A STUDY OF STEM RUST RESISTANCE IN OATS (*AVENA* L. sp.)

by

Alison Margaret Robertson Baillie

A thesis
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Alison M.R. Baillie

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ABSTRACT

Baillie, Alison M.R. M.Sc., The University of Manitoba, July, 1986. A study of stem rust resistance in oats (Avena L. sp.) Major Professor; Dr. P.D. Brown.

The first objective of this study was to screen 171 accessions of the CAV (Canadian AVena) collection to oat stem rust races NA25 and NA27 for sources of new stem rust resistance genes. There were 15 accessions with seedling resistance to both stem rust races. All the plants in an additional 30 accessions were resistant to NA25 only and all the plants in nine accessions were resistant to NA27 only.

The second objective was to determine the nature of inheritance of stem rust resistance in five diploid and nine tetraploid accessions. In the diploid study, resistance was conditioned by a single dominant gene in Saia, SR2838-1-1, SR2840-4-2, SR2842-1-2 and WTR5200. The gene in Saia, conditioning resistance to stem rust race NA25, was allelic to the gene in the other A. strigosa accessions used. For the tetraploid study, stem rust resistance was again conditioned by a single dominant gene in A2957-3-1, A3240-1-1, D145, D203, V2872-1-1, V3255-3-1, V3256-1-1, V3258-3-2, V3261-4-1 and V3304-4-1. The gene in V3304-4-1, conditioning stem rust resistance at both the seedling and adult stages, was

allelic to the gene in the A. abyssinica and the other A. vaviloviana accessions excluding V3261-4-1. The genes found in these accessions were different from the Pg-16 gene found in A. barbata accession D203. The accessions D145 and D203 may have the same gene which would be Pg-16.

The third objective of the study was an attempt to transfer this stem rust resistance from the diploid and tetraploid species to the hexaploid, A. sativa. All F1 diploid/hexaploid plants were sterile, however, resistance to oat stem rust race NA25 was observed in all generations up to and including the F2BC2 generation of the tetraploid/hexaploid progeny. The transmission rate for the rust resistance gene was very low.

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Chapter I

INTRODUCTION

Oats are one of the world's major cereal crops ranking sixth in terms of production. This is exceeded only by wheat, rice, corn, barley and sorghum. The cultivated species include hexaploid Avena sativa L., and A. byzantina C.Koch, tetraploid A. abyssinica Hochst. and diploid A. strigosa Schreb.; A. sativa is the most widely grown. Oats are grown mostly in the temperate zones of North America and Europe. The main countries growing this cereal are the U.S.S.R., U.S.A., China, Poland, West Germany, France and Sweden as well as Canada. In 1983, Canada produced 2.7 million metric tonnes of oats on 1.4 million hectares (Anonymous, 1984a). This is approximately six percent of world production. In Manitoba, 223 000 hectares were sown to oats with the predominant varieties being Fidler and Dumont. Both of these varieties have good rust resistance.

Oats are used for human consumption and animal feed. Regardless of use, the main objectives of an oat breeding program are to increase and stabilize yields. Diseases can cause reductions in grain yield and grain quality resulting in financial loss to the farmer.

Rusts (Puccinia species), are one of the major cereal disease problems and can result in losses equal to ten percent of the world grain crop per year (Agrios, 1978). Rusts decrease foliage and root growth. Rust infected plants have a reduced photosynthetic rate, an increased respiration rate and the plant metabolites are diverted from grain production to spore production. The rust pustules permit uncontrolled water loss from the plant and also weaken the stem causing lodging or breakage. The infected plant produces fewer tillers and fewer seeds per tiller resulting in reduced grain yield. These seeds are usually of poor quality due to the lack of starch and the abundance of cellulosic material which has no nutritional value.

Stem rust (Puccinia graminis Pers f.sp. avenae Eriks. and E. Henn.) is the most widespread and devastating disease of oats in Canada. It attacks cultivated oats, wild oats (A. fatua L.) and meadow fescue (Festuca elatior L.) but not wheat (Triticum aestivum L.) or barley (Hordeum vulgare L.). Crop losses are most frequent in Ontario, Quebec, Manitoba and eastern Saskatchewan. Stem rust can spread by wind currents into Alberta later in the season and has occasionally been found in British Columbia and the Maritimes. In Canada, the rust fungus can have two life cycles, the sexual cycle and the asexual cycle, depending on the presence of the alternate host and the type of environment it inhabits. For Puccinia graminis f.sp. avenae to complete its sexual

life cycle, the alternate host, common barberry (Berberis vulgares L.), is required. This is significant in eastern Canada where the main source of inoculum is from the spores that overwinter and infect barberry plants. In western Canada, barberry is not as common due to eradication programs. Here the main source of inoculum is from the southern U.S.A. and Mexico, where the rust fungus overwinters in oat crops and is carried north by wind currents.

Control of stem rust in North America occurs primarily through the use of resistant varieties. Oat varieties having multigenic resistance to stem rust have been developed. Cultivated varieties have previously been a source of disease resistance but these have been screened extensively. Researchers are currently looking towards the use of wild species as a source of disease resistance. Many of these wild species grow in the centers of origin of Avena species and in areas where virulent races of rust are found. This is mainly in North Africa and the Middle East.

The three goals of the present study were to screen part of the wild oat collection for additional stem rust resistance genes, to determine the nature of inheritance for stem rust resistance in selected diploid and tetraploid species of Avena, and to attempt to transfer this resistance to the hexaploid oat, A. sativa.

Chapter II

OATS

2.1 ECONOMIC IMPORTANCE

Oats are one of the major cereal crops in the world ranking sixth in terms of area seeded and production. This is exceeded by wheat, rice, corn, barley, and sorghum (Anonymous, 1984a).

In 1984, oats were grown on 27 356 000 ha producing close to 46 million metric tonnes. The U.S.S.R. is the largest producer, producing close to 38% of the world production. The U.S.A. and Canada come second and third producing 15% and 6% of the world production respectively. World production has increased since 1980 when 44 million metric tonnes were produced. This is still a lower level of production than for that of 1971 when 56 million metric tonnes were produced (Anonymous, 1984a).

In Canada, oats are the third ranked cereal crop. Data for 1985 show that 1 411 000 ha were sown to oats producing close to 3 million metric tonnes (Anonymous, 1985). The three prairie provinces, Manitoba, Saskatchewan and Alberta, produced the most oats with 73% of the Canadian production. Of the prairie provinces, Alberta has the largest area sown

to oats as well as having the largest production; this is followed by Saskatchewan, then Manitoba.

In 1985, 223 000 ha in Manitoba were sown to oats to produce 555 000 tonnes (Anonymous, 1985). This is 25% of prairie production and 18.6% of Canadian production. In 1985, the area seeded to oats decreased by 8 000 ha to 223 000 ha from the previous year. Total production in 1985 increased 24% over that of 1984 due to an improvement in yield per hectare (Anonymous, 1985). Over a ten year period the average production on 296 000 ha was 565 000 tonnes. Over the same period, the area sown and the production of oats in Manitoba have been decreasing but the yield per hectare has increased. The quality of oats also improved as a higher percentage of oats were in graded in the No. 1 class.

The statistics given are for oats used for grain, both for human consumption utilizing 13% of production and for animal feed. Oats are also used for silage or forage as well as making an excellent nurse crop for newly seeded legumes and other hay crops. In 1984, 32 000 ha of oats were sown in Manitoba and cut for hay production (Anonymous, 1984b).

2.2 CLASSIFICATION

2.2.1 Species classification

The genus Avena forms a polyploid series of diploids, tetraploids and hexaploids with the basic chromosome number of seven. The classification of the genus into species is not well defined. There are many classification systems and as Stanton (1961) stated, it largely depends on the taxonomist. Hackel (1890) reported 50 species, Baum (1977) reported 27 and Rajhathy and Thomas (1974) reported 19 species of Avena. Some of the plant groups that Baum classified as species are classified as subspecies or varieties by some authors.

The definition of species is not clear. One of the definitions given by Van Nostrands Scientific Encyclopedia (1976) is "a species is a group of individuals that resemble each other within established limits". Many classifications are based solely on morphology. Nilsson (1901) used panicle shape, plant colour, number of grains per spike, length of maturing period and form of grain as his basis of classification. Recent classification of species based on morphology includes stem, leaf, panicle, spikelet, lemma, awn and caryopsis as well as physiologic and productivity characteristics.

Another definition of a species, is "a group of individuals that are reproductively isolated from one another"

(Reiger, et al., 1976). This would be properly termed a biological species or genomic species. These would be groups of plants having the same genomic formula and hence would be interfertile. Although Baum (1977) classified A. sativa and A. nuda L. (naked oat) as separate species, others classify A. nuda as a subspecies of A. sativa as there is only a one gene difference between the two groups and, hence, are not reproductively isolated.

A combination of the two definitions would give a more useful definition. Problems could arise if species were grouped entirely by their genomic formula. This would lead to all hexaploids, both cultivated and weedy, being called one species. Therefore, a species is a natural group of individuals which are either similar in form or associated in a reproductive manner.

Rajhathy and Thomas (1974) based their classification on chromosome number, genome and unit of dispersal (Table 1). They classified the genus Avena into 19 species; ten diploid, five tetraploid and four hexaploid.

TABLE 1

Species of *Avena* based on chromosome number, genome and unit of dispersal

Ploidy	Genomes	Diaspore: Floret	Diaspore: Spikelet	Cultivated
Diploid	CpCp	*clauda* Dur.	*pilosa* M. Bieb.	
	CvCv		*ventricosa* Bal.	
2n=2x=14	ApAp	*prostrata* Lad.		
	AlAl	*longiglumis* Dur.		
	AdAd	*damascena* Raj. & Baum		
	AcAc		*canariensis* Baum, Raj. & Sampson	
	AsAs	*wiestii* Steud.		
		hirtula Lag.		*strigosa* Schreb.
Tetraploid	AABB	*barbata* Pott.		*abyssinica* Hochst.
		vaviloviana Mordv.		
2n=4x=28	AACC		*magna* Murphy & Terrell	
			murphyi Lad.	
Hexaploid	AACCDD	*fatua* L.	*sterilis* L.	*byzantina* C.Koch
2n=6x=42				*sativa* L.

- from Rajhathy and Thomas (1974)

2.3 SPECIES

This research deals with the diploid A. strigosa Schreb., tetraploids A. abyssinica Hochst., A. barbata Pott., and A. vaviloviana Mordv., and the hexaploid A. sativa L..

2.3.1 Diploid

Avena strigosa is the cultivated form of the diploid which is still grown to a very limited extent in some parts of Europe. It is native to the Mediterranean area. It has been shown that A. strigosa has very good stem rust resistance (Welsh, et al. 1953). This is especially so with Saia (CD 3820), a selection introduced from Brazil and received by Agriculture Canada Research Station, Winnipeg in 1947. Not only is it resistant to all prevalent stem rust races in North America (Rothman, 1984) but also to smut, halo blight, Victoria blight and the majority of crown rust races (Welsh et al., 1953) as well as leaf blotch (Kunovski and Breshkov, 1981). Saia has the stem rust resistance genes Pg-6 and Pg-7 (Simons et al., 1978).

2.3.2 Tetraploid

The three tetraploid species used in this study belong to the same biological species, that is, share common genomes and are interfertile. Avena barbata originated in the Mediterranean and the Middle East area. This species is used in

California as a range grass. Both A. vaviloviana and A. abyssinica are indigenous to Ethiopia. Avena abyssinica is a cultivated species. Avena vaviloviana and A. barbata are morphologically wild, that is, the florets disarticulate at maturity. Resistance to stem rust has been observed in these tetraploids.

2.3.3 Hexaploid

Avena sativa is the cultivated species more economically important in the northern latitudes, whereas A. byzantina C.Koch is associated more with the Mediterranean and the southern latitudes. Both the cultivated species are inter-fertile with the other two hexaploid species, A. fatua L. and A. sterilis L.. Oat stem rust resistance has been found in these hexaploids.

2.4 THE ORIGIN OF OATS

The origin of oats is not as well understood as it is for other cereals. Oats appear to be of more recent origin than either wheat or barley. The oldest known oats date back to 2000 B.C. in Egypt (Tackholm et al., 1941). These were growing with wheat and barley as weeds, centuries before cultivation.

The cultivated oats were derived from the ancient weedy types. The cultivated diploid oats evolved in Iberia. The

cultivated tetraploid species, A. abyssinica evolved from an A. barbata introduction into Ethiopia with the barley crop. The cultivated diploid and tetraploid species are not grown much today. It is thought that the cultivated hexaploid species, A. sativa and A. byzantina, evolved in western and northern Europe by the end of the first century A.D. (Holden, 1966). These may have originated from the weedy components of primary grain crops such as wheat and barley grown in these areas (Helbaek, 1959).

The origin and evolution of the polyploids cannot be understood unless the genome donors are identified. It is important to know the origin and the relationship of the species in order to facilitate interspecific gene transfer.

2.4.1 Genomes

Nishiyama (1939) designated the genomic formulae for the oat species, where the tetraploids had the genomes AAB'B' and the hexaploids AABBCC. Rajhathy and Morrison (1959) designated the diploid A. strigosa as having the genomic formula AsAs, tetraploids AABB and the hexaploids AACCDD. Karyotypic examination showed that there was no B genome in the hexaploids.

2.4.1.1 Diploids

Holden (1966) stated that the diploid progenitors evolved from an archetype. By mutation and segmental differences affecting the pairing mechanisms, different species evolved.

For the A genome, it is thought that A. prostrata may be the evolutionary link between A. longiglumis and the strigosa-like diploids A. strigosa and A. damascena, or it could, more probably, be the common ancestor to all three species (Ladizinsky, 1973). The sequence of the evolution of these species is still not clear. By chromosome pairing at meiosis, A. prostrata is similar to A. longiglumis but based on plant morphology appears to be more closely related to the A. strigosa relative A. wiestii.

The diploids A. strigosa, A. wiestii and A. hirtula all have AsAs as their genomic formula (Table 1). Holden (1966) called this the AA genome and concluded that A. brevis and A. glabrata also have the AA genome. Rajhathy and Thomas (1974) noted that morphologically the chromosomes of AsAs and AA genomes are identical.

There are other A genome designations in the diploids. These are AcAc (A. canariensis), AdAd (A. damascena), ApAp (A. prostrata) and AlAl (A. longiglumis) (Table 1). All are essentially the same except for some slight modifications. These are considered separate species because they are reproductively isolated. The species A. ventricosa and A. pilosa had the genomic formulas AvAv and ApAp but this was

changed to CvCv and CpCp respectively (Rajhathy, 1966). Avena pilosa was considered as having the A genome only because of similarities of two satellite chromosomes. Avena ventricosa was found to be homologous with A. pilosa, so therefore, it was designated Av because of previous nomenclature. The change was made on the basis of the number of subterminal chromosomes. The As genome has one subterminal chromosome, the Cv and Cp genomes have five subterminal chromosomes. Crosses of A. pilosa and A. ventricosa with either A. strigosa and A. longiglumis have failed in producing viable progeny indicating that A. pilosa and A. ventricosa have a different genome than the As group.

2.4.1.2 Tetraploid

The five tetraploid species belong to two genomic groups AABB and AACC (Table 1). The AABB group includes the species A. abyssinica, A. barbata and A. vaviloviana. The A genome and the B genomes are similar. It is considered that the B genome is a modified form of the A genome. Holden (1966) showed that the origin of these three species would be autopolyploid or segmental allotetraploid, i.e., some pairing between genomes takes place. He proposed the designation AsAsAbAb for the A. barbata species to show the homology between the two genomes. The genomic formula AABB proposed by Rajhathy and Morrison (1959) is an oversimplification as there are different forms of the B genome. Holden

(1966) supports his theory by showing that there is no diploid with the B genome and by stating that the allotetraploids were derived from hybrids between species with partly homologous genomes. Chromosomal, morphological, biochemical and geographical evidence was given to support the autopolyploid theory. It may be reasonable to assume that the B genome is not a discrete entity and that there is no B genome species which would be the progenitor but it is suspected that the diploid A. hirtula-A. wiestii group contains plants which have B genomes or something similar (Holden, 1966).

The other tetraploids, A. magna and A. murphyi, have the genomic formula AACC. It was known that the second genome of these tetraploids was distinctly different from the other tetraploids. This was confirmed by differential pairing behaviour in the hybrids. Rajhathy and Sadasivaiah (1969) proposed AADD as the genomic formula for A. magna. This was changed to AACC by Murray et al. (1970) because of biochemical evidence and a comparison of subterminal chromosomes.

2.4.1.3 Hexaploid

The hexaploids all have the genomic formula, AACCDD (Rajhathy and Morrison, 1959, Table 1).

2.4.2 Origin of the hexaploid oats

It is assumed that A. sterilis is the basic hexaploid type from which the other three hexaploid species have evolved (Coffman, 1946). Avena sterilis is one of the most aggressive and adaptable of the oat species which supports the theory of the origin of the hexaploids. Structural changes and gene mutation may have caused the differences among the four hexaploid species. A change from the "wild" characteristics of shattering grain and a range in dormancy to a non-shattering grain and a reduction in dormancy would be advantageous in cultivation. Avena fatua must have arisen early in the sequence as it possesses more of the "wild" characteristics (Griffiths and Johnston, 1956).

Rajhathy (1966) proposed three alternatives to hexaploid origin:

1. A tetraploid with the formula AsAsCvCv hybridized with a DD species.
2. A tetraploid with CvCvDD formed first then crossed with AsAs.
3. A tetraploid AsAsDD formed an allohexaploid with CvCv.

It is believed that the genomes A and C were mediated from the diploid to the hexaploid, A. sterilis, through A. magna. This would be an allopolyploid origin. Speciation would be promoted by a combination of structural differentiation of

chromosomes and hybridization and doubling, to establish reproductive isolation barriers for biological species.

2.4.2.1 The A genome

The A genome of the diploids is found in the tetraploids and a modification is found in the hexaploid species. Rajhathy (1966) noted that the pairing behaviour of the chromosomes in an A. strigosa by A. sativa cross showed that the As genome is the donor of the A genome in the hexaploids, but there was only partial homology between A and As genomes. Some changes took place between the As and A genomes since A was incorporated in the hexaploid. However, Rajhathy and Thomas (1974) noted that morphologically A. canariensis with the genomic formula Ac is the more probable donor of the A genome for the tetraploid A. magna and the hexaploids. Chromosome pairing between A. canariensis and A. magna, however, does not support this theory.

2.4.2.2 The C genome

Rajhathy (1966) proposed that A. ventricosa is the donor of the C genome. This was determined by karyotypic analysis, morphology, geographic distribution, cross compatability and chromosome pairing in hybrids involving A. ventricosa and the hexaploids.

2.4.2.3 The D genome

The D genome progenitor is currently unknown. By a process of elimination of the A and C genomes, the karyotype of the D genome remains. The progenitor species must have characteristic spikelet morphology found in A. sterilis but this is lacking in all diploid species.

As indicated by the recent discovery of A. atlantica Baum et Fedak, sp. nov. (Baum and Fedak, 1985a), A. agadiriana Baum et Fedak, sp. nov. (Baum and Fedak, 1985b) and A. macrostachya Bal., ex. Cos et Dur. (Baum and Rajhathy, 1976), new species are being discovered and they may provide a better clue to the evolution of the genus Avena. Avena atlantica was classified recently as a diploid with the genomic formula As. The other species, A. agadiriana and A. macrostachya are tetraploids but their genomic formulas are unknown.

2.5 RELATIONSHIP AND CROSSABILITY

Success of crossing depends largely on how closely the species are related. Forsberg and Shands (1969) and Holden (1966) reviewed the relationship between the Avena species.

Rajhathy and Thomas (1974) discussed the cross-incompatability between species. By definition, there is no incompatability within a biological species. It was found that like many other polyploid species, cross-incompatability di-

minishes with increasing ploidy. The hexaploids have good compatability with the tetraploids. The diploid-hexaploid crosses are usually difficult to make and embryo culture is usually required. This is in contrast with the C genome diploids where crosses with some of the A genome diploids and tetraploids have been attempted and failed.

2.6 INTERSPECIFIC CROSSES

In most plant breeding projects the parents used for crossing are within the same species. The cross is easy to make; the hybrid will be fertile and there will be genetic recombination. There are cases in which a desirable characteristic is only found in another species and therefore an interspecific cross will have to be made.

There are four basic reasons for making these wide crosses (Briggs and Knowles, 1967):

1. To transfer one or a few genes from one species to another.
2. To achieve new character expression not found in either parent.
3. To produce a new alloploid species.
4. To determine the relationship of one species to another.

This study deals with the first reason. There are many examples in which interspecific crosses are being made to

transfer desirable characteristics. These are mostly for disease and insect resistance. Wild species are subjected to many diseases and pests in their natural environment and may have some defense mechanisms in order to survive.

2.6.1 Problems associated with interspecific crosses

There are problems associated with interspecific gene transfer which can hinder progress. There are a number of barriers in the interspecific crosses which can prevent seed set. The reproductive isolation mechanisms are divided into two categories: pre-fertilization and post-fertilization barriers, or external and internal barriers. External barriers are those in which there are "physical separations in time, space, environment and specific ecological niches" (Bates and Deyoe, 1973). Internal barriers are either physiological or cytological in nature. The first obstacle is the initial cross which is usually more difficult to make. Fertilization can be prevented when pollen does not germinate on the stigma, or the pollen tube moves slowly or does not grow down the style.

The second obstacle is that of post-fertilization. Embryo development may be terminated when the endosperm degenerates. This can be caused by "disharmonies" between the genomes of the parents or between the genome of one parent and the cytoplasm of the other.

Depending on the direction of the cross, hybrid seed may or may not develop. In a study by Nishiyama and Yabuno (1979) crosses within the ploidy level produced plump seed (6x x 6x, 4x x 4x and 2x x 2x). The 2x x 2x reciprocal crosses gave different results depending on the parental species used. In some cases, between ploidy levels also produced plump seed (4x x 6x), but in wider crosses (2x x 6x) there were only empty or shrivelled kernels. The reciprocal cross (6x x 2x) resulted in poorly developed seeds that were difficult to germinate. Marshall and Myers (1961) got the same results involving crosses with Saia (an A. strigosa accession) and A. sativa in which the hybrid embryo and endosperm appeared normal but aborted shortly after pollination. Brown (1964) reported that in crosses of Saia x Clintland 60 (an A. sativa cultivar) after 24 hours both the embryo and endosperm were normal. After six days the proembryo cells were healthy but there was abnormal endosperm. By ten days the embryo had increased in size but degeneration of the cells had begun.

It has been shown that success in wide crosses is partially limited due to the breakdown of the endosperm. There are a number of theories as to why there is incompatability. These include variation in ratios of genome numbers in the embryo, endosperm and maternal tissue. This is usually of the ratio of 2:3:2, differences result especially when there are differences in ploidy. None of the theories can explain

all the reasons for seed abortion. Nishiyama and Yabuno (1979) working with Avena species presented the hypothesis of polar-nuclei activation. Seed failure is related to the activation index (AI) of the polar nuclei. This is:

$$\frac{\text{Activation value of male nucleus}}{2 \times \text{response value of female nucleus}} \times 100$$

at double fertilization. This is 50% in a selfed plant, any deviation from this usually results in seed abortion due to endosperm breakdown. Nishiyama and Yabuno used 10 species and made 78 reciprocal crosses and found that the AI ranged from 7 to 375%.

If hybrid seed is produced the seed may be inviable due to "disharmonies" between the F1 zygote and the endosperm. The problems do not cease once a hybrid plant is produced. The resulting F1 may die or it may be sterile. This may again be due to the "disharmonies" between the parental genomes or the cytoplasm of one species and the genome of the other. There are two types of sterility depending on the cause: cytological and genetic. Sterility is most often due to the lack of chromosome pairing during late prophase and the first metaphase of meiosis.

The introduction of different germplasm may cause major disturbances in the plant cell. Undesirable characteristics may be transferred, this could result in duplications, deficiencies and lethality. Crossing-over does not occur regularly, and there may be difficulties in breaking linkages

between the desirable genes and the undesirable characteristics.

It is not guaranteed that the gene one is looking for will be transferred or effective. Disease resistance can be lost when it is moved from the diploid or tetraploid species up to the hexaploid species. If it is transferred the expression of resistance is sometimes reduced compared to the parental reaction. This can vary depending on the genetic background.

2.6.2 Overcoming barriers

With increasing knowledge and newer techniques many of these barriers are circumvented. If the problem is the initial cross, one can make reciprocal crosses which may prevent any disharmonies between the genome of one species and the cytoplasm of the other. If the problem is due to a difference in ploidy level, doubling the chromosome number of the lower ploidy species and crossing this autoploid with the higher ploidy level species may result in more normal development. A third species, compatible with the two parents, may be used to bridge the ploidy difference.

Some of the post-fertilization problems can be overcome. When the endosperm degenerates it cannot provide the nutrients required by the embryo for further development. The embryo has to be excised and placed on a nutrient media.

This is the process of hybrid rescue using the technique of embryo culture. Embryo culture has been used in a number of cereal crops to facilitate the cross.

The type of media used in embryo culture varies depending on the species, the type of tissue and its nutrient requirement. It usually consists of organic and inorganic fractions, carbon, vitamins, hormones and minerals. Both Murashige-Skoog (MS) media (Murashige and Skoog, 1962) and Gamborgs B5 media (Gamborg, et al., 1968) have been used in tissue culture of oats. MS media is very high in nitrate, potassium and ammonium. The components are essentially the same as B5 except that the concentration of organic nutrients is higher in B5 than MS. The media composition can be modified to allow specific tissues to grow. Casein hydrolyzate is one of these modifications used for embryo culture. This is a mixture of amino acids acting as a nitrogen source giving a synergistic effect.

To overcome F1 sterility, colchicine can be used to double the chromosome number. This produces amphiploids which have increased vigor and fertility.

2.6.3 The use of wild species in the genus Avena

Even with the problems previously mentioned there have been a number of successes in transferring rust resistance from the diploid and tetraploid species to the hexaploid.

Sadanaga and Simons (1960) succeeded in transferring crown rust resistance from the diploid and tetraploid species using two methods.

1. Producing a synthetic hexaploid by doubling the chromosome number of the hybrid between a diploid and a tetraploid.
2. Producing an autotetraploid of a resistant diploid.

In these cases, the researchers were working with the species A. strigosa, variety Saia.

The transfer of genes can be difficult when the chromosomes involved are not sufficiently alike to permit crossing over. In this case, irradiation can be used to transfer the resistance by causing a translocation. Sears (1956) was the first to use this procedure to transfer leaf rust resistance from Aegilops umbellulata Zhuk. to Triticum aestivum. He also used Triticum dicoccoides (emmer) Korn as the bridge species.

In oats, the use of thermal neutron irradiation has been used to provide stable rust resistance by causing a translocation of that portion of the chromosome with the gene for rust resistance. In one study, the resistant parent was a synthetic hexaploid comprised of A. abyssinica and A. strigosa (Sharma and Forsberg, 1977). Brown (1985) has transferred Pg-16, a stem rust resistance gene, directly from the tetraploid A. barbata, accession D203, to the hexaploid. Gamma irradiation was used to induce a translocation.

Even though these methods are successful in transferring the disease resistance, the rate of success is still very low. Zillinsky and Derick (1960) using the autotetraploid method reported F2 seed set ranging from 0.3 - 0.5%. The use of irradiation still requires much screening to select the resistant plant. These breeding programs require much backcrossing and selecting so as to retain the desirable gene and eliminate other extraneous deleterious material.

2.7 SCREENING AND SELECTING

A source of stem rust resistance must be found before it can be incorporated into the cultivated oat varieties grown in a particular area. Large screening programs have been undertaken to find stem rust resistance genes.

The world collection of cultivars and cultivated oats totalling over 5000 lines is maintained by U.S.D.A. (Anonymous, 1980). These collections have been screened for stem rust resistance to many races of rust. Kunovski and Breshkov (1981), during the period of 1975 - 1977, screened 326 samples of A. sativa and A. byzantina to natural stem rust infection and found that 14.28% of them had some resistance. There are very few genes available in the hexaploids and these have already been used extensively in breeding programs.

For some time, researchers have turned to the wild species of oats for disease resistance genes. These wild species have genes for resistance to powdery mildew (Aung et al., 1977), barley yellow dwarf virus (Comeau, 1984) as well as crown and stem rust (Zillinsky and Murphy, 1967). The use of these genes can sometimes be prohibited due to crossing barriers.

Agriculture Canada has one of the largest collections of wild Avena species in the world. This is known as the CAV collection (Canadian AVena), of which there are about 5000 entries. These have been collected on several expeditions. The major ones being in 1964 to the Mediterranean, 1966 to Israel and 1970 to the Middle East. Other smaller expeditions have been to Kenya, Ethiopia, Tunisia, Algeria, Morocco and the Canary Islands.

2.7.1 Screening the hexaploids

Other than A. sativa and A. byzantina, there are the weedy species A. fatua and A. sterilis. Since all four hexaploid species are interfertile these would be relatively easy to cross and, hence, transfer genes. Over 2200 samples of A. fatua have been collected in the north central United States (Rines et al., 1980). These were screened to stem rust race 94, none of which were resistant. Accessions of A. sterilis have also been screened. Dinoor and Wahl (1963) tested 123 samples from Israel, where there are many viru-

lent races of P. graminis f.sp. avenae, to five different races of rust: 1, 6, 6A, 7A and 8 at both the seedling and adult stage. One resistant accession was found in the greenhouse but no resistance to these races was found in the field. An additional 420 accessions of A. sterilis from the Mediterranean were screened by Zillinsky and Murphy (1967) to stem rust race 6AF. Of these 420 accesssions, only 22 were resistant.

2.7.2 Screening the tetraploids

In the same study by Dinoor and Wahl (1963), 79 accessions of A. barbata were also tested using the same races. Again differences were shown between the greenhouse where 17 accessions were found to be resistant at both the seedling and adult stages and the field where only 8 accessions showed resistance at the same stages. There were two accessions, D145 and D203, that were resistant or very resistant to all races of stem rust at both the seedling and adult stage. D203 also showed excellent resistance to crown rust.

2.7.3 Screening the diploids

Several diploid species have also been screened. It has been mentioned previously that the species A. strigosa has stem rust resistance genes. Accessions of diploids A. clauda Dur., A. pilosa M. Bieb., and A. wiestii Steud. collected in Iraq, Iran and Turkey have been screened but no resistance was found (Martens, et al., 1980).

Chapter III

STEM RUST

3.1 ECONOMIC IMPORTANCE

Stem rust is one of the principal oat diseases occurring throughout the world. When conditions are favourable for the rust, great losses can occur. These losses are expressed in terms of lower yields and lower quality of grain. Plants usually produce fewer tillers and fewer seeds per panicle and these seeds may be small and shrivelled.

The stem rust fungus can affect the plant's growth. Production of photosynthates is decreased due to numerous uredia on the stem and leaves, hence, reducing the photosynthesizing area. The plant also experiences an increase in water loss through the pustules. These effects result in a reduction of nutrients and water available to the plant for seed development and grain filling. If a heavy rust infection takes place early in the season, before the flowering stage of the plant, there is a greater yield reduction than if the infection occurred later.

There have been several epidemics during the 1950's to 1970's in the north central American states and the rust area of the Canadian prairies. In Manitoba in 1970, on 510

000 hectares there was an oat yield reduction of 180 000 tonnes due to stem rust (McKenzie et al., 1971). This was 22% of the total production. In 1977, losses of up to 30% due to stem rust were found in the oat growing region of North Dakota and Minnesota (Roelfs and Long, 1980).

3.2 LIFE CYCLE AND BIOLOGY OF RUST

Rusts are either autoecious or heteroecious basidiomycetes that can occur on trees, ferns, grasses and weeds. The rust fungus belongs to the order Uredinales of the class Basidiomycetes. Puccinia, a heteroecious fungus, is the largest genus of the order with over 4500 species.

There are two distinct cycles, the sexual cycle and the asexual cycle, which oat stem rust can take depending on the environment. The sexual cycle or the complete life cycle has five distinct spore stages. These are basidiospores which have a nuclear condition of 1n, pycniospores (n), aeciospores (n+n), urediospores (n+n) and teliospores (2n).

The most important aspect of the sexual cycle is the potential for variation due to genetic recombination which may result in the establishment of new rust races. In cold climates the fungus overwinters as diploid teliospores on oat debris. The teliospores germinate in the spring to produce haploid basidiospores. These basidiospores are carried short distances by air currents to barberry plants. This is

the alternate host needed for the rust to complete its life cycle. On barberry the basidiospores germinate and penetrate the epidermis. A spermagonium develops and fertilization takes place when the hyphae from this spermagonium comes in contact with hyphae from a compatible spermagonium. The dikaryotic aecial phase is established and the aeciospores are produced on the underside of the leaf. These spores cannot reinfect barberry but are carried by wind currents to infect oat plants in late spring. The aeciospores infect the oat plant through the stomata. A mat of mycelium is formed, additional hyphae develop and at the tip of these there is a dikaryotic urediospore. This growth creates a pressure and pushes the epidermis out to form a pustule. These pustules contain several hundred thousand brick-red coloured urediospores. These spores are easily detached from the hyphae and can be carried great distances by air currents. Urediospores can infect other oat plants and within ten days a new uredium (pustule) and more urediospores are produced. The asexual cycle of uredial infection can occur many times during the summer until the plant matures and the fungus starts producing teliospores which gives the pustules a black colour. The teliospores do not infect oats and are the overwintering stage for the fungus. This is also the stage at which fusion of two nuclei and meiosis takes place resulting in genetic recombination and new rust races. The life cycle repeats itself.

In the southern states and other warm climates the stem rust fungus follows the asexual cycle. The most important aspect of the asexual cycle is the lack of variation due to genetic recombination. The main source of variation and new rust races in the asexual cycle is through mutation. The fungus overwinters as mycelium in the uredial stage on fall sown oats and, therefore, needs no alternate host. Urediospores produced on early spring crops in Mexico and the southern states can be carried by air currents later in the season and can infect the oat crops in the northern states and Canada.

3.3 CONTROL

The use of resistant cultivars is the most effective means of controlling stem rust. Resistant cultivars are currently recommended across Canada. In Manitoba, the varieties Dumont, Fidler and Riel are resistant to the current major rust races.

Barberry eradication programs have also decreased the losses from stem rust by eliminating the early season infections and by preventing new races from developing through genetic recombination. This creates a more stable race population and longer lasting resistant cultivars.

Fungicides can also be used to control stem rust. This is not economical for as many as one to ten applications per season are needed for complete control.

3.4 HOST-PATHOGEN INTERACTION

3.4.1 Stem rust resistance genes

To date, 16 - 17 stem rust resistance genes, Pg-1 to Pg-16 along with Pg-a, have been found and reported (Simons et al., 1978). Differential lines with these genes make up the oat stem rust set which is used for screening and testing. Seven of these genes are excluded from the set for various reasons. The Pg-5 gene is excluded as it may be the same gene as Pg-4. Both Pg-6 and Pg-7 are found in the diploid, A. strigosa. The line with Pg-10 is too temperature sensitive and, therefore, could not be used in the set. Both Pg-11 and Pg-12 are only effective at adult and seedling stages respectively. The line with Pg-14 is not included in the set as not enough is known about it yet.

Five of these Pg genes were found in Avena species other than A. sativa. These are Pg-6 and Pg-7 found in A. strigosa, variety Saia. Both Pg-13 and Pg-15 are found in two accessions of A. sterilis CAV 2667 and CAV 1830 from Tunisia and Turkey respectively. Avena barbata accession D203 from Israel contributes Pg-16 (Martens et al., 1979).

In the current resistant Canadian varieties there are two major stem rust resistance genes per variety. Dumont and Riel have Pg-2 and Pg-13 and Fidler has Pg-1 and Pg-13. There could possibly be a few other genes involved. The genes Pg-13, Pg-16 and Pg-a are effective in controlling

prevalent stem rust races across Canada. In western Canada the genes Pg-9 and Pg-15 are also effective, but in eastern Canada they are not due to the presence of additional races. Across Canada, virulence is high on the lines with Pg-1, Pg-2, Pg-3 and Pg-4 (Harder, 1984).

3.4.1.1 Dominant genes

The dominant genes are Pg-1, 2, 3, 4, 5, 6, 7, 10, 14, 15, and 16.

Pg-1 also called gene S or gene D is found in the cultivar White Russian conditioning resistance to many races of rust (Simons et al. 1978).

Pg-2 also called gene A, found in Green Russian, gives resistance to rust races 1, 2, 3, 5, 7, 7A and 12. This is allelic or closely linked to Pg-1 and Pg-8 (Simons et al., 1978).

Pg-3 also termed gene E is found in Joanette giving resistance to stem rust races 1, 3, 4 and 11. This gene is closely linked or allelic with Pg-9 (Simons et al., 1978).

Pg-4 and Pg-5 may be the same gene, both are found in RL 1225 which is derived from the cultivar Hajira. Pg-4 is also called gene B and Pg-5 is gene C (Simons et al., 1978).

Pg-6 and Pg-7 are both found in the diploid species A. strigosa accession CD 3820. These genes give resistance to races 6, 7, 7A, and 8 (Simons et al., 1978).

Pg-10 is the temperature sensitive gene found in CI 1575 giving a mesothetic reaction to race 13A (Simons et al., 1978).

Pg-14 is a dominant or semi-dominant gene in S81 which conditions resistance to Swedish oat stem rust race Leijerstan 6AB (Simons et al., 1978).

Pg-15 is the partially dominant gene from A. sterilis CAV 1830 (Martens et al., 1980) giving resistance to many races of rust.

Pg-16 is from the tetraploid A. barbata, line D203. Pg-16 is a dominant gene which confers resistance to all stem rust races except NA55. The resistance is expressed up to temperatures of 20 - 22°C (Brown, P.D., pers. comm.).

3.4.1.2 Recessive genes

The recessive genes are Pg-8, 9, 11, 12 and 13.

Pg-8 gives resistance to rust races 1, 2, 6, 6A, 7, 7A, 8, 8A, 10, 13 and 13A at moderate temperatures. This gene was also termed F (Simons et al., 1978).

Pg-9 found in CI 4529 gives resistance to races 6F and 6AF. This gene was termed H (Simons et al., 1978).

Pg-11 is an incompletely recessive gene found in CI 3034, giving resistance to many rust races but at the adult stage only. This gene is associated with discolouration of the plant (Simons et al., 1978).

Pg-12 found in CI 8250, which is the cultivar Kyto, conditions resistance at the seedling stage only (Simons et al., 1978).

Pg-13 is found in A. sterilis accession CAV 2667. This gene confers resistance to many stem rust races but this resistance is only expressed up to 20 - 25°C (Simons et al., 1978).

The nature of the gene Pg-a is unknown.

Pg-a is derived from CI 9139. The exact genetic makeup is unknown, Pg-12 may be present along with one or two genes or modifiers from A. sterilis (Martens, et al., 1979). This gene gives a resistant reaction to all stem rust races but a range of reactions can occur.

3.4.2 Stem rust races

The stem rust fungus consists of several strains differing in their ability to attack certain hosts. The stem rust fungus capable of infecting oats is Puccinia graminis f.sp. avenae, and this is further subdivided into substrains or races. Many designations have been used for race classification. The current nomenclature for oat stem rust is based on host-parasite gene for gene interactions (Martens et al., 1979). For each stem rust race there is an avirulence/virulence formula which shows which genes will be effective/ineffective in controlling stem rust. For example, the race NA25 has the formula 8, 13, 16, a/ 1, 2, 3, 4, 9,

15. The Pg genes 8, 13, 16 and a are effective in preventing infection of this stem rust race while the others listed are not.

There are currently 55 North American races of stem rust designated NA1 to NA55 (Harder, D.E., pers. comm.). Many of these rust races are rarely found in western Canada. In Manitoba and western Canada, NA27 is the dominant race comprising 83% of field isolates in Manitoba and slightly less in Saskatchewan and Alberta. In Ontario and Quebec, NA25 is the dominant race comprising 42% of field isolates (Harder, 1984). The current varieties are resistant to NA25 and NA27 but are susceptible to NA26. In 1983, low levels of NA26 were found but this was confined to eastern Canada.

3.4.3 Stem rust reactions

One of the rating scales used to read rust reactions was devised by Stakman et al. in 1923. He divided stem rust seedling reactions into three classes: R=resistant, S=susceptible and X=mesothetic. The resistant and susceptible groups are further subdivided based on the presence and size of the uredia of the pathogen and on the size and sharpness of the necrosis of the host. On this basis, the resistant class was further divided into three subclasses, 0, 1 and 2. The symptoms of these three subclasses are as follows:

0 - Practically immune. There are no pustules but there are definite hypersensitive flecks.

1 - Extremely resistant. The infection is very light with very small pustules surrounded by sharp necrotic areas.

2 - Moderately resistant. The infection is light with small pustules. There are hypersensitive areas with sharply defined necrosis to pronounced chlorosis.

The susceptible class was divided into two subclasses, 3 and 4 and the expression of these two classes is as follows:

3 - Comparatively susceptible. The infection is moderate. The pustules are mid-sized and tend to merge. There is no hypersensitive reaction but indistinct or poorly chlorotic areas are still seen.

4 - Completely susceptible. The infection is heavy. The pustules are large and numerous with abundant spore production. There are no hypersensitive areas but chlorosis may be present when cultural conditions are unfavourable.

X - This is the intermediate or mesothetic reaction. All infection types may be seen on the leaf, from necrotic flecks to type 4 pustules.

Upadhyaya and Baker (1960) gave a more critical classification for both seedling and adult rust reactions but it was still based on Stakman's scale of 0 - 4. They divided the scale into six categories (Table 2).

TABLE 2

Classification of oat stem rust infections according to Upadhyaya and Baker

Classification	Seedling	Adult	Symbol
Immune	0;	0	I
Highly resistant	;to 1=	1-, 1	R
Resistant	1,1n,1+,2-	1+,2	R
Moderately resistant	2n, 3-cn	1 to 3c	MR
Moderately susceptible	2+,2++,3	3c to 3+	MS
Susceptible	3+c, 4	3+, 4	S

The symbols -,=, + and ++ indicate variations in the type of uredial infection. The ; (semi colon) represents necrotic flecks and "c" and "n" stand for chlorosis and necrosis respectively. Chlorosis is defined as the yellow ring of tissue around the pustule due to chlorophyll destruction. Necrosis is defined as dead or discoloured tissue. In relation to rust resistance, it is a defense mechanism. The necrotic tissue isolates the pathogen from the living tissue on which the pathogen depends for nutrition and growth.

Rating scales based on a percentage of leaf or stem covered with rust have also been devised. Cobb (1892) reported the first diagrammatical rust scale. The scale had five degrees of rust infection, 1%, 5%, 10%, 20% and 50%. These figures were based on the actual percentage of surface area covered with rust.

The modified Cobb scale or U.S.D.A. scale (Melchers and Parker, 1922) has six degrees of infection, 5%, 10%, 25%,

40%, 65% and 100%. The rating of 100% actually has 37% of its surface area covered with rust. This assumes that when pustules cover 37% of surface area the destructiveness of the underlying mycelium is almost at a maximum. The lower percentages were assigned accordingly.

These scales were improved by Peterson et al., (1948). Their scale consisted of 12 degrees of infection (1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100), hence, having smaller regular intervals of rust intensity and a greater range of pustule size. The scale still used 100% as 37% actual surface area covered with rust.

3.4.4 Inheritance of stem rust resistance

It is important to know the nature of the stem rust resistance genes, how many are involved in controlling resistance and whether they are dominant or recessive, in order to fully utilize them in interspecific gene transfer. The first inheritance study data in *Avena* was presented by Garber in 1921. He found a single dominant factor conditioning stem rust resistance in the *A. sativa* cultivar, White Russian. This was known as gene D but is now called *Pg*-1. The early work by Garber with *Pg*-1, Dietz (1928) with *Pg*-2 and Waterhouse (1930) with *Pg*-3 was all with *A. sativa* cultivars. These studies concluded that stem rust resistance was conditioned by a single dominant gene.

3.4.4.1 Diploid

Studies have been conducted on certain accessions of A. strigosa. Murphy et al. (1958) worked with the resistant accession CD 3820 and made reciprocal crosses with the susceptible accession CI 4748. The seedling rust reaction of the F2 material gave a good fit to a 7:8:1 ratio (resistant:segregating:susceptible) which would indicate that two independent dominant genes were involved. Four stem rust races were used, 6, 7, 7A and 8. All families gave the same results, suggesting that the same genes condition resistance to all these races.

These results conflict with those of Dyck (1966). He also worked with CD 3820 and another resistant accession CI 3078. He used three susceptible parents for the crosses with CD 3820 and two susceptible parents for crosses with CI 3078. These were CD 3820/CD 1017, CD 3820/CD 7994, CD 4482/CI 3078, CD 8024/CI 3078 and CI 4748/CD 3820. Segregation in F3 families of the first four crosses gave a good fit to a 1:2:1 (resistant, segregating, susceptible) ratio indicating a single dominant gene. The fifth cross gave a poor fit to a 1:2:1 ratio due to the lack of susceptible lines. In a cross between the resistant accessions CI 3078 and CD 3820 there was no segregation which suggests that the genes are allelic. These plants were tested to a number of races which included the ones Murphy used; 6, 7, 7A and 8 as well as 1, 2, 2F, 6A, 6F, 6AF and 8AF. The reason for the

difference in results obtained by Murphy and Dyck is not totally clear, it was suggested that CD 3820 is not homogenous but heterogenous for rust resistance.

3.4.4.2 Tetraploid and hexaploid

Very little work has been done on the inheritance of stem rust resistance in the tetraploid species. This is in contrast with the many inheritance studies conducted with the hexaploids A. sativa and A. sterilis. As previously mentioned, early research concentrated on A. sativa. Most of the cultivars tested have one dominant gene. Many accessions of A. sterilis have been tested. A recessive gene (Mckenzie et al., 1970) and a partially dominant gene (Martens et al., 1980) have been found.

Chapter IV

INVESTIGATION I

4.1 SCREENING CAV COLLECTION FOR STEM RUST RESISTANCE GENES

4.1.1 Introduction

To breed for disease resistance, one first requires a source of disease resistance. Large programs have been undertaken to screen both the cultivated and wild species of oats for resistance genes.

One of the largest collections of wild Avena species is maintained by Agriculture Canada. This is known as the CAV collection (Canadian AVena), of which there are over 5000 accessions. These have been collected on several expeditions, mainly to the Mediterranean and the Middle East.

The accessions in the collection provides the initial material for oat breeding programs, this is especially so for new sources of disease resistance and quality factors. Parts of the CAV collection have already been screened to stem rust and other diseases.

The objective of this investigation was to screen another part of the CAV collection to two different races of stem rust.

4.1.2 Methods and Materials

4.1.2.1 Parental Material

Many accessions of the CAV collection were rust tested previously. From those accessions possessing resistance, 171 were chosen for further screening. The species and the number of accessions screened in this study are shown in Table 3.

TABLE 3

Avena species and the number of accessions screened to stem rust races NA25 and NA27

Species	Number of Accessions
A. sterilis	79
A. barbata	49
A. vaviloviana	18
A. longiglumis	8
A. abyssinica	7
A. wiestii	4
A. magna	3
A. hirtula	2
A. byzantina	1
	171

4.1.2.2 Inoculation

For the CAV screening study, stem rust races NA25 and NA27 were used. Plants were inoculated at the seedling stage only (1 to 2 leaf).

Inoculation was done by one of three methods:

1. Finger method in which the rust spores were applied by rubbing moistened stems and leaves with rust-covered fingers.
2. Oil suspension in which plants were sprayed with a mixture of rust spores and oil.
3. Talc method in which the rust spores were mixed with talc and were puffed on the leaves which had been coated with a water-surfactant (Tween-20) solution.

To permit spore germination, penetration into the plant and subsequent infection, the plants were incubated in a high humidity atmosphere for 24 hours. After approximately 14 days rust readings were taken. Ratings of ;, 1 and 2 are designated as resistant, whereas, susceptible plants have ratings of 3 and 4.

4.1.2.3 Screening procedure

Five seeds, when five seeds were available, from each accession were treated with 10^{-3} M gibberellic acid for 15 - 20 minutes to improve germination. These seeds were planted in pots in growth cabinets. The growth conditions in the cabinets were 16 hour light period and an eight hour dark period. Temperatures during the light period ranged from 17 - 18°C and during the dark period temperatures ranged from 13 - 14°C. At the 1 and 2 leaf stage, the plants were inoculated with stem rust races NA25 and NA27 respectively. Rust reactions for both stem rust races were recorded. The

upright or prostrate growth habit of the plant was also recorded.

Plants were grown to maturity and the seed was collected from each plant separately. For the plants that shatter, the panicles were bagged with perforated plastic bags to prevent seed loss.

4.1.3 Results and Discussion

The results of this investigation involving screening of the CAV collection, indicated that there are some genes for stem rust resistance in the wild species of Avena. All nine species screened had some resistance except the A. magna accessions which were susceptible to both stem rust races.

Of the 171 (Appendix A) accessions screened to stem rust race NA27, 24 accessions were resistant (having ratings of 0;, 1 or 2), 9 of which were resistant to NA27 but not homozygous resistant to NA25. These are shown in Table 4. Fifty-seven accessions were segregating or had intermediate reactions (ratings of 2 - 3), 70 accessions were completely susceptible (ratings of 3 or 4) and 20 accessions either escaped the rust infection or did not germinate.

Results from the CAV screening to stem rust race NA25 show that 45 accessions were resistant, 30 of which were resistant to NA25 but not homozygous resistant to NA27. This is shown in Table 5. Seventy-six accessions were segregat-

TABLE 4

Avena accessions with seedling resistance to oat stem rust race NA27 but susceptible, intermediate or segregating to NA25

CAV#	Species	Genome	Origin	Stem rust reaction NA27	Growth Habit
526	*barbata*	AABB	Algeria	1+	prostrate
527	*barbata*	AABB	Algeria	1-2	semi-prostrate
528	*barbata*	AABB	Algeria	;1-1+	prostrate
552	*wiestii*	AsAs	Algeria	1=	prostrate
888	*sterilis*	AACCDD	Iran	2	upright
3934	*longiglumis*	AlAl	Morocco	;1-2+	prostrate
3941	*barbata*	AABB	Morocco	;1	prostrate
3952	*longiglumis*	AlAl	Morocco	1+-2-3-	prostrate
4250	*sterilis*	AACCDD	Morocco	1--2+N	prostrate

TABLE 5

Avena accessions with seedling resistance to oat stem rust race NA25 but susceptible, intermediate or segregating to NA27

CAV#	Species	Genome	Origin	Stem rust reaction NA25	Growth Habit
311	*barbata*	AABB	Israel	;1+	prostrate
551	*wiestii*	AsAs	Algeria	1	prostrate
553	*barbata*	AABB	Algeria	1+ - 2-3	prostrate
561	*barbata*	AABB	Algeria	1+-2	prostrate
672	*sterilis*	AACCDD	Iran	1-2	semi-prostrate
826	*sterilis*	AACCDD	Iran	1-2	upright
1355A	*sterilis*	AACCDD	Israel	1--2+	prostrate
1402B	*sterilis*	AACCDD	Israel	;1= - 1-2++	prostrate
2136	*byzantina*	AACCDD	Turkey	2-3-	segregating
2872	*vaviloviana*	AABB	Ethiopia	1-2	upright
2893	*abyssinica*	AABB	Ethiopia	1-2	upright
2907	*vaviloviana*	AABB	Ethiopia	;1	upright
2917	*abyssinica*	AABB	Ethiopia	1-2	upright
2957	*abyssinica*	AABB	Ethiopia	1- -1	upright
3073	*vaviloviana*	AABB	Ethiopia	1-2 - 2-3	upright
3201	*abyssinica*	AABB	Ethiopia	1-2	upright
3232	*vaviloviana*	AABB	Ethiopia	1-2	upright
3240	*abyssinica*	AABB	Ethiopia	1-2	upright
3255	*vaviloviana*	AABB	Ethiopia	1-2	upright
3256	*vaviloviana*	AABB	Ethiopia	1	upright
3261	*vaviloviana*	AABB	Ethiopia	1-2	upright
3268	*vaviloviana*	AABB	Ethiopia	1-1+	upright
3273	*vaviloviana*	AABB	Ethiopia	1-2	upright
3278	*vaviloviana*	AABB	Ethiopia	1	upright
3279	*vaviloviana*	AABB	Ethiopia	1-2	upright
3304	*vaviloviana*	AABB	Ethiopia	1-2	upright
3317	*barbata*	AABB	Turkey	1 - 2-3	prostrate
3322	*barbata*	AABB	Turkey	2 telia	prostrate
3323	*barbata*	AABB	Turkey	1- 2-3-	prostrate
3336	*barbata*	AABB	Turkey	;1 - 1-2	prostrate

ing or had intermediate reactions and 26 accessions were completely susceptible. The accessions that escaped rust infection or did not germinate totalled 26.

There were 15 accessions that had seedling resistance to both NA25 and NA27, this is shown in Table 6. Resistance ranged from 0; to 2-3-. Of the resistant accessions, eight were diploid which may be a problem when trying to transfer the genes to the hexaploid species. Of the resistant diploids, more were found to have resistance to both stem rust races rather than resistance to only one, three were resistant to NA27 and only one had seedling resistance to NA25. Four of the accessions with resistance to both races were tetraploids and three accessions were the hexaploid, A. sterilis.

There were more accessions resistant to NA25 than to NA27. This could be due to the area the accession is from and the stem rust races in that particular area. The accessions that were resistant to NA25 were mainly A. abyssinica and A. vaviloviana from Ethiopia. Other resistant accessions came from Israel and Iran. There are many virulent races of stem rust in the Middle East (Dinoor and Wahl, 1963) and the accessions from these areas must have resistance genes in order to survive. The stem rust race NA25 is one of the most virulent of the North American rust races. Resistance to NA27 was found mostly in the species A. barbata, A. sterilis and A. longiglumis from Algeria and Morocco.

TABLE 6

Avena accessions with seedling resistance to both oat stem rust races NA25 and NA27

CAV#	Species	Genome	Origin	Stem rust reaction NA25	Stem rust reaction NA27	Growth Habit
521	*barbata*	AABB	Algeria	;-1+	;-1	prostrate
539	*hirtula*	AsAs	Algeria	1	1+-2+	prostrate
540	*hirtula*	AsAs	Algeria	;1-	1	prostrate
545	*wiestii*	AsAs	Algeria	1	1-2-	prostrate
546	*wiestii*	AsAs	Algeria	1	1-1+	prostrate
1341A	*sterilis*	AACCDD	Israel	0;-1-	0;	upright
1474B	*sterilis*	AACCDD	Israel	1--2-3-	2--2-3-	prostrate
2850	*vaviloviana*	AABB	Ethiopia	1-2	2-2+	upright
3252	*vaviloviana*	AABB	Ethiopia	1	1+-2+	upright
3260	*vaviloviana*	AABB	Ethiopia	2	1-2	upright
3724	*sterilis*	AACCDD	Tunisia	;	;1	upright
3920	*longiglumis*	A1A1	Morocco	;1-	;	prostrate
3935	*longiglumis*	A1A1	Morocco	1--2	;1	prostrate
3949	*longiglumis*	A1A1	Morocco	;-2	;1--2+	prostrate
3950	*longiglumis*	A1A1	Morocco	;	;1--2	prostrate

Resistance to NA27 is predominantly found in North Africa and not the Middle East, suggesting that different rust races inhabit these areas and, therefore, different resistance genes are needed.

Results indicate that stem rust resistance was less frequent in A. sterilis (9 out of 79 accessions showed resistance) and A. barbata (12 out of 49 accessions showed resistance), than A. vaviloviana, A. wiestii and A. hirtula where almost all accessions screened had some resistance either to one race or to both races. Growth habit and oat stem rust resistance were independent in this study.

4.1.4 Conclusion

This investigation involved screening 171 accessions of nine wild oat species to stem rust races NA25 and NA27 at the seedling stage.

Of these 171 accessions, 15 were resistant to both stem rust races. There were an additional nine accessions homozygous resistant to NA27 only and 30 accessions homozygous resistant to NA25 only. Resistance was found in accessions from all ploidy levels and in all but one of the species tested. The resistance genes found in this study could be used in future attempts to transfer resistance to the cultivated hexaploid species.

Chapter V

INVESTIGATION II

5.1 INHERITANCE OF STEM RUST RESISTANCE GENES IN SELECTED DIPLOID AND TETRAPLOID SPECIES OF AVENA

5.1.1 Introduction

Inheritance studies conducted on F2, F3, F1BC1 and other generations can be used to determine how many genes are involved in the control of a particular characteristic and the nature of the gene(s), whether dominant or recessive.

Inheritance studies for stem rust resistance in oats have been conducted at all three ploidy levels. Most studies have involved A. sativa cultivars. The results of these studies suggest that stem rust resistance is usually conditioned by a single dominant gene. However, in A. sterilis, a recessive gene, Pg-13 (McKenzie, et al. 1970), and a partially dominant gene, Pg-15, (Martens et al., 1980), have been found. Very little work has been done on the inheritance of stem rust resistance in the tetraploid species. As discussed previously, results from studies done at the diploid level have had varying results (Murphy et al., 1958; Dyck, 1966).

The objective of this investigation was to determine the nature of inheritance for stem rust resistance genes, that is, the number of genes involved in resistance, the action of the gene, and whether the resistance genes are the same in selected diploid and tetraploid accessions of Avena.

5.1.2 Methods and Materials

5.1.2.1 Parents

In this study, parents were selected on the basis of their rust reaction. Initial screening of many entries in the CAV (Canadian AVena) collection to many races of stem rust had been done previously (Martens, J.W., pers. comm.). Based on this previous screening, 19 accessions were selected for use in this inheritance study. Accessions used in this study, their origin and their stem rust reaction to NA25 at both the seedling and adult stage are shown in Table 7. Rodney 0 was used as a control for the rust testing.

For both diploid and tetraploid inheritance studies, crosses were made between selected resistant and susceptible accessions. In each of the crosses the male parent was chosen on the basis of its abundant pollen production. For the diploid inheritance study, Saia, with a seedling rust reaction of ;N, was used as the resistant parent and SR2837-3-1, with a seedling rust reaction of 4, was used as the susceptible parent. The diploid crosses made in this study are shown in Table 8. For the tetraploid inheritance study, A.

TABLE 7

Parental species and accessions used in this study

Species	Genome	Accession	Origin	Stem rust reaction to NA25	
				Seedling	adult
A. strigosa	AsAs	Saia	Brazil	;N	2-3
		SR2837-3-1	Portugal	4	4
		SR2838-1-1	Spain	;-3	2-4
		SR2840-4-2	Spain	;1	;1
		SR2842-1-2	Spain	1	;1
		WTR5200	Spain	1	;1
A. abyssinica	AABB	A2957-3-1	Ethiopia	1	2-3
		A3240-1-1	Ethiopia	1-2	2-3
		RL1315	Ethiopia	4	4
		RL1317	Ethiopia	2+	4
		RL1322	Ethiopia	2+-3	3
A. barbata	AABB	D145	Israel	;1-	;1-
		D203	Israel	;1-	;1-
		CAV436	Turkey	;N2	;N2-3
A. vaviloviana	AABB	V2872-1-1	Ethiopia	1	2-3
		V3255-3-1	Ethiopia	1	2-3
		V3256-1-1	Ethiopia	1	2-3
		V3258-3-2	Ethiopia	1	2-3
		V3261-4-1	Ethiopia	;1	2-3
		V3304-4-1	Ethiopia	;1	2-3
A. sativa	AACCDD	Rodney 0	Canada	4	4
		Makuru	New Zealand	4	4

TABLE 8

Crosses used to study the inheritance of oat stem rust resistance at the diploid level

Resistant x Resistant	Resistant x Susceptible
SR2838-1-1/Saia	SR2837-3-1/Saia
SR2840-4-2/Saia	SR2838-1-1/SR2837-3-1
SR2842-1-2/Saia	SR2840-4-2/SR2837-3-1
WTR5200/Saia	SR2842-1-2/SR2837-3-1
	WTR5200/SR2837-3-1

abyssinica RL accessions were used as the susceptible parents in the resistant/susceptible crosses. Avena abyssinica is a cultivated species and has a non-shattering habit. This is beneficial in crosses with wild species; the resulting progeny will have a non-shattering habit which makes harvesting easier. The accessions RL1315, RL1317 and RL1322 were designated as susceptible in previous rust tests but when tested in this study had seedling reactions of 4, 2+ and 2+ - 3 respectively. These accessions were chosen as the susceptible tetraploid parents for use in crosses with the resistant tetraploid accessions on the basis of previous screening. The resistant test parents in the resistant/resistant crosses were D203 and V3304-4-1. Two different resistant tetraploid parents were used as there may be differences in resistance genes due to their diverse origin. The tetraploid crosses made in this study are shown in Table 9.

TABLE 9

Crosses used to study the inheritance of oat stem rust resistance at the tetraploid level

Resistant x Resistant	Resistant x Susceptible
A2957-3-1/D203	A2957-3-1/RL1322
A2957-3-1/V3304-4-1	A3240-1-1/RL1315
A3240-1-1/D203	V2872-1-1/RL1322
A3240-1-1/V3304-4-1	V3255-3-1/RL1317
V2872-1-1/D203	V3256-1-1/RL1315
V2872-1-1/V3304-4-1	V3258-3-2/RL1322
V3255-3-1/D203	V3261-4-1/RL1315
V3255-3-1/V3304-4-1	V3304-4-1/RL1322
V3256-1-1/D203	
V3256-1-1/V3304-4-1	
V3258-3-1/D203	
V3258-3-1/V3304-4-1	
V3261-4-1/D203	
V3261-4-1/V3304-4-1	
V3304-4-1/D203	

5.1.2.2 Inoculation

Stem rust race NA25 was used as the test race. Plants were inoculated at the seedling stage (1 - 2 leaf) and/or adult stage (just after heading). Inoculation was by one of three methods as outlined in Investigation I. Ratings of ;, 1 and 2 were designated as resistant, whereas, susceptible plants had ratings of 3 and 4.

5.1.2.3 Procedure

The F1 seed was grown out in growth cabinets and the plants were allowed to self. The F1 plants were not rust tested at the seedling stage because healthy plants were re-

quired so as to maximize seed production; these plants were inoculated with stem rust race NA25 at the adult stage. In the F2 generation, 100 seeds per cross were sown in greenhouse beds and in pots in growth cabinets. Growth conditions in the growth cabinets were set for a 16 hour light period with a temperature range of 17 - 18°C and a eight hour dark period with a temperature range of 13 - 14°C. Plants were inoculated with stem rust race NA25 at the seedling and adult stage. To get both a seedling and adult reaction for four of the diploid crosses a second set of plantings had to be done as no seedling rust reaction could be read with the initial planting. For the plants that shatter their seeds, the panicles were bagged with a perforated plastic bag to prevent seed loss.

A seedling F3 test was carried out on progeny of the five diploid and one of the tetraploid resistant by susceptible crosses. Approximately 7 - 15 seeds of fifty F2 families were planted in the greenhouse during the winter of 1985 - 1986. There were five replicates. At the seedling stage, each replicate was inoculated with a different stem rust race using the oil suspension method. The races used were NA8, NA26, NA27, NA30 and NA55. Rust reactions were read 14 days later. An additional F3 seedling test was carried out on the diploid resistant by susceptible crosses to verify F2 results. Of the five diploid crosses, approximately 7 - 10 seeds from ten F2 families were sown in the greenhouse. These plants were inoculated with stem rust race NA25.

The avirulence/virulence formulas for the rust races used in this study are shown in Table 10. The oat stem rust race NA25 is one of the most virulent races in Canada and was used as the test race for the F1 and F2 generations in this study. NA25 is avirulent on the stem rust resistance genes Pg-8, Pg-13, Pg-16 and Pg-a. The five stem rust races used for the F3 seedling test were chosen on the basis of economic importance and on the genes they attack. Rust race NA8 will characterize those plants with the oat stem rust resistance gene Pg-13. This gene is also ineffective against NA26; this race is virulent on the recommended resistant varieties grown in Manitoba. Rust race NA27 is the predominant race in western Canada. This race is virulent on Pg-8. NA30 also attacks Pg-8. The stem rust race NA55 is a newly found race which was used in the seedling F3 test as a substitute for NA25. The race NA55 differs from NA25 only in its reaction to Pg-16.

TABLE 10

Avirulence/virulence formulas for the stem rust races used in this study

NA8	1, 2, 8, 16, a / 3, 4, 9, 13, 15
NA25	8, 13, 16, a / 1, 2, 3, 4, 9, 15
NA26	8, 16, a / 1, 2, 3, 4, 9, 13, 15
NA27	9, 13, 15, 16, a / 1, 2, 3, 4, 8
NA30	13, 16, a / 1, 2, 3, 4, 8, 9, 15
NA55	8, 13, a / 1, 2, 3, 4, 9, 15, 16

5.1.3 Results and Discussion

The inheritance of stem rust resistance using the stem rust race NA25 was studied in selected accessions of diploid and tetraploid species of oats. Studies were conducted on the F2 generation at both the seedling and adult stages and on the F3 generation at the seedling stage. The seedling test is usually easier to read and, therefore, has the more reliable results.

5.1.3.1 Diploid inheritance study

Resistant by Susceptible crosses

All F1 adult plants gave a resistant reaction similar to that observed in the resistant parent of the respective cross. The F2 results of the resistant by susceptible crosses using rust race NA25 are shown in Table 11. For both the seedling and adult tests all but one of the crosses segregated with a 3:1 (resistant:susceptible) ratio. Based on the Chi-square statistic, the probability of acceptance for the 3:1 ratio for all but one of the crosses was greater than 0.05. Based on screening with NA25, results indicate that in the selected *A. strigosa* accessions (Saia, SR2840-4-2, SR2842-1-2 and WTR5200) there was a single dominant gene conditioning stem rust resistance at both the seedling and adult stages.

TABLE 11

F2 inheritance study of oat stem rust resistance at the diploid level

Resistant/Susceptible (SR2837-3-1)

Seedling test

	Res	Sus	Total	Ratio	P
SR2837-3-1/Saia	59	12	71	3:1	0.10 - 0.20
SR2838-1-1/SR2837-3-1	72	27	99	3:1	0.50 - 0.70
SR2840-4-2/SR2837-3-1	74	23	97	3:1	0.70 - 0.80
SR2842-1-2/SR2837-3-1	77	19	96	3:1	0.20 - 0.30
WTR5200/SR2837-3-1	78	20	98	3:1	0.20 - 0.30

Adult test

Cross	Res	Sus	Total	Ratio	P
SR2837-3-1/Saia	162	40	202	3:1	0.20 - 0.10
SR2838-1-1/SR2837-3-1	46	50	96	3:1	< 0.01
SR2840-4-2/SR2837-3-1	73	25	98	3:1	0.90 - 0.95
SR2842-1-2/SR2837-3-1	147	51	198	3:1	0.70 - 0.80
WTR5200/SR2837-3-1	72	19	91	3:1	0.30 - 0.50

For the adult stage, the cross SR2838-1-1/SR2837-3-1 did not fit the one gene model (3:1 ratio) found at the seedling stage because of an excess of susceptible plants. This is probably due to a misclassification of the rust reaction. The accession gave good stem rust resistance (; - 3N) at the seedling stage, but at the adult stage the resistance was not as distinct (2 - 4-). For the F2 generation there were several classes of rust reactions. Some F2 plants had resistance (1 - 1+) but many adult plants gave intermediate rust reactions (2+ - 3) and susceptible stem rust reactions (3 - 4). Those plants having ratings of 2+ - 3 were ranked as susceptible.

Resistant by Resistant crosses

All F1 adult plants gave a resistant reaction. This is to be as expected as the parental accessions used in these crosses were resistant. The F2 results of the resistant by resistant crosses using NA25 as the test race are shown in Table 12. The total number of plants tested at the seedling stage and at the adult stage differ. This is because the test had to be repeated due to not being able to read the seedling rust reaction on plants of the first planting. When the test was repeated there were only eight seeds of the WTR5200/Saia cross, therefore, no information was available for this cross at the seedling stage. No rust reactions for the cross SR2838-1-1/Saia were recorded as the two F1 plants died.

TABLE 12

F2 inheritance study of oat stem rust resistance at the diploid level

Resistant/Resistant (Saia)

Seedling test

Cross	Res	Sus	Total	Ratio	P
SR2840-4-2/Saia	103	0	103	no seg.	-
SR2842-1-2/Saia	102	0	102	no seg.	-

Adult test

Cross	Res	Sus	Total	Ratio	P
SR2840-4-2/Saia	202	0	202	no seg.	-
SR2842-1-2/Saia	204	0	204	no seg.	-
WTR5200/Saia	110	0	110	no seg.	-

Results from both the seedling and adult tests show that the plants did not segregate for the stem rust reaction, rather all plants were resistant to rust race NA25. This would suggest that the single dominant gene found in Saia is allelic to the gene in SR2840-4-2, SR2842-1-2 and WTR5200. Saia has the oat stem rust resistance genes Pg-6 and Pg-7 (Murphy et al., 1958). The gene found in the selected accessions could be either Pg-6 or Pg-7.

The single gene model for stem rust resistance at the diploid level agrees with the results found by Dyck (1966). He also worked with Saia and a single gene was found to condition stem rust resistance to many rust races. The results presented here show that the gene found in Saia is allelic to the resistance gene found in other A. strigosa accessions. Both the results presented here and those of Dyck conflict with the results of Murphy et al. (1958). Murphy reported that there were two independent dominant genes involved in conditioning stem rust resistance to many races of stem rust.

5.1.3.2 Tetraploid inheritance study

Resistant by Susceptible crosses

The F1 adult plants gave either a resistant or intermediate reaction similar to the reaction of the resistant parent

in the respective cross. The F2 results of the resistant/ susceptible crosses are shown in Table 13. Two F2 populations, V3256-1-1/RL1315 and V3261-4-1/RL1315 were not obtained as the F1 plants were sterile.

At the seedling stage all crosses of the selected resistant A. abyssinica and A. vaviloviana accessions with the susceptible A. abyssinica accessions segregated with a 3:1 (resistant:susceptible) ratio. Seedling stem rust reactions were distinct with resistant reactions showing a 1 to 2+ type of pustule and the susceptible reaction a 3 to 4 type pustule. Based on the Chi-square statistic, the probability of acceptance for the 3:1 ratio for all crosses was greater than 0.10. These results indicate that in all the crosses studied a single dominant gene was involved in stem rust resistance at the seedling stage to rust race NA25. This single dominant gene would be from the selected resistant A. abyssinica and A. vaviloviana accessions.

Unlike the resistance observed at the seedling stage, the adult rust reactions for the parental accessions were not distinctly resistant or susceptible but rather intermediate. The adult rust reactions for the resistant parents were 2 - 3 and the susceptible parents had an adult stem rust reaction of 3 - 4 (Table 7). This intermediate resistance was reflected in the F2 population. Telia were observed on many of the F2 plants indicating resistance. Due to this intermediate reaction, the F2 adult population was harder to read

TABLE 13

F2 inheritance study of oat stem rust resistance
at the tetraploid level

Resistant/Susceptible (RL accessions)

Seedling test

Cross	Res	Sus	Total	Ratio	P
A2957-3-1/RL1315	74	24	98	3:1	0.90 - 0.95
A3240-1-1/RL1315	70	31	101	3:1	0.10 - 0.20
V2872-1-1/RL1322	70	30	100	3:1	0.20 - 0.30
V3255-3-1/RL1317	76	27	103	3:1	0.70 - 0.80
V3258-3-2/RL1322	77	22	99	3:1	0.50 - 0.70
V3304-4-1/RL1322	78	23	101	3:1	0.50 - 0.70

Adult test

Cross	Int res	Sus	Total	Ratio	P
A2957-3-1/RL1315	77	27	104	3:1	0.80 - 0.90
A3240-1-1/RL1315	97	4	101	3:1	< 0.01
V2872-1-1/RL1322	74	30	104	3:1	0.30 - 0.50
V3255-3-1/RL1317	104	0	104	no seg.	-
V3258-3-2/RL1322	78	25	103	3:1	0.80 - 0.90
V3304-4-1/RL1322	69	30	99	3:1	0.20 - 0.30

than at the seedling stage. Depending on the interpretation of what was classified as resistant and what was susceptible, the genetic ratios may vary. For most of the crosses the resistant class was defined as those plants having rust reactions equal to or better (more resistant) than those of the resistant parent. The susceptible class consisted of those plants having a lower level of resistance (more susceptible) than those of the resistant parent.

When this classification system was used the crosses A2957-3-1/RL1315 and V2872-1-1/RL1322 fit a 3:1 (resistant:susceptible) ratio indicating a single dominant gene for resistance (P = 0.80 - 0.90 and 0.30 - 0.50 respectively). A 3:1 ratio was also observed for the crosses V3258-3-2/RL1322 (P = 0.80 - 0.90) and V3304-4-1/RL1322 (P = 0.20 - 0.30). The 3:1 ratio indicates the presence of a single dominant gene for adult resistance but in these particular crosses the resistance is intermediate. For these four crosses, the plants rated as resistant as seedlings were usually also rated resistant at the adult stage.

When classifying the F2 adult rust reactions based on the parental rust reaction two crosses did not fit any clear genetic ratio. These were V3255-3-1/RL1317 and A3240-1-1/RL1315. For the cross V3255-3-1/RL1317, the parental adult rust reaction for V3255-3-1 was 2 - 3 with predominantly type 3 pustules and RL1317 had a rust reaction of 3 - 4 (Table 7). The F2 population had a stem rust reaction

of 2 - 3 with the majority of the pustules either type 2 or type 3. Classification into resistant and susceptible classes based on the parental reaction resulted in all plants being classified as "resistant". In this case, the resistance was poor and would be more properly termed intermediate resistance. This is similar to the cross A3240-1-1/RL1315 in which the accession A3240-1-1 had an adult rust reaction of 2 - 3 predominantly 3 and RL1315 had a rust reaction of 4. Again the F2 population had an intermediate rust reaction and when classfied based on the parental reaction most were "resistant". However, four plants were susceptible or worse than the A3240-1-1 parent.

For the six tetraploid resistant/susceptible crosses studied, seedling stem rust resistance to race NA25 was conditioned by a single gene. For four of the six tetraploid resistant/susceptible crosses the stem rust resistance to NA25 was also conditioned by a single gene at the adult stage. The gene giving good stem rust resistance at the seedling stage was less effective at the adult stage resulting in poor adult stem rust resistance. The adult plants classified as having intermediate resistance had larger pustules and more urediospore production than resistant plants but the pustule size was inhibited and, therefore, could not be classified as susceptible.

Resistant by Resistant crosses

For the tetraploid species, two different resistant parents were chosen for allelic testing based on their diverse origins. The resistant parental accessions were A. barbata accession D203 from Israel and A. vaviloviana accession V3304-4-1 from Ethiopia. No F2 results for the crosses A3240-1-1/D203, V2872-1-1/D203 and V3304-4-1/D203 were recorded as no hybrid seed was produced. Using resistant parent V3304-4-1, crosses with A3240-1-1 and V2872-1-1 were also not obtained.

Resistant parent D203

All F1 adult plants had resistant reactions as expected in resistant/resistant crosses. The F2 results of the resistant by resistant crosses using D203 as the resistant parent are shown in Table 14. For the seedling test, the crosses A2957-3-1/D203, V3255-3-1/D203, V3256-1-1/D203, V3258-3-2/D203 and V3261-4-1/D203 all segregated to a 15:1 (resistant:susceptible) ratio ($P > .30$). This indicated that two independent dominant genes were involved, each parent contributing one dominant gene.

For the adult test, the crosses A2957-3-1/D203, V3256-1-1/D203, V3258-3-2/D203 and V3261-4-1/D203 behaved the same way as the seedlings, all segregated to a 15:1 ratio which indicated that two independent dominant genes were involved. For the cross V3255-3-1/D203 the rust reaction of

TABLE 14

F2 inheritance study of oat stem rust resistance
at the tetraploid level

Resistant/Resistant (D203)

Seedling test

Cross	Res	Sus	Total	Ratio	P
A2957-3-1/D203	79	7	86	15:1	0.30 - 0.50
D145/D203	98	0	98	no seg.	-
V3255-3-1/D203	87	7	94	15:1	0.50 - 0.70
V3256-1-1/D203	78	7	85	15:1	0.30 - 0.50
V3258-3-2/D203	78	7	85	15:1	0.30 - 0.50
V3261-4-1/D203	64	6	70	15:1	0.30 - 0.50

Adult test

Cross	Res	Sus	Total	Ratio	P
A2957-3-1/D203	66	7	73	15:1	0.20 - 0.30
D145/D203	97	0	97	no seg.	-
V3255-3-1/D203	73	21	94	13:3	0.30 - 0.50
V3256-1-1/D203	86	6	92	15:1	0.90 - 0.95
V3258-3-2/D203	82	7	89	15:1	0.50 - 0.70
V3261-4-1/D203	73	8	81	15:1	0.10 - 0.20

the plants did not segregate to a 15:1 ratio at the adult stage as there were too many plants in the susceptible class. However, the plants did fit a 13:3 ratio. This again indicated that two genes were involved but in this particular cross one of the genes, either from V3255-3-1 or D203, acted as a recessive.

At both the seedling and adult stages plants of the D145/D203 cross did not segregate for the rust reaction; all plants were resistant. This suggests that the gene in D145 is allelic to that gene found in D203.

The A. barbata accession D203 used as the common resistant parent in this study has the stem rust resistance gene Pg-16. This is a single dominant gene. Segregation in crosses with A. abyssinica accession (A2957-3-1) and A. vaviloviana accessions (V3255-3-1, V3256-1-1, V3258-3-2 and V3261-4-1) to a 15:1 or a 13:3 ratio at both the seedling and adult stages suggested that the gene found in the selected resistant accessions is not the same as the gene in A. barbata and, hence, not Pg-16. The ratios suggested that two independent genes were involved in the crosses. One of the genes will be the dominant Pg-16 gene from D203 and the other will be from the A. abyssinica and A. vaviloviana accessions. This gene will be dominant for the crosses giving the 15:1 segregation ratio but will be recessive for the cross giving the 13:3 segregation ratio. Further evidence suggesting that the genes are not the same is their place of

origin. The Pg-16 gene, found in D203, comes from Israel. The A. abyssinica and A. vaviloviana accessions used in this study all come from Ethiopia. It is then possible that the genes are different as there will be different races of rust inhabiting these areas and different genes will be required for resistance to the races in that particular area. The type of rust reaction and the resistance spectrum also differ. The Pg-16 gene conditions a high level of resistance at both the seedling and adult stages (Table 7) to all but one of the 55 North American stem rust races. The resistance found in the A. abyssinica and A. vaviloviana accessions is not as high a level as the resistance found in the A. barbata accessions. This is especially so at the adult stage where the A. abyssinica and A. vaviloviana accessions gave intermediate resistance with rust ratings of 2 - 3 (Table 7). Unlike Pg-16 which confers resistance to all but one of the stem rust races, the gene in the A. abyssinica and A. vaviloviana accessions is effective to some races but ineffective to others. The F2 results of the cross D145/D203 shows no segregation, which suggest that the gene in D145 is allelic to Pg-16 and may be Pg-16. This is quite possible as both accessions come from Israel and are resistant to the same rust races (Dinoor and Wahl, 1963). The resistance in both accessions is a ;1- at both the seedling and adult stages.

Resistant parent V3304-4-1

The F1 adult plants gave resistant or intermediate rust reactions similar to the reaction of the resistant parental accessions used in the respective crosses. The F2 results of the resistant/resistant crosses using V3304-4-1 as the resistant parent are shown in Table 15. At both the seedling and adult stages no segregation for the stem rust reaction was observed for the crosses A2957-3-1/V3304-4-1, V3255-3-1/V3304-4-1, V3256-1-1/V3304-4-1 and V3258-3-2/V3304-4-1. This indicated that the gene in the selected resistant A. abyssinica and A. vaviloviana accessions was allelic to the stem rust resistance gene found in V3304-4-1. Further evidence suggesting that the gene is the same is that all the accessions come from Ethiopia and all are resistant to the same stem rust races. The exception to the absence of segregation is the cross V3261-4-1/V3304-4-1 where F2 results indicated that the genes in V3261-4-1 and V3304-4-1 are not allelic. Segregation for the stem rust reaction to NA25 was observed in the ratio of 15 resistant to 1 susceptible. This suggests that two dominant genes are involved in resistance, one from the V3261-4-1 and one from the V3304-4-1 parent.

As observed in the other tetraploid inheritance studies, the level of stem rust resistance at the adult stage was not as good as the stem rust resistance exhibited at the seedling stage. The parental stem rust reactions were intermediate, that is, having a rust rating of 2 - 3 (Table 7).

TABLE 15

F2 inheritance study of oat stem rust resistance
at the tetraploid level

Resistant/Resistant (V3304-4-1)

Seedling test

Cross	Res	Sus	Total	Ratio	P
A2957-3-1/V3304-4-1	98	0	98	no seg.	-
V3255-3-1/V3304-4-1	100	0	100	no seg.	-
V3256-1-1/V3304-4-1	101	0	101	no seg.	-
V3258-3-2/V3304-4-1	101	0	101	no seg.	-
V3261-4-1/V3304-4-1	90	7	97	15:1	0.50 - 0.70

Adult test

Cross	Res	Sus	Total	Ratio	P
A2957-3-1/V3304-4-1	99	0	99	no seg.	-
V3255-3-1/V3304-4-1	99	0	99	no seg.	-
V3256-1-1/V3304-4-1	103	0	103	no seg.	-
V3258-3-2/V3304-4-1	104	0	104	no seg.	-
V3261-4-1/V3304-4-1	90	11	101	15:1	0.05 - 0.10

This was reflected in the F2 population where most of the plants had an intermediate rust reaction of 2 - 3 with either predominantly type 2 pustules or predominantly type 3 pustules. This made reading and classifying the rust reaction more difficult. Classification of the F2 plants into resistant or susceptible classes was based on the parental reaction. The resistant class was made up of those plants with equal or better resistance than the appropriate parental accessions. Those plants having more susceptible reactions than the "resistant" parent were classified as susceptible.

Very little work has been done on the inheritance of stem rust resistance in the tetraploid species of Avena. The results from this study indicated that stem rust resistance was simply inherited. This means that the stem rust resistance in the selected A. abyssinica, A. barbata and A. vaviloviana accessions is conditioned by a single dominant gene at the seedling and adult stages. In the case of the A. abyssinica and A. vaviloviana accessions, this was illustrated by the 3:1 ratio found in the crosses between the resistant and susceptible accessions (Table 13). Crosses involving the selected A. abyssinica and A. vaviloviana resistant accessions with the resistant D203 accession showed that two dominant genes were involved (Table 14). The exception was V3255-3-1/D203 which segregated for stem rust with a 13:3 ratio at the adult stage (Table 14). This

suggests that two genes are involved but that one of the genes in this particular cross may be a recessive gene. This recessive gene could be either from V3255-3-1 or D203. Based on previous studies (Brown, P.D., pers. comm.) and other crosses in this study, D203 has one dominant gene for stem rust resistance. It is most likely that the accession V3255-3-1 would have the recessive gene as the majority of the F2 plants had a ;1- adult reaction characteristic of D203. The dominant gene would be expressed more frequently. Due to the segregation ratios found in the crosses with D203 it can be concluded that the gene in the selected A. abyssinica and A. vaviloviana accessions is not the same as the gene in D203; hence, not Pg-16. No segregation for the stem rust reaction was observed for the cross D145/D203. This suggests that the stem rust resistance gene in D145 is allelic or the same as the gene in D203. Since D203 has one dominant gene for stem rust resistance, it can be assumed that D145 will also have one dominant gene for resistance; this is probably Pg-16.

In crosses with V3304-4-1 the absence of segregation indicated that the single dominant resistance gene found in the A. abyssinica and A. vaviloviana accessions is the same or allelic to the single dominant gene in V3304-4-1 (Table 15). The exception was V3261-4-1 in which the genes differ at both the seedling and adult stages; the 15:1 segregation ratio in F2 progeny of the V3261-4-1/V3304-4-1 cross indi-

cated that both parents possess one dominant gene for stem rust resistance. Although the gene for stem rust resistance in V3255-3-1 appeared to be a recessive gene for adult resistance in the V3255-3-1/D203 background, this gene appeared to be a dominant gene for adult resistance in the V3255-3-1/V3304-4-1 background.

In the tetraploid inheritance studies conducted, the stem rust resistance gene found at the seedling stage gave good resistance but at the adult stage the gene was less effective. This would account for the intermediate rust reaction observed for the selected accessions at the adult stage. The poor adult resistance conditioned by this gene makes the gene less useful for incorporation in a breeding program unless in combination with another gene.

To determine if a new gene has been found, several approaches can be used. From the results of this study and previous screening of the diploid and tetraploid accessions to many races of rust, one can determine which genes may be involved in rust resistance.

The A. abyssinica accessions A2957-3-1 and A3240-1-1 and the A. vaviloviana accessions V2872-1-1, V3255-3-1, V3256-1-1 and V3304-4-1 may have a stem rust resistance gene similar to Pg-8. All these accessions are resistant to NA25, and four genes, Pg-8, Pg-13, Pg-16 and Pg-a, condition resistance to this race. The accessions mentioned give the

same stem rust reactions as Pg-8 to the nine stem rust races to which they were tested (Brown, P.D., pers. comm.). This was not the case for Pg-13 and Pg-a. Results from the crosses with D203 (which has the gene Pg-16) would indicate that the gene is not Pg-16. Based on the similar avirulence/virulence pattern of Pg-8 and the gene found in these accessions these two genes are similar; however, Pg-8 is a recessive gene and in this study a dominant gene was found to condition resistance to stem rust race NA25. Although results from this study indicate that the resistance genes found in V3258-3-2 and V3304-4-1 are allelic, a different gene may be found in the A. vaviloviana accession V3258-3-2. This is based on previous screening to many races of rust. The accession V3258-3-2 gave a resistant rust reaction of 1 at the seedling stage when screened to stem rust race NA27. The A. abyssinica accessions and the other A. vaviloviana accessions used in this study all gave susceptible rust reactions at the seedling stage when tested to NA27. The A. vaviloviana accession V3261-4-1 may also have a different gene. Results from this study of the resistant by resistant crosses using V3304-4-1 as the resistant parent also indicated that the gene found in V3261-4-1 is different from the gene found in V3304-4-1. There was segregation of a 15:1 ratio at the seedling stage and at the adult stage. This gene seems to be different from all the others including V3258-3-2 as it is resistant to the nine rust races it was tested to (Brown, P.D., pers. comm.). This would eliminate

Pg-8, Pg-13 and Pg-a. Tests with D203 indicated that the gene was not Pg-16.

A further study involving crossing the selected accessions to lines with the known stem rust resistance genes (Pg-1 to Pg-a) would determine if these genes were the same as any existing genes or if they are new genes. There may be problems with this approach as it involves crosses between ploidy levels. The transfer of the gene will be more difficult, the transmission rate will be low and the segregation ratios may be distorted due to the wide crossing.

F3 test

An F3 seedling test was conducted on the diploid and one of the tetraploid resistant by susceptible crosses to verify F2 results and to determine if the genes in the selected resistant accessions were new. However, results from the F3 test did not follow the expected monogenic ratio of 1 homozygous resistant:2 segregating:1 homozygous susceptible observed in the F2 generation. The results are shown in Appendix B. A Chi-square test to fit a 1:2:1 ratio was conducted for the diploid and tetraploid F3 tests but the results were significant based on a probability of acceptance at 0.05. The results for the diploid F3 test did not fit the expected ratio for a one gene difference because of an excess of homozygous resistant and a shortage or absence

of homozygous susceptible F3 lines. In some of the tests there were a shortage of segregating lines as well as susceptible lines. The abundance of resistant lines was noted for all crosses tested to all six rust races. Within segregating F3 lines no expected 3:1 ratio was observed as there were too many resistant plants. Like the diploid test, the tetraploid F3 seedling test did not follow the expected 1 resistant:2 segregating:1 susceptible ratio for a one gene model. But unlike the diploid test where there was an abundance of resistant lines and few susceptible lines, the tetraploid test has a low number of resistant lines and too many segregating F3 lines. The exception was the screening to NA8 where both parents showed resistance. This was reflected in the F3 family where all but two lines were homozygous resistant. It is unclear why two lines should show segregation for the rust reaction unless the rust reaction was affected by environmental conditions. Screening to stem rust races NA26 and NA55 resulted in too many segregating F3 lines and a shortage of homozygous resistant and homozygous susceptible lines, whereas, screening to stem rust races NA27 and NA30 resulted in an excess of both segregating and susceptible lines and not enough homozygous resistant lines. Within segregating F3 lines for all tetraploid families, the number of resistant and susceptible plants did not fit the expected ratio of 3:1 for a single gene model. The distorted ratios found in the F3 results could be due to a number of reasons.

The selection process was not random. As high seed yields were not obtained the plants that had the highest seed yield were used in the F3 test. To test the F2 progeny to five different rust races at least 35 - 50 seeds were required so the diploid and tetraploid F3 lines were selected on the basis of F2 yield. This was on the assumption that homozygous resistant, heterozygous and homozygous susceptible F2 plants would yield the same. This selection process resulted in more resistant than susceptible F2 plants being selected and, therefore, this could have distorted F3 ratios.

A second explanation for the F3 test not following the expected monogenic ratio observed in the F2 generation is that reading the stem rust reaction was more difficult in the F3 generation than in the F2 generation. The resistant and susceptible reactions were not as distinct as in the F2. For the diploid F3 test the majority of the seedling leaves showed signs of necrosis. This necrosis may be due to the plants being hypersensitive to the oil or more likely the necrosis may actually be a defense mechanism against the rust and, hence, be a resistant reaction. The resistant parental accessions showed the necrotic reaction, whereas, the susceptible parental accession did not. In this study, it was assumed that the necrotic reaction was a resistant reaction.

At the tetraploid level there were also difficulties in reading the rust reaction as it was hard to distinguish the three classes (resistant, segregating and susceptible). The parental accessions did not behave as expected to some of the rust races. The accession RL1322, which was used as the susceptible parent in the initial cross, was resistant to stem rust race NA8. It had a stem rust reaction of 1+ - 2, whereas the resistant parent, V3258-3-2, displayed a resistant rust reaction of 1-. The differential set and the controls gave resistant and susceptible reactions as expected. The rust reaction of the F3 lines was rated based on the parental stem rust reaction. For screening the F3 generation to stem rust race NA26, the accession RL1322 used as the susceptible parent again did not show a susceptible reaction. This accession was classified as having a type 2 reaction to race NA26. The resistant accession V3258-3-2 gave a high degree of resistance with a stem rust reaction of ;1-. Rodney 0, the stem rust susceptible hexaploid line used as a control in this study had a stem rust reaction of 2. The differential set showed the expected resistance and susceptibility of the lines but the susceptible reaction was not as complete as expected. This may have been due to the environment or to the rust source. It is unlikely that the environment had any effect as other tests grown at the same time showed differences between resistant and susceptible reactions. Because the V3258-3-2/RL1322 F3 lines were rated based relative to the parental reaction, those plants having

similar reactions to the RL1322 parent were classified as susceptible. For the F3 seedling test screened to stem rust race NA30 both accessions were susceptible. The susceptible accession RL1322 had a rust reading of 3 and the "resistant" accession had a rust reading of 3 - 3+. The F3 generation were rated accordingly. Differences in the parental accessions were clearer when stem rust races NA27 and NA55 were used for screening. The resistant accession V3258-3-2 had a stem rust reading of 1 - 1+ few 2, whereas, the susceptible parent RL1322 had a rating of 2 - 3+.

Different stem rust races were used in the F3 generation than the F2 generation. The distorted ratios may be due to additional genes or the interaction of different genes being expressed by using different stem rust races. The stem rust race NA55 was used as a substitute for NA25 as the only difference is in the reaction to Pg-16. The results showed that there may be differences between the two stem rust races as certain plants gave resistant reactions to NA25 in the F2 generation and were susceptible to NA55 in the F3 generation. This study shows that it is important to screen the population to many stem rust races rather than test to only one.

The environment can sometimes have an effect on the type of rust reactions obtained. This may be the reason for the distorted F3 ratios found in this study. The F2 generation was grown under slightly different conditions than the F3

generation. The majority of the F2 generation was grown in the growth cabinets where temperatures and lighting conditions are more consistent and are more easily controlled. Although not as great, there can be a temperature gradient in the growth cabinet as well as the greenhouse. The F3 generation was grown during the winter in two separate greenhouses differing in lighting conditions. The quality and intensity of the light can affect the rust reactions (Bushnell and Roelfs, 1984). However, the same necrotic reaction was observed under both lighting regimes. The differential set and the susceptible parents did not show any necrotic reaction and within segregating F3 lines there were plants showing symptoms of necrosis and plants that did not.

The distorted F3 ratios occurred for all rust races at both the diploid and tetraploid levels. This resulted in either too many resistant lines, segregating lines or susceptible lines. Within segregating lines the ratio of resistant to susceptible plants did not follow any genetic model. There were usually too many of one class and only one or two of the other. Although there are a number of possible explanations as to why the F3 segregation ratios did not follow the expected ratio, none of these can fully explain the distorted ratios found in this study.

5.1.4 Conclusion

This investigation was conducted to determine the mode of inheritance for selected accessions of the diploid and tetraploid species of the genus Avena. An F2 seedling and adult test was conducted on resistant by susceptible and resistant by resistant crosses. A seedling F3 test was conducted on all the diploid and one of the tetraploid resistant by susceptible crosses. Results indicate that stem rust resistance is simply inherited.

Results from the diploid inheritance study suggests that there is one dominant gene conditioning stem rust resistance to stem rust race NA25 in the selected resistant A. strigosa accessions Saia, SR2838-1-1, SR2840-4-2, SR2842-1-2 and WTR5200. It was also found that the single dominant gene found in SR2840-4-2, SR2842-1-2 and WTR5200 is allelic to the gene found in Saia.

Results from the tetraploid study also show that stem rust resistance to stem rust race NA25 is simply inherited. Results from the resistant by susceptible crosses suggest that one gene is involved in conferring stem rust resistance in the A. abyssinica accessions A2957-3-1 and A3240-1-1 and the A. vaviloviana accessions V2872-1-1, V3255-3-1, V3258-3-2 and V3304-4-1. The gene found in V3304-4-1 is allelic to the stem rust resistance gene in the A. abyssinica and the other A. vaviloviana accessions excluding V3261-4-1.

This gene is different from the Pg-16 gene found in the A. barbata accession D203. Results from the D145 by D203 cross indicates that the genes are allelic. This gene found in D145 may be Pg-16.

Chapter VI

INVESTIGATION III

6.1 INTERSPECIFIC GENE TRANSFER FOR STEM RUST RESISTANCE IN OATS

6.1.1 Introduction

Most interspecific crosses are made to transfer a desirable characteristic such as disease or pest resistance to the cultivated species and eventually to cultivars that are adapted to a particular region. Many of the naturally occurring wild species have disease resistance permitting their survival. Many of the wild species of oats have resistance genes for a number of diseases including stem rust.

If the stem rust resistance gene is in another cultivar or one of the other hexaploid species the cross is easy to make, the hybrid will be fertile and there will be genetic recombination. The few genes found in the hexaploids have been used extensively in breeding programs. The diploids and tetraploids are additional sources of resistance genes to exploit. There are a number of problems associated with using the diploids and tetraploids because of the difference in ploidy and genome composition.

Different methods have been used to successfully transfer rust resistance, these include the use of a synthetic hexaploid made up of resistant diploid and tetraploid species, the use of a bridge species to bridge the ploidy difference and irradiation to induce a translocation.

The objective of this investigation was to determine if the stem rust resistance in the selected diploid and tetraploid accessions could be transferred to the hexaploid.

6.1.2 Methods and Materials

6.1.2.1 Parents

In this study, parents were selected on the basis of their rust reaction. Initial screening of the CAV collection to many races of stem rust had been done previously (Martens, J.W., pers. comm.). The resistant accessions used in this study comprised five accessions of the diploid A. strigosa (Saia, SR2838-1-1, SR2840-4-2, SR2842-1-2, and WTR5200) and ten tetraploid accessions. The tetraploid accessions comprised two A. abyssinica (A2957-3-1 and A3240-1-1), two A. barbata (CAV436 and D145) and six A. vaviloviana (V2872-1-1, V3255-3-1, V3256-1-1, V3258-3-2, V3261-4-1, and V3304-4-1) accessions. The A. sativa hexaploid lines used were Rodney O and Makuru. Rodney O is a stem rust susceptible, near-isogenic line of the cultivar Rodney in which the genes for oat stem rust resistance were eliminated. Makura, a susceptible New Zealand oat variety,

was also used in crossing to the resistant diploid and tetraploid accessions. Two A. sativa lines were used to determine if there was any difference in their potential to cross with the lower ploidy species. The parental accessions, their place of origin and their rust reactions are shown in Table 7.

6.1.2.2 Inoculation

Stem rust race NA25 was used as the test race. Plants were inoculated at the seedling stage and/or adult stage. Inoculation was by one of three methods: fingering, a spore-talc mixture or a spore-oil suspension.

6.1.2.3 Procedure

Crosses were made between the resistant diploid accessions (Saia, SR2838-1-1, SR2840-4-2, SR2842-1-2 and WTR5200) and the hexaploid (Rodney 0 or Makuru) and between the resistant tetraploid accessions (A2957-3-1, A3240-1-1, CAV436, D145, V2872-1-1, V3255-3-1, V3256-1-1, V3256-1-1, V3261-4-1 and V3304-4-1) and the susceptible hexaploid. Rodney O and Makuru were used as the pollen parents because of their abundant pollen production.

Hybrid seed developed normally from the tetraploid by hexaploid crosses but not from the diploid by hexaploid crosses. In this case embryo culture had to be used to rescue the F1 plant.

To improve germination, F1 seed was treated with 10^{-3}M gibberellic acid for 15 - 20 minutes, then dusted with Maneb to provide a manganese supply and prevent fungal growth. After seven days on filter paper, root tips were collected. Seedling rust reactions were not obtained as the plants were frail and could not withstand the rust test. Plants were grown to maturity, inoculated with stem rust and adult rust reactions were taken. The F1 plants were backcrossed to Rodney O; approximately 200 pollinations per cross were made. Because the F1 plants were sterile, colchicine was used to induce chromosome doubling to aid seed development. This was done to different plants from the ones that were used in backcrossing. Colchicine treatment was applied in one of two ways: immersing the crown of the seedling in 0.2% colchicine for two hours followed by several water rinses, or for older plants the soil was removed from the crown, a plastic disc was placed around the crown, cotton wool soaked in 0.2% colchicine was packed around the root and the plant was kept in a closed environment for five days.

The F1BC1 seed was treated similarily to the F1's. Plants were inoculated with stem rust race NA25 at both the seedling and adult stage. The resistant plants, those having stem rust reactions of ;, 1 or 2, were backcrossed to Rodney O using Rodney O as the pollen parent. Both the selfed seeds (F2BC1) and the F1BC2 seeds were planted in the

summer of 1985 in growth cabinets. These cabinets were set at a 16 hour light, 8 hour dark period. Temperatures were maintained at 17 - 18°C during the day and 13 - 14°C at night. The F1BC2 and F2BC1 plants were inoculated with NA25 at the seedling and adult stages and rust reactions were recorded. The plants were allowed to self and percent fertility was recorded. This latter information was obtained by counting the number of seeds and comparing this with the number of florets or potential seeds.

Of the resistant F1BC2 plants, those producing more than 100 seeds were selected. These seeds were planted in the greenhouse during the winter of 1985 - 1986. Attempts were made to keep greenhouse temperature and lighting conditions similar to those of the growth cabinet. The plants were rust tested with stem rust race NA25 at both the seedling and adult stages.

6.1.2.4 Embryo culture

This was carried out in a Laminar flow cabinet using aseptic techniques. Approximately 15 - 20 days after pollination, embryos were extracted from seeds prior to endosperm degeneration. These embryos were placed in vials containing Orchid agar, Gamborg's B5 or Murashige and Skoog's MS media plus 0.2% casein hydrolyzate, and 8g agar/l water. Vials containing an embryo and growth media were kept in a well lit, humid, warm environment. The embryos that did grow

were transferred to vermiculite for a few days to establish a good root system. The seedlings were then transferred to a soil mixture (3 soil: 1 sand: 1 turface) and treated similarily to other F1 hybrids.

6.1.2.5 Cytology

Chromosome counts were made on samples of F1, F1BC1, F1BC2 and F2BC2 material. Seeds were allowed to germinate on filter paper in the light. When the roots were between 1 and 2 cm long the tip portion was cut off and treated in one of two methods: the roots were placed in an ice water bath for 20 hours or placed in a vial containing 0.01% colchicine solution for 1.5 hours. This was done at 8.45 a.m. when best chromosome spread was achieved. After treatment, the root tips were transferred to Farmer's fix (3 parts ethanol: 1 part acetic acid). In preparation for counting chromosomes the root tips were hydrolyzed in 1N HCl at 60°C for six minutes, after which they were transferred to Feulgens stain for approximately 30 minutes or until the root tip was darkly stained. The mitotic growing point of the root tip was cut off, placed on a slide with a drop of aceto-carmine and squashed in preparation for chromosome counting under the microscope.

6.1.3 Results and Discussion

Interspecific crosses were made between the resistant diploid accessions and A. sativa and between the resistant tetraploid accessions and A. sativa. The crosses made, the number of pollinations and the number of seeds produced are shown in Table 16.

The diploid by hexaploid crosses were less successful than the tetraploid by hexaploid crosses. This is shown by the number of pollinations made and the resulting seed. The diploid-hexaploid seed would develop normally up to 15-20 days after pollination then abort. Marshall and Myers (1961) got the same results when working with Saia/A. sativa crosses. The hybrid seed would start to develop normally but would abort shortly after pollination. Brown (1964) noted that abnormal endosperm was evident six days after pollination and after ten days degeneration of the cells had begun. In this study, embryo culture was used to rescue the embryo before endosperm degeneration. Using this technique two F1 plants grew, these were one Saia/Rodney 0 and one SR2842-4-2/Rodney 0 which was later split into two plants. These plants developed normally. Neither backcrossing to Rodney 0 or colchicine treatment resulted in seed development. Without the aid of embryo culture a seed from the diploid-hexaploid cross SR2842-4-2/Makura developed but it was uncertain whether this was a true hybrid. An F1 chromosome number of 28 indicated that this was a hybrid, however, the

TABLE 16

Crosses made between diploid and tetraploid *Avena* species with *A. sativa* and subsequent seed set

accession	*A. sativa* line	number of pollinations	number of F1 seeds produced	%F1 seed set
Diploid				
A. strigosa				
Saia	Rodney 0	376	1*	0.3
SR2838-1-1	Rodney 0	94	0	0
SR2840-4-2	Rodney 0	103	0	0
SR2842-4-2	Rodney 0/Makuru	67	2**	3.0
WTR5200	Rodney 0	174	0	0
Tetraploid				
A. abyssinica				
A2957-3-1	Rodney 0	60	18(3)	30.0
A3240-1-1	Rodney 0	59	46(1)	78.0
A. barbata				
CAV436	Rodney 0	37	25(12)	67.6
D145	Rodney 0	42	31(2)	73.8
A. vaviloviana				
V2872-1-1	Rodney 0	55	24(1)	43.6
V3255-3-1	Rodney 0	45	22(4)	48.9
V3256-1-1	Makuru	56	21(1)	37.5
V3258-3-2	Rodney 0	57	25(2)	43.9
V3261-4-1	Rodney 0	97	31	32.0
V3304-4-1	Makuru	38	13(1)	34.2

* Embryo culture
** 1 Embryo culture and 1 uncertain
(n) number of F1BC1 seeds produced

plant morphology and the rust reaction suggested that it was actually a self. Further testing to stem rust showed no segregation for the rust reaction and reactions were similar to that of the diploid parent.

There was abundant seed set for the tetraploid by hexaploid crosses. As indicated in Table 16 seed set ranged from 30.0 to 78.0%. High seed set for tetraploid-hexaploid crosses has been reported by others (Nishiyama and Yabuno, 1979).

The F1 plants were inoculated at the adult stage only, the rust reactions ranged from 1 - 4- with the majortiy of the readings in the resistant (1 and 2) and intermediate (2 - 3) classes. Stem rust reactions are based on Stakman's scale of 0 to 4. Resistant plants are those plants that have rust reactions of 0, 1 or 2, whereas susceptible plants have rust ratings of 3 or 4.

All F1 crosses were backcrossed to Rodney 0, seed set was low and percent fertility ranged from 0.3 to 2.5% to produce 27 F1BC1 seeds. The F1 hybrids were highly self-sterile. There were only two selfed seeds, these were of the cross CAV436/Rodney 0.

F1BC1

Of the 27 F1BC1 seeds produced (Table 16), 23 germinated. The F1BC1 plants were tested to stem rust race NA25 at both the seedling and adult stages. The data in Table 17 list the eight plants having some resistance as well as their chromosome number and percent fertility. Chromosome counts were available for some of the crosses. This number was approaching that of the normal hexaploid count of 42. Chromosome counts greater than 42 indicate that these plants may have alien addition chromosomes derived from the tetraploid parent. Those plants having fewer than 42 chromosomes are lacking chromosomes, not only from the tetraploid but also from the hexaploid parent.

Resistance was observed in the F1BC1 generation at the seedling stage and although most of the plants had rust ratings of 3 - 4, eight plants did have some resistance. Seedling rust reaction of the resistant plants ranged from 1+ to 2+. Resistance at the adult stage was less pronounced. Rust reactions of the adult plants ranged from 1+ to 3+. This lesser resistance could be due to the nature of the parental accessions. As previously mentioned, the expression of resistance for the adult plants was not as obvious as the resistance at the seedling stage. In the F1BC1 generation, the resistance gene would be in the heterozygous condition, this may also be the cause for the poorer level of resistance shown in the F1BC1 generation than that of the parents. Another reason for the poorer rust reactions is the

TABLE 17

Resistant plants of the F1BC1 generation with their chromosome number, rust reactions and percent fertility

Plant No.	Cross	Chromosome number	Rust reaction to NA25			F2BC1		F1BC2	
			Seedling	Adult leaf	Adult stem	number of seeds	% fertility	number of seeds	% fertility
1	CAV436/2*Rodney O	43	2+	2	3-3+	0	0	4	5.7
2	CAV436/2*Rodney O	*	2+	2	3-3+	33	1.9	20	20.6
3	CAV436/2*Rodney O	*	2+	2	3	0	0	10	30.3
4	V3255-3-1/2*Rodney O	44	1+-2	*	3-3+	2	0.4	0	0
5	V3255-3-1/2*Rodney O	42	2	2	3	3	0.7	5	5.4
6	V3255-3-1/2*Rodney O	40	2	1+-2	2--2	2	0.1	9	14.5
7	V3256-1-1/Makuru//Rodney O	40	2-	*	2	0	0	1	3.0
8	V3258-3-2/Rodney O//Makuru	*	2+	2+-3	3+	0	0	3	4.6

* not available

gene may be sensitive to the genetic background, therefore, the level of resistance is reduced when the gene is transferred from the lower ploidy species to the hexaploid. This has occurred in interspecific crosses involving Triticum monococcum L. and hexaploid wheat in an attempt to transfer stem rust resistance (Kerber and Dyck, 1973). The plants had a rust rating of ;1 at the diploid level, 1+ for the tetraploids and a type 2 reaction at the hexaploid level.

Of the 23 F1BC1 plants that grew, eight were selected as being resistant, as shown in Table 17. The number of selfed seeds from all 23 plants had increased from two in the F1 generation to 271 for the F1BC1 generation. The percent self-fertility ranged from 0 to 8.6%. The highest self-fertility was again with the cross that produced the two selfed F1 seeds (CAV436/Rodney 0). The resistant F1BC1 plants were backcrossed to Rodney 0 to produce F1BC2 seed. Seed set had increased slightly but was still low; this ranged from 0 to 30.3%. There were 52 F1BC2 seeds produced.

F1BC2

Of the 52 F1BC2 seeds produced, 45 germinated and grew into plants. Of these 45 plants, 25 were susceptible, four had seedling resistance only, five had adult resistance only and 11 had both seedling and adult resistance. Plants having resistance at both stages was 24% of the total number of

plants. These plants are shown in Table 18 along with their chromosome number and percent fertility. The chromosome number of the F1BC2 generation was not 42, the normal hexaploid chromosome count, but was approaching it. With the second backcross to A. sativa, the chromosome number either stayed the same or increased with the introduction of A. sativa chromosomes.

The results of Investigation II indicated that in the accessions V3255-3-1 and V3258-3-2 the same gene conditioning stem rust resistance to NA25 was effective at both the seedling and adult stages. Those 11 plants having resistance at both stages suggested that the genes for seedling and adult resistance are not independent. Those plants having either seedling or adult resistance do not agree with one gene conditioning resistance at both stages. This may be due to the background the foreign gene or chromosome may be in. This background may cause the resistance gene to be less effective at either the seedling or adult stages subsequently giving adult or seedling resistance respectively. For the plants that were resistant at both stages the results show that there was resistance at both the seedling and adult stages although resistance at the adult stage was not as good. This is to be expected as the parental accessions show a higher level of resistance at the seedling stage than at the adult stage. Also, a reduced level of re-

TABLE 18

Resistant F1BC2 plants based on their rust reactions to NA25
with chromosome numbers and percent fertility

Plant source	Cross	chromosome number	Rust reaction to NA25			number of F2BC2 seed	% self fertility
			seedling	Adult leaf	Adult stem		
2-1	CAV436/3*Rodney 0	39	2N	1-2	2	14	3.0
2-2	CAV436/3*Rodney 0	40	2	2-2+	2-2+	7	1.0
2-3	CAV436/3*Rodney 0	*	1+-3	2-2+	2+-3	339	44.8
2-4	CAV436/3*Rodney 0	*	1+-2N	*	2-2+	212	32.2
2-5	CAV436/3*Rodney 0	39	1+-2	2	2+-3	243	54.2
2-6	CAV436/3*Rodney 0	*	1+-2	2+	2-3	0	0
3-1	CAV436/3*Rodney 0	41	2	*	1-2	77	22.7
6-1	V3255-3-1/3*Rodney 0	43	1+-2	2+	2+-3	34	4.9
6-2	V3255-3-1/3*Rodney 0	40	1+-2	*	2-2+	212	57.9
6-3	V3255-3-1/3*Rodney 0	*	2-3	1+	2-3	2	0.6
8	V3258-3-2/Rodney 0/2/Makuru/3/Rodney 0	41	N;	2+	2-3	21	3.7

* not available

sistance compared with the parents could be expected because in the F1BC2 generation the resistance gene would be in the heterozygous condition.

The F1BC2 generation was allowed to self. By this generation self-fertility had increased greatly and now ranged from 0 to 84.2% for all plants and from 0 to 57.9% for the resistant plants as shown in Table 18. There appears to be no relationship between chromosome number and percent seed set. This may be due to what chromosomes are present in the chromosome complement of the plant. Certain essential genes and chromosomes necessary for fertility and normal functioning of the plant may be missing from the genome. Having the foreign chromosomes and the A. sativa chromosomes in the same background may affect meiosis in the plant which could lead to partial sterility.

F2BC2

Five crosses of the F1BC2 generation were selected on the basis of their rust reaction and the number of F2BC2 seeds produced. The crosses selected along with their F2BC2 rust reactions, chromosome counts and percent fertility are shown in Table 19.

Chromosome counts of a representative sample had been recorded for the F2BC2 generation. These were either 40, 41, 42 or 43, which is approaching the normal hexaploid chromo-

TABLE 19

Inheritance of oat stem rust resistance in the F2BC2 generation

Cross	Population Source	Chromosome number	Reaction to stem rust race NA25								percent fertility	
			seedling stage				Adult stage					
			Res.	Int.	Sus.	Total	Res.	Int.	Sus.	Total	Res.	Sus.
CAV436/3/Rodney 0*1	2-3	40, 41	0	7	93	100	0	0	98	98	0	24.5
CAV436/3/Rodney 0*1	2-4	42, 43	10	11	64	86	5	3	75	83	14.9	33.0
CAV436/3/Rodney 0*1	2-5	40, 41	6	4	86	96	0	3	85	88	44.7	43.2
CAV436/3/Rodney 0*1	3-1	40, 41, 42	3	1	49	53	3	1	47	51	10.6	48.5
V3255-3-1/3/Rodney 0*1	6-2	40, 41	7	9	86	102	7	7	87	101	9.3	17.8

some number of 42. The plants with 40 and 41 chromosomes were susceptible indicating that neither the resistance gene nor the chromosome carrying the resistance gene had been transferred. The resistant plants having 42 chromosomes could have had the chromosome carrying the stem rust resistance gene substituted into the hexaploid complement, or a translocation had occurred and the gene has been incorporated on to the A. sativa chromosome. The 43 chromosome plants would have an extra chromosome derived from the tetraploid species. Depending on the presence or absence of stem rust resistance genes on the foreign chromosome the plants would be either resistant or susceptible.

The percent fertility was recorded for the resistant plants as well as the representative sample used for chromosome counts. Fertility had increased slightly over that of the previous generation. The range was from 0 to 86.8%, however, the average seed set had increased greatly. There was no relationship between the chromosome number or rust reaction with seed set.

As observed in Investigation II oat stem rust resistance was simply inherited as a single dominant gene. In the F2BC2 generation, assuming monogenic inheritance and normal transmission of the rust resistance gene the expected ratio should be 3 resistant:1 susceptible. This monogenic ratio was not observed for any of the crosses at either the seedling or adult stages. This agrees with other interspecific

gene transfer studies where very few resistant plants were recovered. Distorted segregation ratios have been found in backcross generations of F2 and F3 of derived tetraploid/hexaploid crosses (Sadanaga and Simons, 1960) and in F5 and F6 generations of tetraploid/hexaploid crosses in oats (Forsberg and Nishiyama, 1969). In this study, transmission of the stem rust resistance gene from the lower ploidy species to the hexaploid occurred but was low.

The results from the first cross in Table 19 indicated that the stem rust resistance gene or chromosome was not transferred or transmission was so low that resistance was not observed within the population size studied. This was observed for both the seedling and adult stages. The seven plants classified as intermediate at the seedling stage may have had this reaction due to environmental conditions. This would include the health of the plant. Plants that are stressed in any way or are dying give a more resistant reaction as the growth of the rust pustules is inhibited. The intermediate reaction could also have been due to the interaction of the tetraploid with genes in the hexaploid Rodney 0. An inhibitor has been found in wheat that will suppress the rust resistant reaction (Kerber and Green, 1980). This suppressor, associated with the D genome, acts on the rust resistant gene found in the A and B genomes. This results in the gene giving a lower level of rust resistance when present in the hexaploid background. It is possible that

there is a suppressor, like the one in wheat, but functioning in oats.

For the other four crosses using V3255-3-1 and CAV436 as the rust resistance sources, three distinct classes of rust reactions were observed. The resistant class included plants having rust ratings of 1 - 2+ and the few plants that had necrosis. The necrotic reaction was classified as resistant as several of the parental accessions also had necrosis. This class may have had the stem rust resistance gene in the homozygous condition, therefore, conditioning a higher level of resistance. This resistance was comparable to that of the resistant parental accessions. The plants having rust ratings of 2 - 3 were classified as intermediate. These plants appear to have some resistance as not only the pustule size but also urediospore production were both reduced. The intermediate rust reaction could be conditioned by the same stem rust resistance gene but in the heterozygous condition. This explanation assumes that both stem rust resistance alleles are required to give a high degree of resistance. The presence of one allele would give intermediate resistance. The majority of the plants in the F2BC2 generation gave susceptible reactions (ratings of 3 - 4) (Table 19). These plants would not have the stem rust resistance gene and, therefore, would be the homozygous susceptible class.

In most cases, the plants showing resistance at the seedling stage also showed resistance at the adult stage. This is to be expected if the same gene is effective at both the seedling and adult stages. The adult resistance was not as good as the resistance exhibited at the seedling stage. This reflects the resistance pattern of the parental accessions which behaved in the same way. Sometimes the resistance was not observed at both stages; plants showing resistant reactions at the seedling stage would have susceptible reactions at the adult stage. The opposite was also observed. The reason for this was not clear; it could be due to the interaction of the tetraploid and hexaploid backgrounds.

A small number of resistant plants were observed in the F2BC2 generation (Table 19). This may be due to differential transmission of the rust resistance alleles (Kerber and Dyck, 1973). In the previous generation, F1BC2, the plants were healthy and there was a high level of germination of the F2BC2 seeds. It is, therefore, unlikely that differential genotypic plant viability or differential viability of seeds had any effect on decreasing the number of resistant plants observed. Differential zygotic elimination is expressed in plants as partial sterility or the development of inviable seeds. The percent fertility of the F1BC2 generation had increased from the previous generation (Tables 17 and 18) but this was still low compared to normal seed set.

The partial sterility observed could be due to differential transmission but more likely it is due to cytological abnormalities. The differential transmission of the rust resistance alleles through the male and/or female gametes could have been the cause of the low number of resistant plants observed. This may be a certation effect where the gamete carrying the allele for resistance is less effective in fertilization than the gamete carrying the allele for susceptibility. This would result in a greater number of susceptible plants and a lower number of resistant plants than expected.

Another possibility for the small number of resistant plants in the F2BC2 generation was that the resistant plants in the previous generation, F1BC2, could have been monosomic alien substitution or addition lines. Chromosome counts were not available for some of these crosses. The monosomic alien chromosome derived from the tetraploid accessions would be carrying the resistance gene. This chromosome may have been lost or transferred at a low frequency when the plants were selfed to get the F2BC2 generation. In the addition lines the n + 1 pollen would be less effective in fertilization than the n pollen. Dyck and Rajhathy (1963) working with the autotetraploid A. strigosa in attempts to transfer crown rust resistance found that in the selfed progenies of monosomic addition lines only 10% of the plants were resistant. The transmission of the n + 1 pollen was very low, (3.3%).

6.1.4 Conclusion

The objective of this investigation was to determine if the stem rust resistance of selected diploid and tetraploid accessions could be transferred to the hexaploid A. sativa.

The interspecific crosses involving the diploids were hard to make and embryo culture had to be used to facilitate the cross. In this study, two crosses were achieved this way but because of sterility nothing past the F1 generation was ever accomplished.

The tetraploid by hexaploid crosses were much more successful as abundant F1 seed was produced. Resistance to stem rust race NA25 was observed in the F1, F1BC1, F1BC2 and F2BC2 generations. By the F2BC2 generation, the chromosome number of the plants was approaching the stable hexaploid chromosome count of 42. The self fertility was also improving and would continue to improve with further generations.

Investigation III indicated that stem rust resistance can be transferred from the lower ploidy species to the hexaploid. This transmission was low but resistance was exhibited at both the seedling and adult stages. Adult stem rust resistance was of a lower level of resistance than the resistance found at the seedling stage. For this resistance to be of use it would require that the gene would have to be in combination with another gene to give a higher degree of resistance at both stages. Presently, this resistance is

unstable and further studies would be required before it could be used in a practical breeding program.

Chapter VII

GENERAL DISCUSSION

Plant breeders are always looking for new sources of genetic diversity in crop species. There will always be a need for new genes for disease resistance or insect resistance in order to replace the genes that are no longer effective. The wild species of cultivated crops are not usually important economically but they do form an invaluable reserve of resistance genes.

The genus Avena consists of 19 species based on chromosome number, genome and unit of dispersal (Rajhathy and Thomas, 1974). The 19 species include ten diploid species, five tetraploid species and four hexaploid species. This includes both cultivated and non-cultivated species. Collections of cultivated and non-cultivated species are used to provide genetic variability in oat breeding. Many cultivars and wild species possess characteristics such as quality factors and disease resistance which would be desirable to incorporate into the high yielding cultivars already adapted to a particular area.

Stem rust of oats is a major problem on the Canadian prairies as well as in the United States. New sources of resistance are needed as rust races can arise through genet-

ic recombination or mutation to cause the pathogen to attack the genes that are presently in the recommended varieties. The current resistant varieties recommended for Manitoba and eastern Saskatchewan have two major genes for stem rust resistance.

Investigation I - Screening for stem rust resistance.

In the present study, additional sources of oat stem rust resistance were found by screening 171 accessions of the CAV (Canadian AVena) collection to two stem rust races. The races used for screening were NA25, which is the most prevalent race in eastern Canada, and NA27 which is the prevailing race in western Canada (Harder, 1984). Results indicated that there were 15 accessions (CAV nos. 521, 539, 540, 545, 546, 1341A, 1474B, 2850, 3252, 3260, 3724, 3920, 3935, 3949 and 3950) that were resistant to both stem rust races. In addition nine accessions (CAV nos. 526, 527, 528, 552, 888, 3934, 3941, 3952 and 4250) were resistant to rust race NA27 but susceptible, intermediate or segregating to NA25 and 30 accessions (CAV nos. 311, 551, 553, 561, 672, 826, 1355A, 1402B, 2136, 2872, 2893, 2907, 2917, 2957, 3073, 3201, 3232, 3240, 3255, 3256, 3261, 3268, 3273, 3278, 3279, 3304, 3317, 3322, 3323 and 3336) were resistant to rust race NA25 but not homozygous resistant to NA27. It would be possible to select additional resistant plants from the segregating accessions. Further screening would be required before this resistance could be used in a breeding program.

Those accessions classified as having intermediate rust reactions do not have a high level of resistance that would be desirable in a breeding program. At present, emphasis should be on the 15 accessions with resistance to both stem rust races. The number of resistant accessions, the level of resistance and the species possessing resistance may be due to the area the accession and species are from and the stem rust races inhabiting that particular area. Different races are found in different areas which means the plants indigenous to the area require different stem rust resistance genes in order to survive. The resistance was found at all three ploidy levels; diploid, tetraploid and hexaploid and in all but one of the nine Avena species tested. The resistance found at the diploid level is going to be hard to transfer no matter what diploid species is used. The difficulty in gene transfer is due to the ploidy difference. Doubling the chromosome number of the lower ploidy species and crossing this autoploid with the hexaploid may have some success. This has been used by Sadanaga and Simons (1960) to transfer crown rust resistance from A. strigosa to A. sativa. This would also apply to the resistance at the tetraploid level, although, transfer could be achieved with a straight cross.

The accessions possessing resistance to both stem rust races NA25 and NA27 should be screened to many stem rust races to determine if they are resistant to a wide spectrum

of races. Crosses involving the resistant accessions to resistant and susceptible plants will determine how many genes are involved in resistance, the nature of inheritance of the gene(s) and whether the genes are the same, as shown in Investigation II. If a new resistance gene is found, one could then transfer it to the cultivated species. This would be relatively easy for the resistance found at the hexaploid level but transfer may cause some problems when the resistance is at the diploid or tetraploid levels as shown in Investigation III.

In Investigation II and Investigation III the parental tetraploid accessions had variable rust reactions. The rust reaction, primarily at the adult stage, appeared to be unstable. The variable rust reactions could be due to environmental conditions, the host plant or the pathogen (Bushnell and Roelfs, 1984).

The main environmental factor would be temperature. Many of the stem rust resistance genes are sensitive to temperature. For some resistance genes the stem rust reactions can be either resistant or susceptible depending on the temperature. Changes in a few degrees can cause this change in the rust reaction especially if the threshhold temperature is near the optimum temperature for plant growth. This is shown with Pg-10 which is a temperature sensitive gene (Simons et al., 1978). The correct temperature is important during incubation and latent period.

The lighting regime, both natural and artificial, may also influence the rust reaction. The intensity, quality and duration of the light can affect the degree of infection (Bushnell and Roelfs, 1984). The artificial lights can cause an increase in temperature of the leaves resulting in the leaves having a higher temperature than the surrounding air.

The health of the plant may influence the rust reaction. If the plants had nutrient deficiencies or the soil were waterlogged the plants would be more prone to different diseases. Under drought stressed conditions, the plants would be less receptive to the stem rust infection. Another possibility for the variable rust reactions is that the parental accessions were not true breeding, that is, not homozygous resistant. Some of the accessions may be segregating for stem rust resistance.

The pathogen may be the source of the variable rust reactions. The rust for the different tests did not come from the same rust source. There may be differences in virulence between the sources due to the age of the rust which may result in a decrease in the viability of the spores. Variations in virulence could also be due to the storage conditions of the rust and minor genetically controlled differences in virulence as different rust cultures were used.

Investigation II - Inheritance Study

In many inheritance studies, rust resistance has been due to a single completely dominant gene. Rust resistance due to a single incompletely dominant gene is less common. Single recessive genes conditioning stem rust resistance are known in A. sativa and A. sterilis. Results from this study show that resistance to stem rust race NA25 was simply inherited at both the diploid level and tetraploid level.

There was a single dominant gene found in the A. strigosa accessions Saia, SR2840-4-2, SR2842-1-2 and WTR5200 conditioning resistance to stem rust race NA25 at the seedling and adult stages. A single dominant gene was also observed for the accession SR2838-1-1 at the seedling stage. The single gene model based on testing with one rust race agrees with the results found by Dyck (1966) but conflict with those of Murphy et al. (1958) who reported two dominant genes conditioning resistance. Murphy et al. (1958) used different and additional stem rust races from what was used in this study. The possibility that the gene in Saia is the same as the gene in the other A. strigosa accessions as suggested in this study decreases the number of potential new genes available at the diploid level. Saia (Murphy et al., 1958) has the stem rust resistance genes Pg-6 and Pg-7. The gene conditioning resistance to stem rust race NA25 found in the other A. strigosa accessions could be either Pg-6 or Pg-7.

Monogenic inheritance of stem rust resistance was also found at the tetraploid level. Very little work has been done on the inheritance of stem rust resistance in the tetraploids. Resistance in the selected A. abyssinica accession (A2957-3-1) and A. vaviloviana accessions (V2872-1-1, V3258-3-2 and V3304-4-1) was conditioned by a single dominant gene at both the seedling and adult stages. The accessions A3240-1-1 and V3255-3-1 also showed that a single dominant gene was present at the seedling stage. The adult rust reactions of the parental accessions had a lower level of resistance than those found at the seedling stage. This made reading the F2 adult plants more difficult.

In allelic studies at the tetraploid level, two different resistant parents were used due to their diverse origins. The A. barbata accession, D203, has the stem rust resistance gene Pg-16. Segregation for the stem rust reaction in the ratios of 15:1 and 13:3 was observed in crosses between D203 and the selected resistant accessions (A2957-3-1, V3255-3-1, V3256-1-1, V3258-3-2 and V3261-4-1). This suggests that the gene in the selected accessions is different from the gene found in D203 and, hence, not Pg-16. Although not studied in a resistant by susceptible cross, the two gene difference indicated by segregation of progeny of the V3256-1-1/D203 cross suggests that V3256-1-1 also have one single dominant gene for stem rust resistance. In the cross involving the two A. barbata accessions D145 and D203 no segregation was

observed in the F2 generation. This would indicate that the gene in D145 is allelic to the gene in D203 and is probably Pg-16. The basis for this conclusion is that both accessions have similar origins and rust reactions.

The use of the second resistant parent, A. vaviloviana accession V3304-4-1, in crosses with the selected resistant A. abyssinica (A2957-3-1) and A. vaviloviana (V3255-3-1, V3256-1-1, V3258-3-2 and V3261-4-1) accessions determined if the genes are the same. Evidence suggesting that the gene in V3304-4-1 might be the same as the gene in the resistant accessions was that all the accessions used in this study come from Ethiopia and all are resistant to the same stem rust races. Results from this study indicated that the stem rust resistance gene in the A. vaviloviana accession, V3304-4-1, was allelic to the stem rust resistance gene in the A. abyssinica accession A2957-3-1 and in the A. vaviloviana accessions V3255-3-1, V3256-1-1 and V3258-3-2. This was shown by the absence of segregation of the stem rust reaction in the F2 generation at both the seedling and adult stages. The F2 segregation ratios from the cross V3261-4-1/V3304-4-1 indicated that the gene in these two accessions was different. The ratio of 15:1 was observed at both the seedling and adult stages indicating that there were two independent dominant genes involved.

In the tetraploid inheritance study, stem rust resistance was observed at both the seedling and adult stages. The

stem rust resistance genes in the A. abyssinica and A. vaviloviana accessions conditions a high level of resistance at the seedling stage. At the adult stage the genes were not as effective; therefore, conferred intermediate resistance.

The F3 seedling test was conducted to verify F2 results and to determine if the genes in the selected resistant accessions were new. However, the results from the F3 tests at both the diploid and tetraploid levels did not follow the expected monogenic ratio of 1:2:1 as found in the F2 generation. At the diploid level there were too many homozygous resistant lines and too few segregating and susceptible lines. Within the segregating lines there were too many resistant plants compared with the number of susceptible. In contrast, at the tetraploid level, too many F3 lines were segregating or homozygous susceptible. There was no consistent segregation ratio found within the tetraploid segregating F3 lines.

The distorted ratios found in the F3 results could be due to a number of reasons. First, there was non-random selection of the F2 plants. As seed yield was low for the F2 generation, only those plants producing enough seed for screening to five stem rust races were put into the F3 test. This resulted in seed from many more resistant than susceptible plants being chosen; therefore, this could have distorted F3 ratios. Secondly, reading the stem rust reaction of the F3 generation was harder than reading the rust reac-

tion of the F2 generation. At the diploid level the majority of the F3 seedling leaves were necrotic. This could have been due to the plants having a hypersensitive reaction to the oil or the necrosis may actually be a resistant reaction. In this study, the necrotic reaction was assumed to be the resistant reaction as the parental accessions showed the same symptoms. The necrotic reaction also occurred if the rust inoculation was done without using oil as the carrier. At the tetraploid level it was difficult to distinguish the resistant class from the susceptible class. The difficulty in reading the rust reaction was partly due to the parental accessions which did not give the expected rust reactions. Another possibility for the distorted ratios found in the F3 generation is that different stem rust races were used for screening. The F3 generation was screened to five stem rust races; whereas, the F2 generation was screened to one stem rust race. The use of different stem rust races may reveal the expression of additional genes or interactions between different genes. The distorted F3 ratios could have been due to the type of environment in which the plants were grown. Most of the F2 generation was grown in growth cabinets, whereas all the F3 generation was grown in the greenhouse. Growth conditions are more precise and easier to control in the growth cabinets. Lighting regimes and gradients in the greenhouse can affect the rust reaction, although the differential set grown with the F3 lines did not appear to be affected by the environment. None of

the reasons mentioned can fully explain the distorted F3 ratios.

Since oat stem rust resistance was found to be simply inherited in both the diploid and tetraploid species of Avena it would be relatively easy to transfer this resistance to the hexaploid species. However, practical use of the gene in plant breeding may be limited due to the gene being in the diploid or tetraploid backgrounds.

Investigation III - Interspecific gene transfer study

Having selected the source of resistance and determined how many genes are involved in the resistance, the next step would be to transfer the resistance gene to the hexaploid oats. If the two species are closely related to allow chromosome pairing then crossing over and recombination can occur permitting the stem rust resistance gene to be transferred. If the two species are not closely related so no chromosome pairing and crossing over may occur then other techniques such as irradiation can be used to break the chromosomes and induce a translocation. In this study, the wild species used had one genome in common with the hexaploid. Avena strigosa has the genomic formula AA (Rajhathy and Morrison, 1959). The tetraploid species A. abyssinica, A. barbata and A. vaviloviana have the genomic constitution AABB (Rajhathy and Morrison, 1959). The hexaploids have the genomic formula AACCDD (Rajhathy and Morrison, 1959). Par-

tial homology of the A genomes has been observed in A. strigosa/A. sativa, A. abyssinica/A. sativa and A. barbata/A. sativa crosses (Rajhathy and Thomas, 1974). The degree of chromosome pairing was similar for the diploid-hexaploid and the tetraploid-hexaploid crosses. For both interploidy crosses chromosome pairing was low. This implies that the frequency of transmission of the rust resistance gene would also be low.

There are difficulties involved when attempting to transfer the stem rust resistance found at the lower ploidy levels to the hexaploid. This was illustrated in the diploid by hexaploid crosses where the endosperm degenerated shortly after pollination. The hybrid embryo had to be rescued and placed on an artificial media so continued development could take place. Two diploid-hexaploid F1 plants developed this way. Colchicine was used in an attempt to double the chromosome number and produce the amphiploid. This ensured that every chromosome had something to pair with. This may result in improved fertility. In this study, the use of colchicine or backcrossing to the hexaploid parent did not result in seed development. Investigation III dealt mainly with the tetraploid by hexaploid interspecific crosses as abundant F1 seed was produced.

Resistance to stem rust race NA25 was observed in the F1, F1BC1, F1BC2 and F2BC2 generations at both the seedling and adult stages of tetraploid-hexaploid crosses. The level of

resistance observed in these generations was not as high a level as the resistance of the parental accessions. This may be due to the nature of the parental accessions as mentioned previously. Another possibility for the lower level of stem rust resistance in the F1, F1BC1 and F1BC2 generations is that the stem rust resistance gene would be in the heterozygous condition. This assumes that both alleles conditioning resistance are needed to give a high level of resistance. One allele would still give resistance but at a slightly lower level. The gene would then be incompletely dominant in the heterozygous condition. In the F2BC2 generation where one expects homozygous resistant, heterozygous and homozygous susceptible plants, there were distinctly three classes of rust reactions. At both the seedling and adult stages there were resistant plants, those having rust reactions of 1 - 2+. This is a high level of stem rust resistance; therefore, the resistance gene may have been in the homozygous condition. These plants could be studied further to determine their potential in a breeding program. A progeny test would determine if the resistance gene was transferred and if this was in the homozygous condition. If these plants were substitution or addition lines then other methods are required to transfer the gene from the alien chromosome to an A. sativa chromosome. Irradiation is one method that could be used to induce a translocation of that portion of the chromosome with the gene for rust resistance to an A. sativa chromosome. Plants having intermediate rust

reactions of 2+ - 3 may have the stem rust resistance gene in the heterozygous condition. Homozygous susceptible plants having stem rust reactions of 3 - 4 were also observed. The lower level of stem rust resistance could also be due to the genetic background. The level of resistance may be reduced when the gene is moved from the diploid or tetraploid backgrounds to the hexaploid. This has occurred in wheat interspecific crosses (Kerber and Dyck, 1973). It was suggested that the rust resistance may be modified by genetic factors found in the hexaploid. Kerber and Green (1980) working with wheat concluded that there was a suppressor gene found in the D genome which prevented the expression of rust resistance associated with the AABB genomic constitution. This may occur in oats. The resistance found in the lower ploidy species having the A or B genomes may be suppressed by a gene found in the C or D genomes of the hexaploid.

As previously mentioned, stem rust resistance was observed in the F1, F1BC1, F1BC2 and F2BC2 generations. Assuming monogenic inheritance and normal transmission of the stem rust resistance gene, a 3 resistant:1 susceptible ratio would be expected in the F2BC2 generation. This did not occur as a small number of resistant plants and a large number of susceptible plants were observed. The abnormal transmission of the resistance gene may have been due to differential transmission of the gamete carrying the rust resistant

allele through the male or female gametes. Another possiblity for a low frequency of resistant plants found in the F2BC2 generation, is that some crosses of the previous generation, F1BC2, may have been monosomic substitution or addition lines. The monosomic chromosome carrying the stem rust resistance gene would be lost or transferred at a reduced frequency when the plants were selfed.

By the F2BC2 generation, the plants were approaching the normal 42 chromosome number of the hexaploids. Self fertility was also improving.

Investigation III illustrates that stem rust resistance can be transferred to the hexaploid level from the tetraploid species but that this resistance is unstable. Further studies are required before this resistance can be of practical use in a breeding program.

Chapter VIII

CONCLUSION

There were three major objectives to this study. The first part of the study was undertaken to screen part of the CAV (Canadian AVena) collection to two races of oat stem rust for a source of new stem rust resistance genes. An inheritance study was conducted to determine the number of genes involved in stem rust resistance and the nature of inheritance of these genes in selected resistant accessions of diploid and tetraploid species of oats. An interspecific gene transfer study was conducted to determine if the resistance in the diploid and tetraploid accessions could be easily transferred to the hexaploid A. sativa.

There were 171 CAV accessions screened to stem rust races NA25 and NA27 at the seedling stage. These accessions were made up of nine species of oats which included diploids, tetraploids and hexaploids. Of the 171 accessions there were 15 accessions which had seedling resistance to both stem rust races NA25 and NA27. Resistance was found in the diploid species A. hirtula, A. longiglumis and A. wiestii, tetraploid species A. barbata and A. vaviloviana and hexaploid A. sterilis.

Results from the inheritance study indicated that stem rust resistance was simply inherited. This would apply to both the diploid and tetraploid species. It was found that there was a single dominant gene conditioning resistance to stem rust race NA25 at both the seedling and adult stages. At the diploid level the single dominant gene in the A. strigosa accessions SR2840-4-2, SR2842-1-2 and WTR5200 was found to be allelic to the stem rust resistance gene in Saia. Results from the tetraploid inheritance study indicated that stem rust resistance was conditioned by a single dominant gene in A. abyssinica and A. vaviloviana accessions. This gene is not the same as the gene found in D203, Pg-16. The two A. barbata accessions D145 and D203 may have the same gene; this would be Pg-16. Results suggested that the single dominant gene found in the A. vaviloviana accession V3304-4-1 was allelic to the stem rust resistance gene in the A. abyssinica accessions and all but one of the other A. vaviloviana accessions.

Transferring the stem rust resistance gene from the lower ploidy species to the hexaploid species may be difficult. Embryo culture had to be used to facilitate the diploid by hexaploid crosses. Two hybrid plants were produced in this manner but sterility in the F1 plants prevented further study in later generations. This was in contrast to the tetraploid by hexaploid crosses where abundant F1 seed was produced. Resistance to stem rust races NA25 was observed

in the F1, F1BC1, F1BC2 and F2BC2 generations of the tetraploid by hexaploid crosses. The level of resistance was decreased as the resistance was moved from the lower ploidy species to the hexaploid. In the F2BC2 generation there was a low frequency of resistant plants, probably the result of abnormal transmission of the stem rust resistance gene.

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Appendix A

Summary of *Avena* accessions screened to oat stem rust races NA25 and NA27

CAV#	Species	Stem rust reaction NA25	NA27	Growth Habit
166	*barbata*	S	S	prostrate
297	"	Seg	S	"
311	"	R	S	"
317	"	Seg	S	"
435	"	S	S	"
447	"	Seg	Seg	"
448	"	S	S	"
452	"	S	S	"
478	"	S	Seg	"
521	"	R	R	"
526	"	Int	R	"
527	"	Seg	R	semi-prostrate
528	"	Int	R	prostrate
535	"	Int	Seg	"
539	*hirtula*	R	R	"
540	"	R	R	"
545	*wiestii*	R	R	"
546	"	R	R	"
551	"	R	S	"
552	"	Seg	R	"
553	*barbata*	R	S	"
554	"	Int	S	"
559	"	Seg	S	"
561	"	R	S	"
565	"	Seg	Seg	segregating
600	*sterilis*	Int	Int	upright
603	"	Int	Seg	semi-upright
617	"	Int	Int	upright
618	"	S	S	semi-upright
628	"	Seg	Int	upright
666	"	Int	S	prostrate
671	"	Int	S	semi-upright
672	"	R	S	"
694	"	Int	Seg	prostrate
753	"	Int	S	"
757	"	Int	S	semi-upright
794	"	Seg	S	"
806	"	Int	Int	prostrate
817	"	Int	Seg	segregating
826	"	R	Seg	upright
831	"	Int	S	prostrate
836	"	Int	S	"
839	"	Int	S	"
861	"	Int	Int	"
863	"	Int	Seg	"
887	"	Int	Int	segregating

Appendix A (cont.)

CAV#	Species	Stem rust reaction NA25	NA27	Growth Habit
888	sterilis	Int	R	upright
890	"	Seg	S	prostrate
898	"	Int	Int	upright
899	"	Int	Seg	"
902	"	Int	Seg	prostrate
910	"	Int	S	"
912	"	Int	Int	segregating
925	"	Int	Int	"
934	"	S	Seg	"
954	"	Int	Seg	upright
961	"	Int	Int	"
966	"	Int	S	prostrate
967	"	Int	Int	segregating
1000	"	Int	S	upright
1114	"	Int	S	prostrate
1126	"	Int	Int	"
1139	"	Int	S	"
1195	"	Int	Int	"
1263	"	Int	S	upright
1341A	"	R	R	"
1355A	"	R	Int	prostrate
1402B	"	R	Int	"
1411B	"	Seg	Seg	"
1474B	"	R	R	"
1479	"	Int	S	"
1678	"	Int	Int	"
1733	"	Int	S	"
1871	"	Int	Int	"
2136	byzantina	R	Seg	segregating
2850	vaviloviana	R	R	upright
2872	"	R	Seg	"
2893	abyssinica	R	Seg	"
2907	vaviloviana	R	Int	"
2917	abyssinica	R	Seg	"
2957	"	R	Seg	"
3073	vaviloviana	R	S	"
3090	abyssinica	Seg	Int	"
3176	vaviloviana	Seg	S	"
3201	abyssinica	R	Int	"
3232	vaviloviana	R	Seg	"
3239	abyssinica	Seg	Int	"
3240	"	R	S	"
3252	vaviloviana	R	R	"
3255	"	R	Int	"
3256	"	R	Int	"
3259	"	Int	Int	"
3260	"	R	R	"
3261	"	R	Seg	"
3268	"	R	Int	"
3273	"	R	Seg	"
3278	"	R	Int	"

Appendix A (cont.)

CAV#	Species	Stem rust reaction NA25	NA27	Growth Habit
3279	vaviloviana	R	Int	upright
3304	"	R	Int	"
3309	"	Seg	Seg	"
3311	barbata	Int	S	prostrate
3312	"	S	S	"
3315	"	Int	Seg	segregating
3316	"	Int	S	prostrate
3317	"	Seg	S	"
3322	"	R	Int	"
3323	"	R	Int	"
3325	"	R	S	"
3328	"	Int	S	"
3329	"	Int	S	"
3330	"	S	S	"
3331	"	S	S	"
3333	"	Int	S	"
3334	"	Int	S	"
3335	"	Seg	S	"
3336	"	R	S	"
3339	sterilis	S	S	"
3661	"	E	S	segregating
3698	"	S	S	prostrate
3702	"	S	S	"
3715	"	E	S	upright
3724	"	R	R	"
3767	barbata	Int	S	prostrate
3784	"	Int	S	"
3920	longiglumis	R	R	"
3929	magna	S	S	"
3934	longiglumis	Seg	R	"
3935	"	R	R	"
3939	barbata	S	S	semi-upright
3941	"	Seg	R	prostrate
3944	longiglumis	Seg	Seg	"
3946	"	Seg	Seg	"
3949	"	R	R	"
3950	"	R	R	"
3952	"	Seg	R	"
4035	sterilis	S	S	segregating
4054	"	S	S	prostrate
4079	magna	E	S	"
4194	barbata	Int	S	"
4242	sterilis	E	S	"
4250	"	S	R	"
4349	"	E	S	"
4396	"	S	S	"
4406-1	"	S	Seg	"
4406-2	"	E	S	"
4466	"	S	S	"
4510	"	S	S	"
4576	"	S	S	"

Appendix A (cont.)

CAV#	Species	Stem rust reaction NA25	NA27	Growth Habit
4642	barbata	S	S	prostrate
4674	"	S	S	"
4834	"	Int	S	"

R = resistant
Seg = segregating
Int = intermediate
S = susceptible
E = escape

Appendix B

F3 inheritance study at the diploid level

Saia/SR2837-3-1

Race	Res.	Seg.	Sus.	Total
NA8	26	23	1	50
NA26	26	23	1	50
NA27	28	21	1	50
NA30	24	26	0	50
NA55	34	16	0	50
NA25	4	5	1	10

WTR5200/SR2837-3-1

Race	Res.	Seg.	Sus.	Total
NA8	20	28	2	50
NA26	22	26	2	50
NA27	27	20	3	50
NA30	27	22	1	50
NA55	28	19	3	50
NA25	4	6	0	10

SR2838-1-1/SR2837-3-1

Race	Res.	Seg.	Sus.	Total
NA8	21	28	1	50
NA26	24	25	1	50
NA27	34	16	0	50
NA30	30	20	0	50
NA55	36	14	0	50
NA25	5	5	0	10

SR2840-4-2/SR2837-3-1

Race	Res.	Seg.	Sus.	Total
NA8	21	28	1	50
NA26	24	25	1	50
NA27	34	16	0	50
NA30	30	20	0	50
NA55	36	14	0	50
NA25	5	5	0	10

Appendix B (cont.)

SR2842-1-2/SR2837-3-1

Race	Res.	Seg.	Sus.	Total
NA8	28	20	2	50
NA26	32	16	2	50
NA27	41	8	1	50
NA30	35	14	1	50
NA55	37	13	0	50
NA25	7	3	0	10

F3 inheritance study at the tetraploid level

V3258-3-2/RL1322

Race	Res.	Seg.	Sus.	Total
NA8	48	2	0	50
NA26	7	35	8	50
NA27	4	31	15	50
NA30	3	29	18	50
NA55	5	39	6	50