2
Progeny testing required	No	Yes	Yes

In the event that chromosome doubling is not successful, the amphiploid method may be modified by taking advantage of the rare formation of female restitution gametes (n=21) by the triploid (ABD) hybrid to obtain derived hexaploid progeny. This method requires an additional cross between the triploid hybrid and a susceptible hexaploid genotype which requires many pollinations to obtain progeny. The progeny so obtained would be heterozygous for the target gene and must therefore be self-pollinated to obtain homozygous resistant S_1 progeny which must be identified by progeny testing. Thus, this method may permit the salvage of unsuccessfully doubled triploid hybrids, and therefore complements the amphiploid method.

The direct introgression method is considerably more laborious, involving three successive generations of controlled crosses. All of these crosses are between parents differing in ploidy or chromosome number, and so require many pollinations in order to obtain progeny. The success rate of the initial crosses in this study (Table 6) was greater using "Chinese Spring" as the seed parent (3.0%), followed by "Marquis" (2.3%). The success rate of the corresponding cross using TetraCanthatch was only 0.7%, which is lower than with the hexaploids, although not to a degree that would have an effect on a gene transfer program. Gill and Raupp (1987) found that the initial seed set was improved if the diploid parent was used

as the seed parent; however, embryo abortion occurred at 10 to 12 days after pollination (DAP). These authors also reported that the recovery of embryos and the frequency of cultured embryos developing into plants were both very low (approximately 30% and 10%, respectively), and they consequently recommended using the hexaploid plant as the seed parent. This study confirms that the use of the hexaploid as the seed parent results in delayed embryo abortion (18 to 22 DAP), allowing increased development of the embryo prior to excision. This improves the frequency of regeneration of plants from cultured embryos (22.8% using "Chinese Spring" as the seed parent, and 50% using either "Marquis" or "TetraCanthatch" - see Table 6).

Both the amphiploid and direct introgression methods require a large number of pollinations in order to recover hybrid progeny. However, in the direct introgression method, a subsequent backcross is also required. The success rates of the backcrosses in Table 14 are also very low (0.7% for "Chinese Spring" and 0.8% for "Marquis"), again requiring a large number of pollinations to recover viable backcross progeny. Since the hybrid parent is heterozygous for the target gene, not all of the backcross progeny will carry the gene (see Table 16), further increasing the number of pollinations required and necessitating selection in the progeny for the target gene. Since the BC_1 plants are not likely to be euploid, they must be backcrossed as pollen parents to a hexaploid plant. Using an aneuploid as a pollen

parent again requires a large number of pollinations as aneuploid gametes are not expected to function at a high frequency. However, as certation is expected to favor euploid or near euploid gametes, using the aneuploid as the pollen parent increases the probability of obtaining euploid progeny (Gill and Raupp, 1987). The average success rate for crosses between such aneuploids and "Marquis" in this study was 2.1% (Table 18). Again, since the aneuploid is heterozygous for the target gene, not all progeny will carry the gene, thus requiring a second cycle of selection. Euploid progeny carrying the target gene should be recovered in this generation and may then be self-pollinated to produce plants homozygous for the target gene. These plants may be identified by progeny testing as in the derived hexaploid method.

Although the direct introgression method is more laborious, it does have several advantages. Progeny may be obtained through this method from crosses with diploid genotypes which are not compatible with the tetraploid genotypes available. Secondly, the two cycles of selection for the target gene offer an opportunity to select (in the D genome) for a background genotype maximizing the expression of the target gene (see Figure 3). This may not be effective for all genes transferred in this manner as some genes are not influenced by the background genotype. An additional advantage to this method of gene transfer is that it may allow

the introgression of the target gene into an adapted cultivar. This may facilitate recovery of progeny of good agronomic type, and may also provide the opportunity for selection in the D genome for other characteristics such as quality. These are important considerations in a breeding program (Gill and Raupp, 1987). Nonetheless, this method would appear, from the results of this study, to have its major use in transferring genes from diploid genotypes which will not form hybrids with the tetraploid genotypes available.

4.3 Mapping the transferred genes

The monotelosomic method (McIntosh 1987) was used to assign some of the transferred genes to a specific chromosome or chromosome arm and, where possible, calculate a map distance from the centromere. Although some crosses were made involving the gene *ASrA*, only the seedling-effective genes were mapped due to time constraints.

In order to detect any colchicine-induced mutations affecting the chromosomal location of the seedling-effective genes, lines containing *LrA* and *SrA* derived from both the derived hexaploid method and the direct introgression method were used to assign the genes to a specific chromosome.

4.3.1 Adult-plant stem rust resistance

The gene *ASrA* confers adult-plant stem rust resistance that was transferred by the amphiploid method. Crosses were made between the amphiploid RL5865 carrying this gene and several D-genome monosomics of "Chinese Spring" (Table 21). Progeny were not obtained from crosses involving "Chinese Spring" monosomics 1D, 4D, and 7D.

Table 21. Segregation for resistance to stem rust race C10 of F_2 progeny from crosses between amphiploid RL5865 (*ASrA*) and "Chinese Spring" monosomics[1]

Seed parent	Segregation R : S
CS-disomic	25 : 11
CS-2D	16 : 6
CS-3D	19 : 5
CS-5D	17 : 7
CS-6D	25 : 4

[1] Monosomics are designated by the chromosome which they lack.

Although the population sizes are small, the high proportion of susceptible plants in all crosses suggests that *ASrA* is not located on any of the chromosomes tested. This implies that *ASrA* is on one of chromosomes 1D, 4D, or 7D. Further research is required in this area.

4.3.2 Seedling leaf rust resistance

The seedling-effective genes *LrA* and *LrB* were mapped using the monotelosomic method (McIntosh 1987). Crosses were made between lines homozygous for *LrA* and *LrB* as pollen parents and the D-genome aneuploids of "Chinese Spring" shown in Table 22 as seed parents. Both monosomic (2n=41) and monotelodisomic (2n=41+t) F_1 progeny were recovered from these crosses. The F_2 progenies derived from monosomic F_1 plants were analyzed first to assign these genes to specific chromosomes. The results from these F_2 populations are shown in Table 22 for *LrA* and Table 23 for *LrB*.

The control crosses between the *LrA* lines and disomic "Chinese Spring" segregated in the expected 3:1 ratio (resistant : susceptible), confirming that a single gene was involved (Table 22). The results were similar using either the direct introgression lines or the derived hexaploid lines. In both cases the critical chromosome was concluded to be 2D because the F_2 progeny from this chromosome did not fit the expected 3:1 ratio. Progenies from crosses to all other aneuploids and the disomic fit the expected ratio, and the $\chi^2_{\text{Homogeneity}}$ was non-significant for these progenies, indicating that they could be considered to be derived from the same population. The susceptible plants from the critical family were examined

cytologically and determined to be nullisomic, confirming the conclusion from the segregation data.

Table 22. Segregation for reaction to leaf rust race 1 of F_2 progeny derived from monosomic F_1 hybrid plants from crosses between plants homozygous for *LrA* and "Chinese Spring" aneuploids[1]

Seed parent	Segregation R : S	$P_{(\chi 2)}$
A : Pollen parent is direct introgression line		
CS-1D	67 : 29	0.10-0.25
CS-2D	93 : 5	<0.01
CS-3D	73 : 20	0.25-0.50
CS-4D	72 : 26	0.50-0.75
CS-5D	70 : 17	0.50-0.75
CS-6D	77 : 22	0.50-0.75
CS-7D	78 : 21	0.25-0.50
CS disomic	78 : 22	0.25-0.50
B : Pollen parent is derived hexaploid line		
CS-1D	72 : 21	0.50-0.75
CS-2D	96 : 2	<0.01
CS-3D	67 : 28	0.25-0.50
CS-4D	38 : 8	0.10-0.25
CS-5D	no progeny	
CS-6D	77 : 21	0.25-0.50
CS-7D	39 : 9	0.25-0.50
CS disomic	71 : 24	0.90-0.95

$\chi^2_{\text{Homogeneity}} = 7.169$, df = 12 , $0.75 < P_{\chi 2} < 0.90$

[1] Aneuploids are designated by the chromosome lacking in the F_1 hybrid.

Raupp *et al.* (1989) also report the transfer of a seedling-effective gene conferring resistance to leaf rust from *T. tauschii* to hexaploid wheat that has also been mapped to chromosome 2D. Since *LrA* is apparently not a rare gene, being found in five of the twelve accessions in this study, it is possible that *LrA* is the same gene that was transferred by Raupp *et al.*(1989). This illustrates the utility of crosses at the diploid level to determine the identity of any genes under consideration for transfer to hexaploid wheat prior to their actual transfer.

Table 23. Segregation for reaction to leaf rust race 1 of F_2 progeny derived from monosomic F_1 hybrid plants from crosses between plants homozygous for *LrB* and "Chinese Spring" aneuploids[1]

Seed parent	Segregation R : S	$P_{(\chi 2)}$
CS-1D	72 : 24	1.00
CS-2D	35 : 14	0.50-0.75
CS-3D	62 : 31	0.05-0.10
CS-4D	36 : 14	0.50-0.75
CS-5D	128 : 7	<0.01
CS-6D	36 : 12	1.00
CS-7D	65 : 22	0.50-0.75
CS disomic	74 : 24	0.90-0.95

$\chi^2_{\text{Homogeneity}} = 3.13$, df = 6 , $0.75 < P_{\chi 2} < 0.90$

[1] Aneuploids are designated by the chromosome lacking in the F_1 hybrid.

The control crosses between the *LrB* lines and disomic "Chinese Spring" segregated in the expected 3:1 ratio (resistant : susceptible), confirming that a single gene was segregating. The critical chromosome in this case was 5D. Again, all non-critical F_2 progenies were non-significant and homogeneous. Since monotelodisomics for 5D were not available, *LrB* could not be mapped to a specific chromosome arm. *LrB* was transferred to hexaploid wheat only by the derived hexaploid method.

The F_2 populations derived from two monotelodisomic F_1 plants from the cross between the *LrA* direct introgression line and "Chinese Spring" monotelosomic 2DS were then grown and analyzed for leaf rust resistance and chromosome number. Since no differences were noted between the F_2 populations from the two F_1 plants the data are combined in Table 24.

Since both recombinant (resistant) and parental (susceptible) ditelosomic (2n=40+2t) plants were recovered (Table 24), *LrA* must be on chromosome arm 2DS. χ^2 analysis of the data indicated that *LrA* segregated independently of the centromere (χ^2 = 10.37, $0.05<p<0.10$). The map distance between *LrA* and the centromere is therefore greater than 50 cM. On the basis of the results in Table 24 the pollen transmission rate of the telosome may be calculated using equation [4] (section 3.6.1) as x = 0.20 $\pm$ 0.059.

Table 24. Segregation for reaction to leaf rust race 1 and chromosome number in the F_2 population derived from monotelodisomic F_1 plants from the cross CS-2DS/*LrA*

Chromosome number	Rust reaction and number of plants	
	Resistant	Susceptible
42	24	4
41 + t	37	10
40 + 2t	9	10

There are several other known markers on chromosome 2DS (McIntosh and Cusick 1987), including several genes for leaf rust resistance. For example, the adult-plant leaf rust resistance gene *Lr22* is also on chromosome 2DS, more than 50 cM from the centromere. Crosses should be made to obtain linkage estimates with other known genes on chromosome 2DS. The genes tenacious glumes (*Tg*) and waxy foliage (*W2*) would be suitable for this purpose. As previously stated, crosses should also be made between the *LrA* stock and stocks containing the gene transferred by Raupp *et al.* (1989).

4.3.3 Seedling stem rust resistance

The seedling-effective stem rust resistance gene *SrA* was also mapped using the monotelosomic method (McIntosh 1987). The F_2 progenies derived from monosomic F_1 plants were analyzed first to assign *SrA* to a specific chromosome. The results from these F_2 populations are shown in Table 25.

The control crosses between the *SrA* lines and disomic "Chinese Spring" segregated in the expected 3 resistant :1 susceptible ratio, confirming that a single gene was involved. Although the χ^2 value for the cross CS-6DS X *SrA*(direct introgression) was significant, the deviation from the 3:1 ratio was not as large as that of the critical cross (CS-1DL), and the corresponding cross with the derived hexaploid line was not significant ($0.75<P<0.90$). The deviation in this cross was therefore considered to be due to chance, and the gene *SrA* was assigned to chromosome 1D. The χ^2 values for all the other aneuploid progenies and for the disomic progeny were non-significant. The $\chi^2_{Homogeneity}$ for all progenies except the critical chromosome was also non-significant. The assignment of *SrA* to chromosome 1D was confirmed by cytological analysis of the single susceptible plant in the critical family. This plant was confirmed to be nullisomic (2n=40).

Table 25. Segregation for reaction to stem rust race C10 of F_2 progeny derived from monosomic F_1 hybrid plants from crosses between plants homozygous for *SrA* and "Chinese Spring" aneuploids[1]

Seed parent	Segregation R : S	$P_{(\chi 2)}$
A : Pollen parent is direct introgression line		
CS-1D	99 : 1	<0.01
CS-2D	52 : 9	0.05-0.10
CS-3D	72 : 16	0.10-0.25
CS-4D	no progeny	
CS-5D	31 : 6	0.10-0.25
CS-6D	82 : 13	0.01-0.05
CS-7D	71 : 20	0.50-0.75
CS disomic	74 : 14	0.05-0.10
B : Pollen parent is derived hexaploid line		
CS-1D	no progeny	
CS-2D	60 : 24	0.25-0.50
CS-3D	66 : 26	0.25-0.50
CS-4D	65 : 23	0.75-0.90
CS-5D	62 : 26	0.25-0.50
CS-6D	64 : 20	0.75-0.90
CS-7D	77 : 20	0.25-0.50
CS disomic	70 : 29	0.25-0.50

$\chi^2_{\text{Homogeneity}} = 18.03$, df = 12 , $0.10 < P_{\chi 2} < 0.25$

[1] Aneuploids are designated by the chromosome lacking in the F_1 hybrid.

The F_2 populations derived from two monotelodisomic F_1 plants from the cross between the *SrA* direct introgression line and "Chinese Spring"

monotelosomic 1DL were then grown and analysed for stem rust resistance and chromosome number (Table 26). As no difference was noted between the F_2 populations from the two F_1 plants they are combined in Table 26. Three plants with unexpected chromosome complements were observed in this F_2 population. All were resistant to race C10 of stem rust. Two plants had a somatic chromosome number of 2n=41+2t and the third was 2n=41+3t. On the basis of these unusual chromosome complements these plants were excluded from the subsequent analysis.

Table 26. Segregation for reaction to stem rust race C10 and chromosome number in the F_2 population derived from monotelodisomic F_1 plants from the cross CS-1DL/*SrA*

Chromosome number	Rust reaction and number of plants observed	
	Resistant	Susceptible
42	34	0
41 + t	48	0
40 + 2t	0	12

It is evident from the lack of recombinant resistant plants with 2n = 40 + 2t that the gene *SrA* is either not located on the long arm of chromosome 1D or is very closely linked with the centromere (less than 1 cM). Jones *et al.* (1991) have assigned *Sr33* to chromosome 1DS, and although *SrA* also appears to be located on chromosome 1DS (Table 26), crosses at the diploid level indicate that these two genes are not allelic (E.R. Kerber, unpublished data). This further underscores the utility of identifying genes prior to transfer in order to avoid duplication of effort. On the basis of the results in Table 26, the pollen transmission rate of the telosome may be calculated using equation [4] (section 3.6.1) as $x = 0.26 \pm 0.064$.

During the evaluation of the F_2 population described in Table 26 two plants were observed which appeared to contain adult-plant resistance to stem rust race C10. While the first two leaves of these plants were susceptible, the third leaf was resistant (IT 11+). Pending future research to characterize this resistance, a second adult-plant stem rust resistance gene is postulated.

To summarize the results of the gene mapping portion of the project, the seedling-effective leaf rust resistance gene *LrA* was mapped to the short arm of chromosome 2D, more than 50 cM from the centromere. The gene

LrB was mapped to chromosome 5D but the arm location was undetermined. The seedling-effective stem rust resistance gene *SrA* was tentatively associated with the short arm of chromosome 1D, although the distance from the centromere was not determined. It is also possible that *SrA* could be located on chromosome arm 1DL, very closely linked with the centromere. The adult-plant stem rust resistance gene *ASrA* was not assigned to a specific chromosome, but preliminary results indicate a location on one of chromosomes 1D, 4D, or 7D.

4.4 Variability in the rDNA repeat unit

The diversity at the ribosomal DNA locus (rDNA) was investigated in 11 of the 12 *T. tauschii* accessions in Table 1. RL5663 was omitted from this portion of the study. Total genomic DNA was cleaved using the restriction endonucleases Bam HI and Eco RI, and Southern Blots were probed with pMF2.

4.4.1 Eco RI restriction

Since Eco RI has only one cleavage site in each repeat unit, this enzyme will cleave the rDNA array into fragments corresponding to the repeat unit length. As expected, each of the 11 accessions tested produced a single band corresponding to the total repeat unit length. This band

resulted from cleavage at the unique Eco RI site in the 26S rDNA coding region. This site is highly conserved (Appels *et al.* 1980). The repeat unit lengths determined by Eco RI digestion are listed in Table 27.

4.4.2 Bam HI restriction

Two distinct types of restriction fragment patterns resulted from digestion of genomic DNA with Bam HI. Eight of the accessions yielded one type (Type I, see Fig. 3 lanes 5 and 7; Maps A and B, Table 27), and three accessions yielded the other type (Type II, see Fig. 3 lanes 1 and 3; Map C, Table 27). Type I is composed of two fragments as this endonuclease has two cleavage sites, one each in the 18S and 26S rDNA coding regions (Fig. 4). These highly conserved sites are 3.9 kb apart (Appels *et al.* 1980), producing a 3.9 kb fragment in all Bam HI digestions. The DNA fragment located between the 3' end of the 26S Bam HI site and the 5' end of the 18S Bam HI site and encompassing the intergenic region (IGR) was either 5.1 kb (Map A) or 5.4 kb (Map B) and accounted for the variation in total repeat unit length (Table 27). The variable length of this fragment, and thus the total repeat unit, is due to variation in the number of subrepeat units in the IGR (Rogers and Bendich 1987).

The Bam HI restrictions of Type I genotypes also yielded a third DNA fragment which corresponded to the total repeat unit length (Fig. 3, lanes 5 and 7). These fragments result from lack of cleavage of the 26S Bam HI site due to methylation or absence of this site (Appels *et al.* 1980; Kim 1990; Kim *et al.* 1992).

In Type II genotypes, four fragments (6.0 kb, 3.9 kb, 2.5 kb, and 2.1 kb) hybridized to the pMF2 probe (Fig. 3, lanes 1 and 3). As shown in Fig. 4 (Map C), two additional Bam HI sites are present in the IGR, 1.1 kb apart. The 1.1 kb fragment in the IGR does not hybridize to the pMF2 probe, and is therefore not

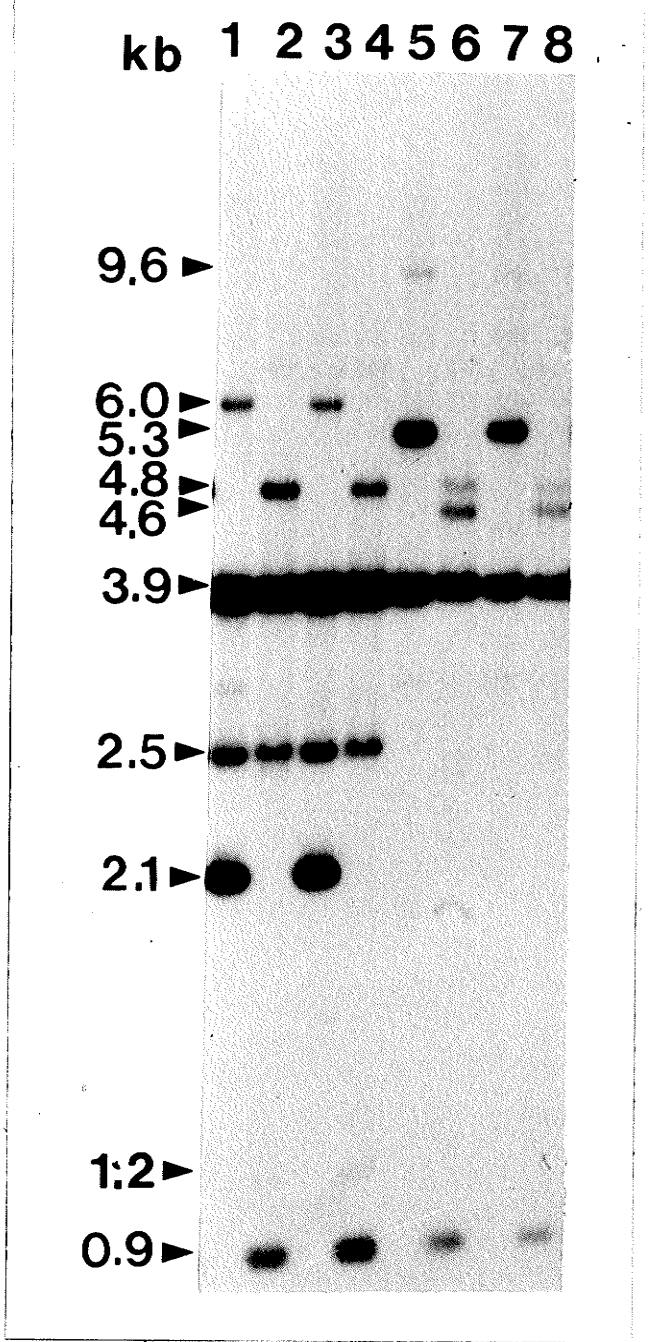

Figure 3. Autoradiograph of Bam HI and Bam HI plus Eco RI restriction fragments probed with pMF2. Lanes 1 and 2 - RL5686 Bam HI and double digestion respectively; Lanes 3 and 4 - RL5688; Lanes 5 and 6 - RL5689; Lanes 7 and 8 - RL5781-1.

detected. However, when the Southern blots were probed with the IGR probe pHbR26a, this fragment was detected. Bam HI sites in the IGR have been reported in barley (Molnar and Fedak 1989, Molnar *et al.* 1989), but such sites have not previously been reported in *Triticum* species. The fragment containing the two additional Bam HI sites in the IGR could have arisen from an insertion event as suggested by Molnar and Fedak (1989). The 6.0 kb fragment in Type II results from lack of cleavage of the 26S Bam HI site, combining the 3.9 kb and 2.1 kb fragments (Fig.4, Map C).

Table 27. Characteristics of the rDNA repeat units of *T. tauschii* accessions

Accession	Repeat unit length (kb)	Map
RL5662	9.3	B
RL5683	9.6	C
RL5686	9.6	C
RL5688	9.6	C
RL5689	9.3	B
RL5764	9.3	B
RL5766	9.3	B
RL5767	9.3	B
RL5778	9.0	A
RL5781-1	9.3	B
RL5782-1	9.3	B

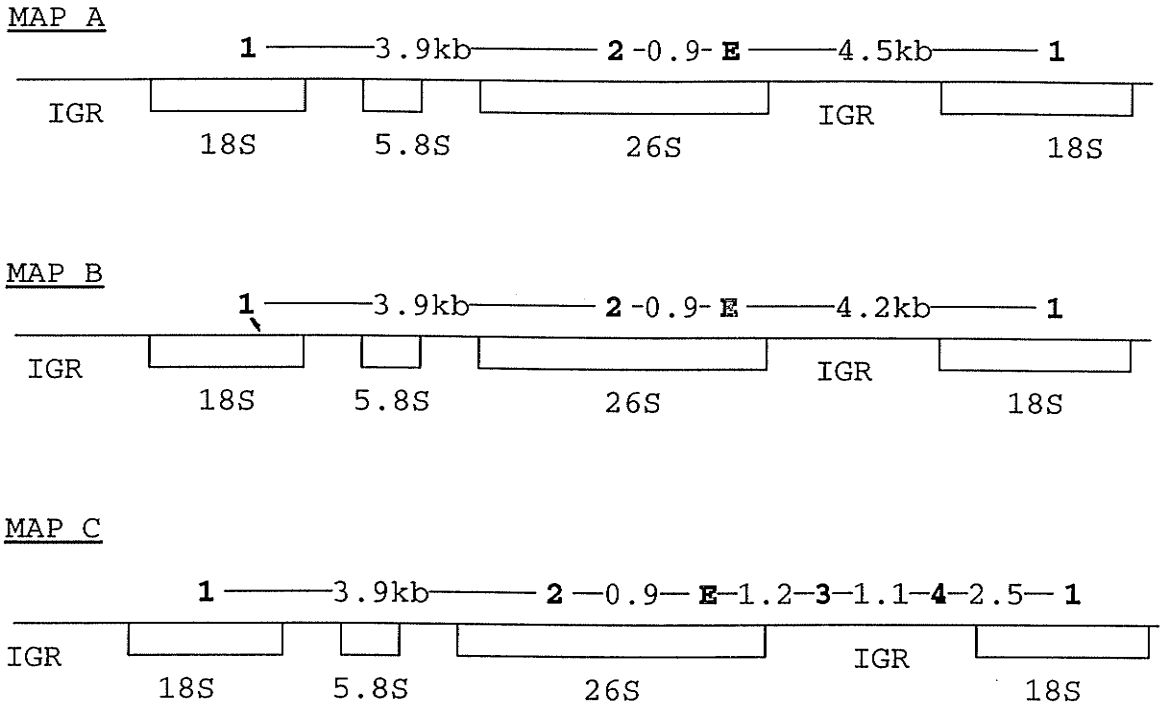

Figure 4. Restriction maps of the three rDNA genotypes found in this study. The 18S, 5.8S and 26S coding regions are indicated by boxes. The unique Eco RI site is indicated as **E**, and the Bam HI sites in the three maps are numbered. Distances are in kb.

4.4.3 Bam HI plus Eco RI double digestion

To further examine the apparent lack of cleavage at the 26S Bam HI site, total genomic DNA of each accession was simultaneously restricted with Bam HI and Eco RI. Since the highly conserved Eco RI site is located 0.9 kb downstream from the 26S Bam HI site (Appels *et al.* 1980), Eco RI cleaves the 5.1 kb and 5.4 kb fragments of Type I and the 2.1 kb fragment of Type II. This produces a constant 0.9 kb fragment in all accessions plus

a variable length fragment in Type I (Fig.3, lanes 6 and 8). The length of this fragment depends on the size of the IGR. In Type II a 1.2 kb fragment is produced (Fig. 3, lanes 2 and 4). A 4.8 kb fragment was also observed in all digestions (Fig. 3, lanes 2, 4, 6, and 8). This fragment is the combined length of the 3.9 kb and 0.9 kb fragments between the 18S Bam HI site and the Eco RI site, and results from lack of cleavage of the 26S Bam HI site. Kim *et al.* (1992) have estimated that cleaveage at this site fails to occur in 5% to 20% of rDNA repeat units.

The Bam HI restriction sites in the coding region of the rDNA repeat unit in *T. tauschii* are highly conserved, which is consistent with related cereals (Appels *et al.* 1980; Molnar *et al.* 1989; Kim *et al.* 1992). Polymorphism was detected in three accessions of *T. tauschii* ssp. *strangulata*, RL5683, RL5686, and RL5688 (Map C, Table 27). RL5683 and RL 5688 were collected from Turkmenia USSR, and RL5686 was collected from Azerbaijan USSR (Table 1). Although these two locations are separated by the Caspian Sea, these accessions share a common rDNA genotype (Table 27) and *Lr* genotype (Table 4). *Triticum tauschii* ssp. *strangulata* accessions RL5781-1 and RL5782-1, both collected from Iran, and RL5689 (collected from Armenia USSR), and *T. tauschii* ssp. *eusquarrosa* accessions RL5662 (geographical origin unknown), RL5764, RL5766 and RL5767 (all from Iran) also share a common rDNA genotype

(Map B, Table 27) which is distinct from Map C. Map A, found in *T. tauschii* ssp. *strangulata* accession RL5778 (geographical origin unknown) differs from the Map B accessions only in the length of the IGR (Figure 4). These findings suggest that *T. tauschii* includes a more diverse group of genotypes than is apparent based on morphological characters.

The classification of *T. tauschii* has been based primarily on morphological characters (Kimber and Feldman 1987) which have proven inadequate to distinguish the botanical varieties. Molnar and Fedak (1989) suggest that variation in rDNA restriction sites may be used as a taxonomic character. Although the length of the repeat unit has no phylogenetic value, Molnar and Fedak (1989) speculate that in barley, the Type I map with two Bam HI sites represents the ancestral state, and that the other maps with more than two Bam HI sites evolved from it. The tendency of rDNA maps with more than two Bam HI sites to have longer repeat units than the two site maps (Molnar and Fedak, 1989) suggests that these maps evolved by insertion of DNA sequences into the IGR. This tendancy is also evident in the three maps found in this study, although it is not evident in other diploid *Triticum* species (Kim *et al.* 1992). Either an insertion or a point mutation in one repeat unit would be either rapidly propagated throughout the array, or eliminated (Dvorak *et al.* 1987). From the genotype of the rDNA repeat unit and the heterogeneous Bam HI sites, the

three accessions with Map C could be considered a separate botanical variety of *T. tauschii* ssp. *strangulata*. It is conceivable that only the ancestral rDNA map containing two Bam HI sites is represented in hexaploid wheat, as this species has only the two sites (Appels *et al.* 1980).

Lack of cleavage at the 26S Bam HI site in some rDNA repeat units is a feature common to *Triticum* species as well as related cereals (Appels *et al.* 1980; Molnar *et al.* 1989; Kim *et al.* 1992). At present it is not known if this site is methylated or absent in some repeat units. The possibility of methylation may be tested by restricting DNA generated by polymerase chain reaction (PCR). PCR-generated DNA has an identical sequence to native DNA but is not methylated. Preliminary work in this area indicates that while all PCR-generated fragments from the 26S rDNA region were cleaved by Eco RI, not all were cleaved by Bam HI (W.K. Kim, unpublished results). Further research is required to confirm the suggested absence of the 26S Bam HI site in some rDNA repeat units and to determine the frequency of such repeat units.

CHAPTER V

Summary and Conclusions

1. This investigation detected four genes for seedling resistance to race 1 of leaf rust in the 12 accessions of *T. tauschii*. These genes have been given the temporary designations *LrA*, *LrB*, *LrC*, and *LrD*. Only *LrA* and *LrB* are expressed in hexaploid wheat.

2. A single gene conferring seedling stem rust resistance to race C10 was also detected in one of the accessions of *T. tauschii*. This gene has been temporarily designated *SrA*. Three of the original accessions of *T. tauschii* were resistant to a broad spectrum of stem rust races at the adult stage of growth. This resistance is due to a single gene temporarily designated *ASrA*.

3. When the seedling-effective leaf rust resistance genes *LrC* and *LrD* were suppressed at the hexaploid level, three adult-plant leaf rust resistance genes were detected. These genes have been temporarily designated *ALrA*, *ALrB*, *ALrC*. Similarly, adult-plant resistance to stem rust race C10 was observed in segregants susceptible at the

seedling stage of growth. The existence of an additional adult-plant gene for stem rust resistance is therefore suspected.

4. The following genes have been transferred to and are expressed in hexaploid wheat : *LrA*, *LrB*, *SrA*, *ALrA*, *ALrB*, *ALrC*, *ASrA*.

5. The seedling genes have been associated with the following chromosomes :

   - *LrA* on chromosome 2DS, more than 50 cM from the centromere.
   - *LrB* on chromosome 5D.
   - *SrA* on either chromosome 1DS or 1DL less than 1 cM from the centromere.

   Additionally, *ASrA* has been located to one of chromosomes 1D, 4D or 7D.

6. The production of amphiploids was found to be the most efficient method of gene transfer from *T. tauschii* to *T. aestivum*. It was also flexible in that in the case of failure to obtain chromosome doubling of the triploid (ABD) hybrid, restitution gametes may still be combined with pollen from hexaploid wheat to yield meiotically stable hexaploid progeny. Although the direct introgression method required more

time and more manipulations, it also offered the opportunity to select for the maximum expression of resistance.

7. Three different rDNA Bam HI restriction phenotypes were observed in these accessions and designated as restriction maps A, B, and C. Maps A and B differ only in the length of the IGR. Map C has two additional Bam HI restriction sites in the IGR. This is the first report of Bam HI sites in the IGR in the genus *Triticum*.

CHAPTER VI

Suggestions for future research

1. This study found three accessions of *T. tauschii* ssp. *strangulata* containing Bam HI restriction sites in the IGR. A larger sample of *T. tauschii* genotypes should be screened to determine the prevalence of these sites in the species.

2. The spectrum of race specificity of the various rust resistance genes identified in this study should be determined.

3. The chromosome arm location of *LrB* on chromosome 5D should be determined.

4. The putative adult-plant stem rust resistance gene *ASrB* should be further characterized by crossing it with *ASrA*.

5. The three adult-plant leaf rust resistance genes should be crossed with stocks containing *Lr22* to ensure that they are different from *Lr22*. If they are indeed new genes, these three and the adult-plant stem rust resistance gene(s) should be mapped.

6. *LrA* should be mapped with respect to the markers *Tg* and *W2* on chromosome 2DS.

7. The Bam HI restriction site in the 26S subunit of the rDNA repeat unit does not appear to be consistently cleaved. This could be due to absence or methylation of this site in some repeat units. This should be investigated by restricting PCR products containing this region, or by digestion with the methylation-insensitive endonucleases Sau3A I or Bst I.

CHAPTER VII

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