Stem Rust Resistance in *Avena strigosa* Schreb.: Inheritance, Gene Transfer, and Identification of an Amplified Fragment Length Polymorphism (AFLP) Marker

by

Taye Zegeye

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements of the degree of

Doctor of Philosophy

Department of Plant Science

University of Manitoba

Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION

Stem Rust Resistance in *Avena strigosa Schreb.*: Inheritance, Gene Transfer, and Identification of an Amplified Fragment Length Polymorphism (AFLP) Marker

BY

Taye Zegeye

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

Of

Doctor of Philosophy

ACKNOWLEDGEMENTS

Throughout the duration of the study, many people helped me out in one form or another. Some offered their time and provided me with their valuable inputs while others gave me the moral support that I needed and encouraged me all the way. It will be difficult to list the names of all the people who have contributed to the success of this project. I appreciate all the help that was offered to me and thank you all for the same. However, there are few people who went extra miles to see me complete my studies and they deserve a special thank you.

First of all I like to thank my advisor Dr. Thomas Fetch Jr. for taking me as his student. Dr. Fetch was very willing to take me as his first Ph.D student when I was looking for an advisor after my first advisor informed me that he was not in a position to be my advisor due to health reasons. I appreciate the advice Dr. Fetch offered, the time he devoted to me, and also the patience he had with me when the project was going at a slow pace. His support helped me to get going and complete the project. Thank you Dr. Fetch.

I like to thank professor Lakhdar Lamari for agreeing to be my co-advisor and for all the helpful inputs he provided me throughout the study. The much relaxed and friendly environment whenever I approached Dr. Lamari has indeed helped me to boost the much needed energy to bring the project back on track.

Dr. P.B.E. McVetty, Dr. Daryl Somers, and Dr. Brent McCallum agreed to be members of my advisory committee at the first request and I thank them all for that. The

valuable inputs and constructive suggestions they provided me whenever I approached them and during advisory committee meetings are highly appreciated.

Dr. Jim Bole, the former director of the Cereal Research Centre (CRC), provided me a huge help when the project was initiated. It was Dr. Bole who facilitated my smooth transition from a technician position to being a full time student. He also played a major role in finding me a supervisor. Thank you Dr. Bole for all the support.

Dr. James Chong deserves a special thank you for motivating me and agreeing to take me as his student until he began having health problems. I am also very grateful for the positive suggestions he offered me whenever I approached him.

I also like to thank the staff at the CRC in Winnipeg, particularly the rust laboratory technicians, Ken Dunsmore, Courtney Leclerc, Rebecca Dueck, Wayne Mayert, Janet Gruenke, Pat Seto-Goh, and Laura Trump for their moral support and technical inputs. The patience and support of technicians in the molecular biology laboratory Leslie Bezte, Yanfen Zheng, Mira Popovic, Zlatko Popovic, Monica Eng, and Debbie Miranda is greatly appreciated. I am very grateful to them for accommodating me in the laboratory and teaching me the techniques while I was doing the molecular work. Technicians in the doubled haploid laboratory, Debbie Jones, Elaine Martineau, and Tobi Malasiuk, deserve a special thank you. Had it not been for their help and willingness to accommodate me in the doubled haploid laboratory I wouldn't have been able to produce the octaploid oat plants. I also like to thank technicians in the molecular cytogenetics laboratory at the CRC, particularly Jadwiga Budzinski, for the help she offered in the cytogenetics work.

I thank Dr. Axel Diedrichsen, Plant Gene Resource Canada (PGRC) in Saskatoon, for providing the original *Avena strigosa* seeds that were used in this study.

I take this opportunity to thank graduate students in the Department of Plant Science, particularly those at the CRC, for all the support they gave me. A special thank you goes to Colin Hiebert, a graduate student in the Department of Botany, who was very helpful whenever I needed his help in cytogenetics.

It was a great honor and privilege to be offered the opportunity to undertake this study by being granted a leave of absence with a financial support. The support of Agriculture and Agri-Food Canada (AAFC) in this regard is much appreciated. The financial support of Western Grains Research Foundation (WGRF) that partially funded the study is also gratefully acknowledged. It was a great pleasure and privilege to have worked with two great institutions, the Department of Plant Science at the University of Manitoba and the Cereal Research Centre of Agriculture and Agri-Food Canada.

Last but not least, I take this opportunity to thank my family, my wife Aikaterini, our daughter Hagere Selam, and our son Theodoros for being so patient and understanding while I was working on this project at the expense of the family time. Had it not been for the understanding and full support of my family, the project wouldn't have come to its conclusion. I owe my family a lot.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

°C	degree centigrade (Celsius)
1M	1 molar
1N	1 normal
2,4-D	2,4-dichlorophenoxyacetic acid
2x	diploid
4x	tetraploid
5B	chromosome 5B
6x	hexaploid
AAFC	Agriculture and Agri-Food Canada
AC	Agriculture Canada
AD	anno Domini (after the birth of Christ)
AFLP	amplified fragment length polymorphism
ai	active ingredient
A_s	the 'A' genome of *A. strigosa*
ATP	adenosine tri-phosphate
BC	backcross
BC*	before Christ
bp	base pairs
BSA	bulked segregant analysis
BSA*	bovine serum albumin
BYDV	barley yellow dwarf virus
C_1	Seeds from colchicine-treated plants
CI	Canadian introduction
cM	centi-Morgan
cm	centimetre (10^{-2} meter)
CN	Canadian National
CRC	Cereal Research Centre
CTAB	cetyltrimethylammonium bromide
cv.	cultivar
Cw57	a diploid oat (*A. longiglumis*)
DH	doubled haploid
DL	differential line
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic Acid
dNTPs	deoxyribonucleoside-5' triphosphates
dpi	days post inoculation
DTT	dithiothreitol
E	*Eco*RI
EDTA	ethylenediaminetetraacetic acid
f. sp.	*forma specialis*
F_1, F_2, F_3	first, second, and third filial generations
g/L	grams per litre
GA_3	gibberellic acid

GC	growth cabinet
GH	greenhouse
GS	growth stage
h d^{-1}	hours per day
h, hr	hour
ha	hectare
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
I	intermediate
IAA	indole-3-acetic acid
IT	infection type
K	potassium
KCl	potassium chloride
kg	kilogram
kPa	kilopascal
KS	Kansas
L	Linnaeus
l	litre
M	*Mse*I
m^{-2} s^{-1}	meter square per second
MAS	marker assisted selection
MB	Manitoba
Mg	magnesium
mg	milligram (10^{-3} gram)
mg/L	milligram per litre
min	minute
ml	millilitre (10^{-3} litre)
mM	millimolar (10^{-3} moles solute per litre of solvent)
mm	millimetre (10^{-3} metre)
MO	Missouri
MR	moderately resistant
MS*	Murashige and Skoog
MS	moderately susceptible
Mt	metric ton
NA67	North American oat stem race TJJ
NaCl	sodium chloride
NaOH	sodium hydroxide
ng	nanogram (10^{-9} gram)
ng/µl	nanogram per microlitre
NIL	near isogenic lines
nm	nanometer (10^{-9} meter)
N-P-K	nitrogen – phosphorous – potassium
P	*Pst*I
PCR	polymerase chain reaction
Pg1, Pg2...	oat stem rust resistance genes *(Puccinia graminis avenae)*
Ph gene	pairing homoeologous gene

pH	potential hydrogen
pmole	picomole (10^{-12} moles solute per litre of solvent)
psi	pound per square inch
P-value	probability value
QTLs	quantitative trait loci
R/Res.	resistant
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RH	relative humidity
RILs	recombinant inbred lines
RMR	resistant to moderately resistant
s	seconds
S/Sus.	susceptible
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SK	Saskatchewan
SNP	single-nucleotide polymorphisms
TE buffer	tris/EDTA buffer
Tris-HCl	tris (hydroxymethyl) aminomethane
tsp	tea spoon full
UK	United Kingdom
USA	United States of America
USSR	Union of Soviet Socialist Republics
v/v	volume to volume
WGRF	Western Grains Research Foundation
X	mesothetic reaction, variable sized uredia on a single leaf
Y	variable sized uredia with larger pustules at leaf tip
Z	variable sized uredia with larger pustules at leaf base
µl	microlitre (10^{-6} litre)
µm	micrometre (10^{-6} metre)
µmole	micromole (10^{-6} mole)
χ^2	chi-square

ABSTRACT

Zegeye, T. Ph.D., University of Manitoba, February, 2008. Stem Rust Resistance in *Avena strigosa*: Inheritance, Gene Transfer, and Identification of an Amplified Fragment Length Polymorphism (AFLP) Marker. Major advisors: Dr. Thomas Fetch Jr. and Dr. Lakhdar Lamari.

Oat (*Avena sativa* L.) is an important crop grown world wide. Stem rust, caused by the fungus *Puccinia graminis* Pers. f. sp. *avenae* Eriks. and E. Henn., is a major disease that can significantly reduce yield and quality of oat. In North America, the disease is mainly controlled through the use of host resistance. However, race TJJ (NA67) which currently is predominant in the *Puccinia graminis avenae* population, is virulent on most *Pg* genes and on most Canadian oat cultivars. A genetic study was conducted to characterize the resistance in seven diploid *A. strigosa* accessions (CN21996, CN21997, CN21998, CN22000, CN22001, CN55115, and CN57130) to race TJJ by crossing each one of them to a susceptible accession (CN56979). Chi-square analyses of F_2 populations and F_3 families indicated that the accessions possess a single dominant gene that was also effective at a range of incubation temperature (12˚C to 29˚C). BC_1F_2 progeny line test results supported the single dominant gene hypothesis. Intercrosses among the seven accessions and to CN56818 (a source of *Pg6*) indicated that the resistance in the seven accessions is at the same locus. Multi-pathotype test results using 23 races showed that the resistance in CN21997, CN57130, and CN56818 is the

same gene (*Pg6a*), while the pattern of reaction was clearly different in the other accessions and were proposed as alleles *Pg6b*, *Pg6c*, *Pg6d*, and *Pg6e*.

Four of the accessions (CN21997, CN22000, CN22001, and CN57130) were crossed to five hexaploid cultivars ('Drummond', 'Triple Crown', 'AC Medallion', 'Hokonui', and 'Sun II') to transfer the resistance from the diploid into a hexaploid background. Using an embryo rescue technique, octaploids were produced and were back-crossed to 'Sun II', producing BC_1F_2 populations from which resistant progeny lines with 43-47 chromosomes were selected. Phenotypic data of the BC_1F_2 seedlings tested with race TJJ showed that resistance was transferred from CN21997 and CN57130 into 'Sun II'. However, further crosses and back-crosses are required to stabilize the resistance in the hexaploid background.

Two F_2 populations (CN56979/CN22000 and CN56979/CN57130) were analyzed to identify molecular markers linked with the resistance allele. Using the amplified fragment length polymorphism (AFLP) finger-printing technique with 256 *Pst*I+*Mse*I primer pair combinations, 12 AFLP markers spread over a total distance of 94.7 cM and flanking the resistance gene were identified for the CN56979/CN57130 F_2 population. The closest marker (*P-acg+M-cga-370*) was 10.9 cM away from the resistance gene. A genetic map was produced for this population. Further work is required to saturate the genetic map and identify closer markers flanking the resistance gene. No useful AFLP marker was identified for the second population (CN56979/CN22000).

FORWARD

This thesis is written in a manuscript style as outlined by the Department of Plant Science, University of Manitoba. The thesis is presented as three manuscripts each containing an abstract, introduction, materials and methods, results, discussion, and a conclusion. A general introduction and review of literatures preceded the manuscripts and a general discussion, a general conclusion, significance of the study and future work, and a list of references cited followed the manuscripts.

Dedicated to My Late Parents

1.0 GENERAL INTRODUCTION

Oat (*Avena sativa* L.) is a very important cereal grown mostly in the temperate regions world wide. Although cultivated diploid (*Avena strigosa* Schreb.) and tetraploid (*Avena abyssinica* Hochst.) oat species exist, the predominant cultivated oat are from the hexaploid species, *Avena sativa* L. (common oat) and *Avena byzantina* Koch (red oat). In 2006, over 11 million hectares were seeded to oat with total worldwide production of 23 million tons (Mt) (FAO statistics, 2007). Oat is the seventh most important cereal in the world after wheat, rice, maize, barley, sorghum, and millet. In Canada, it is the fourth after wheat, barley, and maize with total production of 3.6 million tons from 1.4 million hectares. Canada is the second highest producer of oat next to the Russian Federation (FAO statistics, 2007). Oat is mainly used as feed grain for animals and also fed as fodder. Humans also consume oat in the form of porridge, oat-meal, and rolled oats (Findlay, 1956). Recently, oat snacks and beverages are becoming popular (Ranhotra and Gelroth, 1995). Oat production is affected by several diseases, including stem rust.

Stem rust, caused by the fungus *Puccinia graminis* Pers. f. sp. *avenae* Eriks. and E. Henn., is a major disease in oat that can cause significant yield and quality losses. Stem rust epidemics were frequent in the early 1900s in the USA, and in the 1940s in western Canada (Martens, 1985). One of the early reports on yield loss was made by Greaney (1936), where he indicated that the average annual loss was 128, 694 tonnes in Manitoba and Saskatchewan. Losses of up to 25% were reported in 1953 in the USA (Roelfs, 1978), while in Manitoba the most severe epidemic year caused a loss of 35% in 1977 (Martens, 1978). Martens and Harder (1983) reported severe epidemics in

Manitoba, Canada, in the years 1977 and 1981, and a moderate epidemic of stem rust in 1982 in Manitoba caused loss of 28,000 tonnes, which had an estimated value of over two million dollars. Oat stem rust can wipe out the entire crop under favourable conditions for the disease and when susceptible hosts are grown. As reported by McKenzie et al. (1971), under heavy disease conditions no grain harvest could be done. The disease can effectively be controlled with host resistance or with fungicides. Although fungicides can be used to control the disease, at $35/hectare currently it is an expensive option for producers. Therefore, the most efficient, economical, and environmentally sound method of controlling the disease is through the use of host resistance.

The host-pathogen interaction in the oat-rust pathosystem confirms Flor's (1955) classic gene-for-gene model, which for each gene conditioning resistance in oat there is a specific gene conditioning avirulence in the fungus. Thus, to control the disease through host resistance it is important to understand the effectiveness of the resistance genes and incorporate useful genes into adapted oat. However, the problem is that there are few resistance genes available in cultivated oat. Simons et al. (1978) reported that 16 genes confer resistance to stem rust. Some are allelic or tightly linked (Brown, 1984), thus the number of available for plant breeding is reduced. Martens and Dyck (1989) reported that oat stem rust resistance genes were rare, and as few as ten genes currently provide protection against oat stem rust. Currently, 17 numbered *Pg* genes and the *Pg-a* complex have been described (Adhikari and McIntosh, 2001). These genes originated from both cultivated and wild oat (Martens, 1978).

Most of the resistance genes are in a cultivated oat background, yet it is a continuous battle for oat breeders to bring oat stem rust under control as new virulent stem rust races appear. For example, in Australia genes *Pg8, Pg13,* and the *Pg-a* complex were used to confer resistance to oat stem rust. However, oat cultivars became susceptible when new races that were virulent on *Pg8* and *Pg13* appeared (Adhikari, 1996). In North America, the highly virulent race TJJ (NA67) was reported for the first time in 1998 (McCallum et al. 2000). This race soon became predominant in western Canada comprising 28, 35, 60, 51, 66, 81, 56, 60, 61, and 56 % of the population found on cultivated oat annually between 1998 and 2007 (Fetch and Dunsmore, 2004; Fetch personal communication). Since most registered Canadian cultivars rely on *Pg2*, *Pg9,* and *Pg13* for stem rust protection, and since race TJJ (NA67) is virulent on these genes, most Canadian cultivars grown in western Canada are susceptible. Most of the known oat stem rust resistance genes originated from the western Black Sea and North Africa region (Martens and Dyck, 1989; Martens, 1985). As there is lack of genetic variability for stem rust resistance in cultivated oat, oat stem rust is a potential threat and it is a major concern to oat breeders. Finding new sources of resistance or effectively utilizing the resistance genes available in the lower ploidy level is important due to the prevalence of virulent races such as TJJ (NA67).

In pursuit of new oat stem rust resistance genes, a large number of entries have been evaluated at different times. In the 1960s, 4,500 entries from cultivated oat collections in the USA and Canada were evaluated, with no success of finding additional oat stem rust resistance genes (McKenzie et al. 1971). Rines et al. (1980) also evaluated 1,600 accessions of wild oat (*Avena fatua* L.) and did not find a single resistant accession

to race 94 (race TJD or NA30). Harder (1999) suggested that there are no more oat stem rust resistance genes available in hexaploid oat. Harder's conclusion was supported by Gold Steinberg et al. (2005) when no resistant accession was identified after evaluating nearly 7,000 hexaploid accessions to race TJJ (NA67). In fact, after evaluating 10,000 accessions in total from 22 species (diploid, tetraploid, and hexaploid oat), they found that 98.8% of them were susceptible to the virulent oat stem rust race TJJ (NA67). However, Gold Steinberg et al. (2005) identified several diploid *Avena strigosa* Schreb. accessions with a high level of resistance to this race.

The resistance in *A. strigosa* needs to be characterized and the mode of inheritance understood. Once the resistance is characterized, new sources of resistance may be useful in oat cultivar development if they can be incorporated into a hexaploid background. However, the transfer of the resistance from lower ploidy oat into a hexaploid background has been a challenge. This is mainly due to cross-incompatibility and sterility in F_1 hybrids (Rajhathy and Thomas, 1974). Nevertheless, overcoming this challenge may be possible based on reports that crown rust resistance has been successfully transferred from diploid species into cultivated oat (Zillinsky and Derick, 1960; Dyck and Zillinsky, 1963; Sadanaga and Simons, 1960, 1967; Aung et al. 1996; and Rines et al. 2007). Rothman (1984) reported the successful transfer of oat stem rust resistance gene *Pg6* from *A. strigosa* into a hexaploid cultivar. However, evaluation of 'Delredsa' at the CRC in Winnipeg indicated that the *Pg6* resistance was not present (Fetch, unpublished). In addition to the classical genetic characterization of diploid resistance, molecular markers associated with the resistance would be very useful in marker assisted selection (MAS) for oat breeders.

In the past, molecular markers were developed using different methods and DNA finger-printing techniques. Howes et al. (1992) and Chong et al. (1994) used storage proteins to identify molecular markers, while Penner et al. (1993a and 1993b) employed random amplified polymorphic DNA (RAPD) to identify molecular markers. Chong et al. (2004) used sequence-characterized amplified region (SCAR) to identify molecular markers while Chen et al. (2006) used single nucleotide polymorphism (SNP). Hoffman et al. (1990) employed amplified fragment length polymorphism (AFLP) to identify molecular markers. AFLP was the finger-printing technique that was used in this project to identify molecular markers associated with an oat stem rust resistance gene in *A. strigosa.*

The three major objectives of this research project were to:

1. characterize oat stem rust resistance in seven *A. strigosa* accessions conferring resistance to race TJJ (NA67);
2. transfer the resistance from the diploid *A. strigosa* into a hexaploid background; and
3. identify molecular markers associated with the resistance in the diploid *A. strigosa* using the AFLP finger-printing technique.

2.0 LITERATURE REVIEW

2.1 OAT

Oat (*Avena sativa* L.), both cultivated and wild forms, consists of a polyploid series of diploids (2n=2x=14), tetraploids (2n=4x=28), and hexaploids (2n=6x=42) with a basic chromosome number of seven (x=7) (Rajhathy and Thomas, 1974; Thomas, 1992). The evolution of cultivated oat has been under discussion for a very long time. Cultivated oat is a natural allopolyploid that combined three not clearly known diploid genomes. According to Thomas (1992), two steps were followed to obtain hexaploid species. First, two diploid species produced a tetraploid via hybridization. In the second step, the tetraploid was further hybridized with another diploid to produce the hexaploid oat. This seems to be a valid hypothesis because chromosomes of some diploids have the affinity to pair with chromosomes of only one of the genomes in cultivated oat and not with the others. Some workers suggested that cultivated *Avena sativa* L. was derived from *Avena byzantina* C. Koch (Coffman, 1961), while Jones and Clifford (1983) indicated that the evolution of *A. sativa* to be from *Avena sterilis* L. due to its wider distribution and aggressiveness. Based on the genomic structure, Leggett and Thomas (1995) agreed with Jones and Clifford (1983) that cultivated oat is the result of evolution from weedy oat species, most probably from *A. sterilis*.

2.1.1 Origin and cultivation

The exact origin of oat is not definitely known. Several writers indicated that oat was first grown in a warmer climate of south-east Europe, central or western Asia, or in North Africa where ancient civilizations began (Findlay, 1956). The greatest diversity of oat is found between 25°N and 45°N latitude and between 20°W and 90°E longitude, extending from the Canary Islands through the Mediterranean basin and the Middle East to the Himalayas. Thus, there is a general agreement that the Mediterranean region is the origin of oat. However, Murphy and Hoffman (1992) citing Renfrew (1969) indicted that wild oat dating back to 7000 BC was identified in southern Jordan.

The date and place of domestication of oat is not clearly known either. Coffman (1961) indicated that oat cultivation was first recorded in northwest Europe, earlier than in other parts of the world. However, the oldest oat seeds found were from Egypt in 4000 year-old remains (Stevens et al. 2004). The oldest record indicating that wild oat was cultivated by cave-dwellers in Switzerland dates back to only 1000 BC. In fact, Helback (1959) reported that it was around 1000 BC that the first cultivated oat appeared. Archaeological evidence of carbonized oat indicated that it was being utilized in the later prehistoric period (Moore-Colyer, 1995). Samples of carbonized grains from the Iron Age (400 to 250 BC) were found mixed with wheat and barley in Somerset and Dorset, and again a century later in Glasgow in the United Kingdom. In Denmark and Germany, impressions of cultivated oat were found at a much earlier date suggesting that oat was cultivated for human consumption before oat cultivation began in the United Kingdom (Findlay, 1956). In any case it is a fact that oat has been cultivated for at least 2000 years (Coffman, 1961; Murphy and Hoffman, 1992).

Oat was cultivated in Britain as early as in the first century AD (Moore-Colyer, 1995), while according to Nakao (1950) cited by Baum (1977), cultivation of oat in China began at a much later date (386-534 AD). In the writings of Theophrastus (4th century BC), it was mentioned that both cultivated and wild oat exhaust the soil. He also stated that one type of wheat (spelt) changed to oat. Along the same line, in the first century AD, Pliny suggested that the noxious weed oat is the result of degenerated barley (Findlay, 1956). Although it is not clear when oat became an established crop in different parts of the world, it became an important crop by 1000 AD (Baum, 1977). As oat is not indigenous to North America, it was reportedly brought to North America in 1602 AD (Coffman, 1961).

2.1.2 Distribution

The origin of oat is in a warm environment, but it spread to the temperate area as a weed of emmer wheat (*Triticum dicoccoides*) and adapted to a cool and moist climate where it produces a higher yield. As a cool climate crop, most of world's oat is produced in the northern hemisphere between latitudes of 35°N and 65°N and in the southern hemisphere between 20°S and 46°S (Schrickel, 1986; Hoffman, 1995; Stevens et al. 2004). However, oat has adapted to a range of climatic conditions and does well in temperatures between 16°C and 23°C with an annual rainfall of 500 mm to 1000 mm. Oat requires more moisture than other cereals (except rice) to produce a certain unit of dry matter (Coffman, 1961). The growing period of spring-sown oats ranges from 90 days in North America to 180 days in northern Europe. Oat can be grown on many soil types and

performs relatively better in poor soils compared to other small grains (Murphy and Hoffman, 1992).

2.1.3 Classification

Classification of oat has been a subject of controversy for a long time. Nevertheless, there is a general consensus that oat belongs to the tribe *Aveneae,* the family *Gramineae*, and the genus *Avena*, the term designated by Linnaeus around 1753 (Baum, 1977). Based on chromosome numbers, genome, and unit of dispersal, Rajahathy and Thomas (1974) classified oat into 19 taxonomic species (10 diploids, five tetraploids, and four hexaploids). Baum (1977) classified them into 28 species, while Thomas (1992) classified them into 31 species with 13 diploids, six tetraploids, eight hexaploids, and another four species whose ploidy levels were not determined. Kirilov (2004) indicated that there are about 70 species of cultivated and wild oat. In any case, of the 19 taxonomic species classified by Rajhathy and Thomas and the 31 species classified by Thomas, there are four cultivated species: a diploid *(Avena strigosa* Scherb.), a tetraploid (*Avena abyssinica* Hochst.), and two hexaploids (*Avena sativa* L. and *Avena byzantina* C. Koch). Except for *A. macrostachya* Bal. ex Coss. et Dur., which is an out-breeding autotetraploid perennial oat species, the rest are inbreeding annuals (Leggett and Thomas, 1995).

2.1.4 Oat crossing

Oat is a self-pollinated crop with a very low percentage (0.25%) of out-crossing (Morey, 1949). In comparison with other cereals, oat crossing (especially interspecific cross) is very difficult. As Poehlman (1995) explains, "Plant breeding is the art and the science of improving the heredity of plants for the benefit of humankind". The art part is the keen observation and the skills involved in how one handles the florets during emasculation and pollination procedures. The science part is the conscious decision on what to cross, with a certain expectation of the outcome to get the desired result and analysis of the cross. Initially, oat improvement was accomplished by simple selection without involving crossing. The first recorded single oat plant selection was made in 1788 from a potato field in England that resulted in the variety 'Potato' (Hunter, 1924 citing Lawson, 1836). Although the first oat cross was made by Shirreff in 1873 (Stuthman, 1995) and the variety 'Pringles Progress' was released (Poehlman, 1979), oat hybridization was not widely practiced as an oat improvement strategy until 1925. Hybridizing oat is a very delicate procedure that requires careful handling of florets. Nevertheless, the flower can tolerate a certain level of mutilation (McDaniel et al. 1967).

There are two methods of crossing oat, the conventional method and the approach method. Although emasculation procedures are the same in both methods, pollination procedures are different. To cross oat florets in the conventional method, anthers of the primary florets together with secondary and tertiary florets are removed. Emasculated panicles are covered with crossing bags to avoid out-crossing. A day or two after emasculation and depending on the availability of viable pollen, emasculated florets are pollinated using the desired male parent by carefully shedding the pollen onto the stigma

of the emasculated floret. Pollinated panicles are labeled and covered with crossing bags. When checking pollinated florets 3-4 days after pollination, a wilted stigma and enlarged ovary are indications of successful seed set (Fehr, 1980). Temperature is very critical for germination of pollen grains and growth of the germ tube, which enters the style within five minutes (Brown and Shands, 1957). Crossing bags are removed 8-10 days after pollination.

The second method is the approach method. The approach method of crossing wheat and barley was first described by Rosenquest (1927). The first oat crossing using this method was reported by Curtis and Croy (1958). In this method, oat florets are emasculated like the conventional method and then emasculated florets are cut straight across the glumes, lemma, and palea just above the stigma. The panicle of the desired male parent and the emasculated female panicle are then enclosed in a bag made from dialysis tubing. The panicle of the pollen donor is positioned above the emasculated female panicle so that pollen lands directly onto the upward facing stigma. The enclosed bag needs to be tapped twice a day so that pollen will shed on the emasculated florets. Using the approach method, up to 74% seed set success rate was reported (McDaniel et al. 1967). Both the conventional and the approach methods can be used to produce hybrid oat seeds, but the advantage of the approach method is that it saves time when several crosses are to be made at the same time. Pollination of individual florets by hand is eliminated and that is why it is a time saving procedure. The approach method also has been used to hybridize greenhouse and field grown plants by cutting pollen donor culms from field plants, bringing them to the greenhouse and placing them in test tubes tied to

bamboo stakes (McDaniel, et al 1967), and then enclosing them in a bag with the female plant as described above.

2.1.5 Oat production

Cultivated oat is a coarse grain, grown worldwide and constituting 5-7% of world coarse grain production. Oat is ranked seventh in cereal production after wheat, rice, maize, barley, sorghum, and millet (FAO statistics, 2007). During the period between 1960 to 1990, 13 countries produced 90% of total world oat crop with Canada, Germany, Poland, the USA, and countries of the former USSR supplying about 75% of world's grain and industrial grade oat (Stevens et al. 2004). Even though Canada is the second largest producer of oat next to the Russian Federation, oat is the fourth most important cereal after wheat, barley, and maize in Canada (FAO statistics, 2007).

The total area seeded to oat worldwide was greater than that of wheat up to 1910, but area seeded to oat and thus production has been declining. Area harvested for grain decreased from 87% in 1950 to 47% in 1986 (Murphy and Hoffman, 1992). In the 1960s, a total area of 35.3 million hectares were annually seeded to oat (Hoffman, 1995). This area declined to 11 million hectares in 2006 (FAO statistics, 2007). World oat production was 49.6 thousand metric tons (Mt) in the 1960s, increased slightly to 51.9 thousand metric tons in the 1970s, and then went down to 38.7 thousand metric tons in the 1990s, which was 25% lower than that of the 1960s (Hoffman, 1995). In 2006, total oat production was only 23 thousand metric tons, which was less than half of what was produced in 1970s (FAO statistics, 2007). The decline in oat production has been attributed to the continuous decrease of the number of horses (from 3.5 million to

444,000), because oat was mainly produced as animal feed, particularly for draught animals (Small, 1999). The other reason for the decrease of oat production was the decline in profitability relative to other cash crops such as soybean and corn (Murphy and Hoffman, 1992).

Yield of oat per unit area has dramatically increased since the Second World War. This is because of improved production practices and the use of disease resistant varieties. Yield per unit area in North America is low compared to many European countries. For example, yield is about double in the United Kingdom (4.96 Mt/ha), Germany (4.41 Mt/ha), the former Czechoslovakia (4.28 Mt/ha), and Sweden (4.26 Mt/ha) compared to the USA (1.99 Mt/ha) and Canada (2.33 Mt/ha). The increase in yield in the European countries is attributed to the use of good varieties, a long growing season, and excellent cultural management, while in North America yield has not significantly improved due to less intensive management practices (Hoffman, 1995).

2.1.6 Uses of oat

Oat is primarily used for livestock feed in the form of grain. In Argentina it is mainly used as forage to feed cattle, while in Australia it is mainly used for feed grain. More recently, oat is used as winter cover crop (Schrickel, 1986; Suttie and Reynolds, 2004). In addition to feed grain, oat is also grown for pasture, forage, bedding, and as a rotation crop because it suppresses weeds not only due to high biomass production,but also possibly due to allelopathy.

Oat has been part of the human diet for a very long time. Hippocrates, the father of modern medicine, wrote that oat was made into porridge as early as in the 5th century BC in Greece. Dieuches, in the 4th century BC, wrote that oat flour was better than barley flour suggesting that oat has been used for human consumption (Findlay, 1956). Oat is prepared for food in the form of rolled oats, steel-cut groats, quick oats, baby oats, instant oat flakes, oat flour, and oat bran. The major way in which it is consumed by humans is as a breakfast cereal. Instant oatmeal that requires a short period of cooking time is very convenient. It is also consumed as ready-to-eat cereal like a granola bar from oat alone or in combination with other cereals or soybean flours. It is also used in baby foods because of its healthy contribution to the diet of infants. Whole oat is a very nutritional cereal that is high in fat and protein. There is also an interest in producing oat pasta. Oat can also be used as a beverage, beer, or in distilled products in combination with other cereals. Oat protein concentrate and oat bran beer are already in the market (Ranhotra and Gelroth, 1995).

Oat consumption by humans differs from place to place. In the beginning of the nineteenth century, oat consumption in Scotland was 500g per person per week, which was 20% of the total cereal consumption. At about the same time, consumption in England and Wales was only 150g per person per week (Welch, 1995). In the 1970s and 80s, of the total oat produced worldwide, 74% was used as animal grain feed and 22% was used for food, seed, and industrial use (Hoffman, 1995). The food and industrial use share has increased from 16.3% in 1975 to 17.9% in 1984 as reported by Schrickel (1986). In North America, 1.7 million tonnes of oat (57%) is used by the food industry while 1.3 million tonnes (43%) is used to feed race horses (Stevens et al. 2004). There

also are limited industrial uses of oat such as for fuel, filler, antioxidant, cosmetic/pharmaceutical, and starch/gum (Pomeranz, 1995).

The consumption of oat has been known to lower the level of high blood cholesterol. The major component in lowering cholesterol level was attributed to its high soluble fibre content from oat bran, which is rich in β-glucan (Anderson et al. 1984; Anderson and Gustafson, 1988; Small, 1999). However, in a study conducted by Swain et al. (1990), it was concluded that oat bran has minimal cholesterol lowering effect, which was not in agreement with previous reports. Swain et al. (1990) also concluded that both high and low dietary fibre reduce serum cholesterol levels. In any case, due to the medical benefit attributed to oat bran consumption (lowering cholesterol level), products such as bread, biscuits, cakes, and muffins that contain oat bran are becoming popular (Ranhotra and Gelroth, 1995).

2.1.7 Cytogenetics of Avena

Oat is a polyploid cereal with diploid, tetraploid, and hexaploid species. Chromosome morphology differences were established based on the position of the centromere and secondary constrictions in diploids (Thomas, 1992). Within tetraploids, there are differences in forms and symmetry of the karyotypes and the number of satellite chromosomes (Sadasiviah and Rajhathy, 1968). Rajhathy (1963) grouped standard karyotypes in *A. sativa* into four forms based on the position of the centromere and presence of secondary constriction. In monosomics, they were described based on differences in chromosome morphology, phenotype of the monosomics and nullisomics, and the transmission of deficiency of chromosomes (Thomas, 1992).

Oat, although polyploid, behaves like a diploid. In polyploid wheat it has been established that it behaves like a diploid because of the presence of the *Ph* locus on chromosome 5B (Sears, 1956). Similarly, regular diploid-like chromosome pairing in hexaploid oat is preferential and not random, hence it is genetically controlled (Hutchinson et al. 1983; Thomas, 1992). However, a locus similar to that of wheat has not yet been identified in oat. The disomic mode of inheritance is proof that only homologous pairs form chiasmatic associations, and not the three constituent genomes (Thomas, 1992).

2.1.8 Avena strigosa

Avena strigosa Schreb. is a diploid species primarily found in western Europe and in countries of the former USSR. *A. strigosa* is known to do very well in poor soils and is more common in sandy soil. This species was widely cultivated for grain-fodder (Holden, 1976) and is currently grown to a limited extent in Germany, the UK (Wales), and Australia. It is also used as a fodder oat in Brazil (Martinelli, 2004). This species is a good source of stem and crown rust resistance genes (McKenzie et al. 1971, Aung et al. 1977). Crown rust resistance from *A. strigosa* was transferred into hexaploid oat through the use of an autoteteraploid (Zillinsky and Derick, 1960; Dyck and Zillinsky, 1963). Crown rust resistance gene *Pc94*, which is immune to all current crown rust isolates in western Canada, was transferred from *A. strigosa* to adapted oat 'Sun II' (Aung et al. 1996). More recently, crown rust resistance from CI6954SP was also transferred from *A. strigosa* into hexaploid oat (Rines et al. 2007). The stem rust resistance gene *Pg6*, which is resistant to most oat stem rust races in western Canada except races BLD (NA1) and

CLD (NA70), is from *A. strigosa* (Fetch and Jin, 2007). Several stem rust resistant accessions have been recently identified in *A. strigosa* (Gold Steinberg et al. 2005). Nevertheless, since *A. strigosa* is a diploid species, it is a major challenge to make interspecific crosses to adapted hexaploid species and transfer the resistance into a hexaploid background.

2.2 THE RUST

Cereal rusts have co-existed with their hosts for a very long time (Wahl et al. 1984). The oldest rust spore reported (Kislev, 1982) was wheat stem rust from the late Bronze Age II (1400-1200 B.C) that was found in Israel on the inner concave face of wheat lemma fragments. Several diseases threaten oat production. Barley Yellow Dwarf Virus (BYDV), bacterial blight, loose and covered smut, and leaf blotches caused by *Pyrenophora* and *Septoria* species cause sporadic damage on oat. Fusarium head blight and ergot are diseases that produce toxins in oat grain making it unfit for human and animal consumption. However, stem and crown rusts are the most damaging diseases affecting both yield and quality of oat grain and forage.

2.2.1 Oat stem rust

Oat stem rust is present in all oat growing regions of the world. It is caused by *Puccinia graminis* f. sp. *avenae* Eriks. and E. Henn., which is an obligate parasite and heteroecious fungus with two hosts. The primary host is oat (*Avena* spp.) and the secondary or alternate hosts are the common barberry (*Berberies vulgaris* L.) and *Mahonia* species. Rust distribution in North America has been divided into three sub-

regions. These are the Great Plains region that extends from the Gulf coast to the northern limit of oat production where common barberry is not a significant factor (about 4000 km from the gulf of Mexico), the eastern region where barberry is important, and the western region with a different rust population (Martens, 1985). The discovery of new and virulent stem rust races such as TJG (NA76) and TJJ (NA67), found in Manitoba, Canada, in 1998 (McCallum, 2000), raised the threat of oat production in the Prairies and the Great Plains to a higher level. This is because these races are virulent on most cultivars currently grown in the Prairies that get their protection from *Pg2, Pg9*, and *Pg13* (Harder, 1999).

An oat crop could be worthless for fodder if both stem and crown rusts infect the plant (Harder and Haber, 1992). Oat grown throughout the year for fodder outside the traditional oat growing season can increase rust disease. The availability of the host for the fungus makes it possible for production of inoculum all year. The rust will easily spread from this source once the spring crop starts growing.

2.2.2 Classification of oat stem rust

Oat stem rust belongs to the division Eumycota (eumycetes), sub-division Basidiomycotina (basidiomycetes), class Hemibasidiomycetes (teliomycetidae), and order Uredinales, the genus *Puccinia*, and species *graminis* (Agrios, 1988). Eumycota produce mycelium, and the sexual spores of the club fungi (basidiomycetes) are produced on one or four-celled spore producing structures. *Puccinia graminis* f. sp. *avenae* is a fungus specific to oat causing stem rust. Cereal stem rusts, although morphologically similar, are specialized parasites infecting only certain hosts, hence *forma specialis*. This

term was first used by Eriksson in 1894 (Anikster, 1984). Within *forma specialis*, there are different physiologic races (Stakman et al. 1962; Anikster, 1984) infecting certain varieties and not others. Through the use of single-gene differential lines (DL), physiologic races are identified from samples collected from surveys conducted yearly to monitor virulence changes in the oat stem rust population.

2.2.3 Life cycle of Puccinia graminis avenae

The oat stem rust fungus, like some other cereal rusts, has five different spore stages. These are urediniospores, teliospores, basidiospores, pycniospores (spermatia), and aeciospores. Urediniospores, also called red spores, are elliptical, thick-walled, and covered with spines. The size of a stem rust urediniospore is about 20 μm x 30 μm compared to that of the spherical urediniospores of *Puccinia coronata*, the causal agent of crown rust, which has a diameter of 30 μm (Harder and Haber, 1992). Urediniospores are dikaryotic with two nuclei in each cell. Urediniospores infect the cereal host, and depending on weather conditions they can re-infect for several cycles as long as green tissue is available. When environmental conditions are not favourable for continued rust infection and development, or towards the end of the growing season, the fungus changes to the over-wintering black spore form called teliospores. At this stage, the two nuclei in each cell fuse together to form a diploid nucleus, which quickly starts dividing meiotically. Before meiosis is completed the teliospores go into a dormant stage for a period of over six months. In early spring, meiosis resumes and the two-celled teliospores germinate, each of them producing four haploid basidiospores. The haploid basidiospores infect only the alternate host, the common barberry plant (*Berberies*

vulgaris), on which they produce pycniospores with the two ('+' and '-') mating types. When the germinating mycelium (requires a thin film of moisture on the leaves to germinate) from one pycniospore comes in contact with the mycelium of the opposite mating type, exchange of nuclei takes place once again establishing dikaryotic cells (Craigie, 1927). Inter-mixing of pycniospores of one mating type with the opposite mating type is accomplished more effectively and efficiently by insects while collecting nectar from different pustules. The dikaryotic mycelium then produces aecia and aeciospores on the under side of barberry leaves, which upon release infect the cereal host, hence completing the life cycle (Craigie, 1940).

2.2.4 Environmental factors

The most critical environmental factor that determines germination of rust spores is moisture. Urediniospores require dew or a thin film of moisture on the leaf surface to germinate. For successful infection, stem rust requires a minimum of 6-8 hours of dew period followed by three hours of light. It is reported that the longer the dew duration, the higher the infection level (Sharp et al. 1958). Maximum infection occurs with 8-12 hours of dew period at 18°C, followed by at least three hours of light period and then with a temperature that can gradually be raised to 30°C (Roelfs et al. 1992). Another important environmental factor, which plays a significant role in spreading rust spores long distance, is wind. Wind currents carry rust spores to distant places and over a large area where infection may be initiated in the new areas. In this method of rust spread, wind can carry urediniospores to a distance of over 1,500 kilometres (Craigie, 1940).

2.2.5 Stem rust damage

Stem rust of oat, which infects leaves, leaf sheaths, and stems, debilitates and kills the host. If the host is not killed, then it is weakened because of reduced foliage and decreased root growth (Agrios, 1988). In addition to reduced foliage and root growth, the total leaf area for photosynthesis is reduced due to the damage caused by the fungus to leaf tissues. This in turn results in a reduced rate of photosynthesis, decreased translocation of photosynthates, increased rate of respiration, diversion of nutrients into infected tissue, disruption of water and nutrient transport, and lodging and stem breakage (Roelfs, 1985; Agrios, 1988). The net effect of all this, under conducive environmental conditions for the disease, is that oat stem rust causes significant yield and quality losses. Roelfs (1978) reported that in the USA in 1953, some oat growing states reported losses of up to 25%. In Manitoba, Canada, it was reported that the most severe epidemic year caused losses of 35% (Martens, 1978).

2.2.6 Oat stem rust resistance genes in North America

There were three phases of resistance gene deployment in North America (Martens, 1985). The first phase was from 1942 to 1955, where *Pg1* and *Pg2* were the major genes providing protection against oat stem rust races of the time. The next phase was when *Pg4* was introduced, and it became important individually or in combination with *Pg2*. It was used in the period between 1956 and 1978. The final phase was the period after 1978, where combinations of three genes (*Pg2, Pg4,* and *Pg9; Pg1, Pg9* and *Pg13;* or *Pg2, Pg9, Pg13*) were used.

2.2.7 Adult plant resistance

There are only two known adult plant resistance genes that have been described. The first one is *Pg11* from the line CI3034, which is also known to carry *Pg1* (McKenzie and Martens, 1968). The second adult plant resistance gene is *Pg17*, which was obtained from the wild oat *A. sterilis* in Spain (Harder et al. 1990). *Pg11* is the first known adult plant resistance gene, and conferred resistance to all prevailing races in the early 1960s (McKenzie and Martens, 1968). Harder et al. (1971) reported that as seedlings with gene *Pg11* become older, the color of the leaves deteriorates rapidly compared to other plants without the gene. Hence *Pg11* might be tightly linked to a gene affecting chlorophyll levels. Alternatively, Harder et al. (1971) argued that *Pg11* might be a chlorophyll deficient gene resulting in stem rust resistance and not a true resistance gene. McKenzie and Martens (1974) also indicated that this gene is associated with weak straw and yellow-green leaves.

Another resistance gene that displays a unique reaction when inoculated with oat stem rust is *Pg10* (Harder, 1999). This gene was from a selection of 'Illinois hullless' from *A. sativa* line CI2824. It was also identified in the Wisconsin line X1588-2 (Pavek and Myers, 1965; Harder et al. 1995). Plants with *Pg10* resistance display a seedling infection type of 2 to 3 with a small area of chlorosis surrounding the uredinia, which is surrounded by a zone of dark brown necrosis. This unique reaction with a brown, somewhat watery-appearing ring of necrosis around the uredinia, is a typical infection response of this gene to stem rust infection. Lines with *Pg10* did not show similar response when inoculated with the crown rust fungus (Harder, 1999). The resistance

gene *Pg10* has been used in rust pathotyping for over 20 years and none of the 4000 isolates tested were fully virulent on this gene (Harder, 1999).

2.2.8 Gene-for-gene hypothesis

The host-parasite relationship in the oat-rust pathosystem follows the classic gene-for-gene system in flax described by Flor (1955). The original definition of the gene-for-gene relationship was "for each gene conditioning rust reaction in the host, there is a specific gene conditioning pathogenicity in the parasite". The gene-for-gene concept was also supported by Person (1959), but he referred to the genes in the host and the parasite as corresponding genes. Subsequently, Person et al. (1962) explained the gene-for-gene concept as "a gene-for-gene relationship exists when the presence of a gene in one population is contingent on the continued presence of a gene in another population, and where the interaction between the two genes leads to a single phenotypic expression by which the presence or absence of the relevant gene in either organism may be recognized". The gene-for-gene relationship is also true in other systems such as in lettuce (Paran et al. 1991), maize (Hulbert and Bennetzen 1991), wheat (Milne and McIntosh 1990), and barley (Wise and Ellingboe 1985).

Utilizing the gene-for-gene relationship may be very helpful in developing cultivars with durable resistance. Johnson (1984) defined durable resistance as "resistance that remains effective during its prolonged and widespread use in an environment favourable to the disease". Durable resistance can be condioned by a single gene, as was demonstrated by the resistance gene *Rpg1* introduced in barley in the 1940s and remaining effective to date (Steffenson, 1992). However, it usually has been linked

to either use of adult plant resistance or as a result of pyramiding several resistance genes into a cultivar. In oat, though virulence on resistance gene *Pg2* and *Pg13* exists in the prairie region, the *Pg2* and *Pg13* combination was effective for nearly 20 yeas. This was made possible because races that were virulent on *Pg13* were avirulent on *Pg2* (Harder, 1999).

2.2.9 Differential infection in oat

Different oat genotypes display differential reactions in response to different oat stem rust races. Stakman et al. (1923) reported distinct biological forms of *Puccinia graminis* f. sp. *avenae* due to differential response of three oat genotypes. As a result of differential responses, Stakman et al. (1923) developed a method of rust rating, which is still in use with some modifications. Infection types (ITs) are basically divided into two categories, low reaction type (resistant) and high reaction type (susceptible). The resistant group ranges from an immune response (0) where there is no visible sign of infection, to nearly immune (;) where there are no uredinia but necrotic tissues were present at the point of infection, to very resistant (1) where small uredinia surrounded by chlorotic tissue is visible, to moderately resistant (2) with the presence of small and medium sized uredinia. It is also possible to see combinations of the above ITs (0;, ;1-, 12,...etc) in the low (resistant) category. The susceptible category has only two groups, the type 3 IT with medium sized uredinia that may be associated with chlorosis, and the type 4 IT which has large uredinia without chlorosis or necrosis. Additionally, the presence of random distribution of variable-sized uredinia with different infection types ranging from low to high could be present on a single leaf. This type of host response is

called the mesothetic reaction and is designated with the letter 'X'. Orderly distributed uredinia of variable size with bigger uredinia at the tip of a leaf are designated as 'Y' reaction type, while the reverse (bigger uredinia at the base) is designated as 'Z' reaction type (Roelfs, 1984). It is difficult to designate resistance or susceptibility when the mesothetic reaction is observed. Martens (1985) indicated that the designation of resistance and susceptibility with the mesothetic reaction is a matter of judgement where X^- is considered as resistant and X^+ is considered susceptible.

2.2.10 Control methods

Environmental conditions favouring growth and development of the host also favours development of the disease (Knott, 1989). Since the destructive oat stem rust fungus develops under favourable conditions for the crop, it has to be controlled without damaging the crop so that high quality oat with very good yield can be produced. The following are some of the control measures.

2.2.10.1 Cultural practices

There are essentially two cultural practices that have been used to control oat stem rust. These are early seeding and eradicating the alternate host (Harder and Haber, 1992). Early seeding is done so that the plants escape infection before the rust arrives later in the season on southerly wind currents. Since early seeding is a weather-dependent operation and the farmer cannot go into the field to seed unless field conditions permit to work in the field, this is not a very reliable method of controlling the disease.

The second cultural control method is eradicating the alternate host (barberry). Eradication of the alternate host reduces local rust epidemics (Roelfs, 1985; Harder and Haber, 1992). This method, as the experience in the USA showed, helps to reduce the initial inoculum and also minimize the appearance of new races that arise from sexual recombination on the alternate host. In the USA, the federal barberry eradication program that continued for over 50 years in wheat growing states since it was initiated in 1918, destroyed 98% of the barberry bushes by 1972 (Leonard, 2001). This program minimized stem rust epidemics but did not bring oat stem rust under control. Hence, other methods of controlling the disease should be sought. One other method that is being used is chemical control.

2.2.10.2 Chemical control

Chemical control is an effective method of controlling oat stem rust. Contact and systemic fungicides can readily control the fungus. For example, Tilt 250EC 1-[[2-(2,4-dichlorphenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1*H*-1,2,4-triazole) with 41.8% active ingredient (ai) of propiconazole, is commonly used to control the disease in Manitoba. However, at $35 per hectare, it is an expensive option and an additional cost to producers. It is estimated that 1-5 million dollars is spent on fungicides to control stem rust and other diseases on oat (Fetch, 2003). Although chemical control is effective, it is not only an additional cost to producers but also environmentally not a sound option. Therefore, an effective, cheap, and environmentally sound measure needs to be considered. This will leave producers with the option of using genetic resistance.

2.2.10.3 Genetic host resistance

Genetic resistance is the most effective, cheap, and environmentally sound method of controlling stem rust. According to Agrios (1988), resistance is "the ability of an organism to exclude or overcome, completely or to some degree, the effect of a pathogen or other damaging factor". Resistance genes in oat effectively reduce expression of the stem rust fungus and reduce its damaging effect. Most resistant genes conditioning resistance in cultivated oat were obtained from hexaploid oat, both cultivated and wild (Martens 1978). Oat cultivars grown in western Canada mostly carry the resistance genes *Pg2* and *Pg13*, and possibly *Pg9*.

Over the last several decades, virulence in the oat stem rust fungus in North America was stable compared to other cereal rusts (Harder, 1994). However, as new races appear through recombination or mutation, some are virulent on existing resistant cultivars. This likely occurs because the cultivars currently grown in western Canada have a narrow genetic base for resistance (Martens and Dyck 1989). In fact, they reported that stem rust resistance in oat is rare and only about 10 genes for resistance (currently 17 numbered *Pg* genes and *Pg-a* complex) display the entire range of genetic expression. In other words, resistance in the host is conditioned by a few effective genes. For example, race TJJ (NA67) was virulent on all cultivars grown in western Canada in 1998 (McCallum et al. 2000). The frequency of this race in Manitoba jumped from 0% in 1997 to 45% and 55% on wild oat, and to 66% and 81% on cultivated oat, in 2002 and 2003, respectively (Fetch, 2005). In the same two years (2002 and 2003), the frequency of this race in Saskatchewan increased to 28% and 43% on wild oat and to 36% and 25% on cultivated oat, respectively. Subsequently in Manitoba, the frequency went up to 56,

60, 61, and 56 % on cultivated oats in the years 2004 to 2007, respectively (Fetch personal communication). This was very alarming considering the fact that race TJJ (NA67) was not detected on either wild or cultivated oat up to 1997. Thus, it is very important to look for new sources of resistance.

There are useful stem rust resistance genes in diploid and tetraploid oat (Martens, 1985; Martens and Dyck, 1989; Gold Steinberg et al. 2005). However, transferring the resistance from lower ploidy into an adapted hexaploid background was found to be very challenging. Nevertheless, Zillinsky and Derick (1960), Sadanaga and Simons (1960, 1967), and Dyck and Zillinsky (1963) reported that crown rust resistance genes were transferred from diploid species into cultivated oat. In a relatively recent report, crown rust resistance gene *Pc94* has been transferred from the diploid *A. strigosa* into hexaploid through the use of *A. longiglumis* (Cw57), a diploid line that was reported to induce homoeologous chromosome pairing (Aung et al. 1996). *Pg16* was also successfully transferred from autotetraploid *A. barbata* into hexaploid oat by irradiation (Brown, 1984). Although resistance was transferred both from diploid and tetraploid into adapted oat backgrounds, the transfers were accomplished with great difficulty. A method should be sought to address this challenge so that the resistance available in lower ploidy would be exploited in oat cultivar development. One such tool that was used in the process of transferring the resistance from lower ploidy into hexaploid background was the use of a doubled haploid technique (Aung et al. 1996). Once resistance genes are identified, characterized, and transferred into hexaploid background, the use of biotechnology to identify markers associated with the resistance would be very useful for oat cultivar development.

2.3 AMPLIFIED FRAGMENT LENGTH POLYMORPISM

Amplified fragment length polymorphism (AFLP) is one of the DNA finger-printing techniques available to develop molecular markers. It is a very powerful technique in detecting DNA polymorphism co-amplifying 50 to 100 restriction fragments in each reaction. AFLP detects a higher number of polymorphisms than restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) (Mackill et al. 1996; Maughan et al. 1996; Powell et al. 1996; Lin et al. 1996; Ma and Lapitan, 1998; Nakajima et al. 1998; Baker et al. 1999; Mueller and Wolfenbarger 1999; Xu et al. 1999).

There are several steps in AFLP. The three main ones are digestion of genomic DNA and ligation of adapters; selective amplification of the restriction fragments; and analyses of amplified fragments. Genomic DNA is digested with restriction enzymes and amplified with AFLP primers in the pre-amplification reaction. In the selective amplification step, pre-amplification products are used as a template for AFLP primers. Selective amplification is amplification of a DNA sequence using primers and thermostable *taq* DNA polymerase. The three parts of AFLP primers are core sequence, enzyme specific sequence, and selective extension.

In AFLP, it is important that genomic DNA is completely digested. If it is only partially digested, the presence of a band could wrongly be interpreted as polymorphism. The complexity of genomic DNA and the number of selective nucleotides in the primers determine polymorphism or the number of amplified DNA fragments. An AFLP marker is very sensitive for minor genetic differences. It can resolve a small region in the entire genome, and is not limited to a particular region of chromosomes but has wide coverage.

AFLP has the capacity to screen many different DNA regions simultaneously; however, AFLP markers can not detect DNA mixture of up to 10% (Zhu et al. 1998).

Several factors such as pure high molecular weight DNA, concentration of deoxyribonucleoside-5' triphosphates (dNTPs), magnesium chloride ($MgCl_2$), primers, DNA polymerase, and PCR cycling temperature (cycling temperatures and length of cycle) affect reproducibility of AFLP. On the other hand, genomic DNA concentration has no effect on the DNA pattern. Zhu et al. (1998) reported that AFLP banding pattern was not particularly sensitive to the initial concentration of template DNA, which is consistent with the report of Vos et al. (1995). This insensitivity helps to avoid making mistakes in wrongly scoring bands from DNA contamination. In addition, good reproducibility was obtained in selective amplification when the ^{32}P-labelled to unlabelled primers was in a 6:1 ratio (Vos et al. 1995). However, Hayashi et al. (2005) reported that equal concentration of labelled to unlabelled primers produced results with no sacrifice of quality or reproducibility.

In AFLP, the choice of restriction enzymes or primers affects the number of polymorphisms. As reported by Ridout and Donini (1999), more polymorphism was detected in barley with the *Pst*I/*Mse*I primer pair than with the *EcoR*I/*Mse*I combination. Also, different crops respond differently with respect to polymorphism. For example, the level of polymorphism in the 96 oat cultivars released from 1886-2001 was low compared to that in other crops such as soybean, barley, rice, mulberry, and sugarcane (Fu et al. 2004). Polymorphic bands were found both at higher frequency (indicating that allelic diversity resulted from introduction of exotic germplasm) and lower frequency

(suggesting that there is residual heterozygosity in the different genomic regions) (Fu et al. 2004).

2.3.1 Application of AFLP

Amplified fragment length polymorphism can be used for may different studies such as population genetics, molecular evolution, and breeding (Hayashi et al. 2005) investigation of genetic variation (Russell et al. 1999) introgression and hybridization (Rieseberg et al. 1999) distribution of species and their hybrids (Beismann et al. 1997) paternity analysis and gene flow (Krauss and Peakall 1998) and phylogenetic analysis of allele frequency (Heun et al. 1997; Aggarwal, 1999). Kardolus et al. (1998) reported that AFLP was an efficient method for evolutionary studies. AFLP also produces fairly consistent results. Winfield et al. (1998) reported 98.9%, 97.6%, and 100% similarity of banding pattern.

One other important application of AFLP is its use for production of genetic maps (Ridout and Donini, 1999). AFLP markers have been used to produce genetic maps for QTLs for resistance to barley stripe rust (*Puccinia striiformis* Westend. f. sp. *hordei* Eriks.) (Toojinda et al. 1998), flax wilt (*Fusarium oxysporum* Schlechtend Fr. f. sp. *lini* [Bolley] W.C. Snyder and H.N. Hans.) (Spielmeyer et al. 1998), and barley net blotch (*Drechslera teres* Sacc. Shoemaker) (Richter et al. 1998). In wheat, AFLP was used to identify markers associated with powdery mildew (*Erysiphe graminis* DC. f. sp. *tritici* Ém. Marchal) resistance in common wheat (Hartl et al.1999), and loose smut (*Utilago tritici* [Pers.] Rostr.) in durum wheat (Knox et al. 2002).

2.4 BULKED SEGREGANT ANALYSIS

Bulked segregant analysis (BSA) is a quick method of screening for loci that differ in specific regions between groups of lines selected for a particular trait. In BSA, a marker showing polymorphism between the two parents and between their respective bulks with different scoreable phenotypes for a particular trait is potentially linked to the trait (Michelmore et al. 1991). To find a marker associated with disease resistance, a resistant bulk is made up of resistant F_2 individuals and a susceptible bulk of susceptible F_2 plants. The bulks should be similar in disease reaction with their respective parents, and linked to a polymorphic marker at a specific band size. This method uses DNA samples pooled from individuals expressing similar phenotypic reactions, so that marker(s) can be related to the trait of interest.

3.0 INHERITANCE OF OAT STEM RUST RESISTANCE IN SEVEN *AVENA STRIGOSA* ACCESSIONS.

3.1 ABSTRACT

Stem rust, caused by *Puccinia graminis* Pers. f. sp. *avenae* Eriks. and E. Henn., is an important disease that can significantly reduce yield and quality of oat (*Avena sativa* L.). In North America the disease is mainly controlled through the use of host resistance. However, most currently registered oat cultivars in Canada are susceptible to race TJJ (NA67), a race which is predominant in the eastern Prairie region of Canada. Seven *Avena strigosa* Schreb. accessions (CN21996, CN21997, CN21998, CN22000, CN22001, CN55115, and CN57130) displaying a high level of resistance to race TJJ were previously identified. The objective of this study was to investigate the mode of inheritance of stem rust resistance in the seven *A. strigosa* accessions. A genetic study was conducted by crossing the seven accessions to a susceptible *A. strigosa* accession (CN56979). Intercrosses among the resistant accessions were also made to determine whether or not the gene(s) in the seven accessions were the same. Allelism tests with *Pg6* were conducted by crossing each of the seven accessions to *A. strigosa* accession CN56818 (a source of *Pg6*). Chi-square analyses of seedling tests with race TJJ on F_2 populations, F_3 families, and BC_1F_2 progeny lines indicated that each of the accessions possesses a single dominant gene. The gene(s) in each accessions was effective across a range of incubation temperature from 12°C to 29°C. Approximately 300 F_2 progeny from the 21 intercrosses among the seven accessions were tested and not a single susceptible seedling was recovered. Also, F_2 populations from the allelism tests with *Pg6* in all the

crosses failed to segregate, resulting in all progeny displaying resistance to race TJJ. This result indicates that the resistance gene in each of the accessions is either *Pg6*, or alleles of *Pg6*, or very tightly linked to it at the same locus. Multi-pathotype tests with 23 stem rust races showed that the gene in CN21997 and CN57130 is *Pg6* (*Pg6a*) while the genes in CN21996; CN21998 and CN22000; CN22001; and CN55115 likely are alleles of *Pg6* (*Pg6a*) and are designated as allele '*Pg6b*', '*Pg6c*', '*Pg6d*', and '*Pg6e*'respectively.

3.2 INTRODUCTION

Oat (*Avena sativa* L.) is an important cereal grown world wide. Production of oat is affected by several diseases, among which stem rust (caused by the fungus *Puccinia graminis* Pers. f. sp. *avenae* Eriks. and E. Henn.) is the most devastating. In the presence of a virulent race and under favourable environmental conditions for the fungus where a susceptible cultivar is grown, the entire crop could be lost. However, the disease can be controlled by growing resistant varieties.

The host-pathogen interaction in the oat-rust complex follows the gene-for-gene model in which for each gene conditioning resistance in oat there is a specific gene conditioning pathogenicity in the fungus (Flor, 1955; Person, 1959). Therefore, it is necessary to characterize resistance genes in the host before attempting to incorporate the stem rust resistance into cultivated oat to control the disease.

Oat stem rust resistance genes are rare (Simons et al. 1978; Martens and Dyck 1989). Presently, 17 numbered genes and the *Pg-a* complex have been described (Adhikari, 1996). Some are linked (*Pg1*, *Pg2* and *Pg8*; *Pg3* and *Pg9*; and *Pg4* and *Pg13*) and may actually be alleles at the same locus (McKenzie, et al., 1970; Brown, 1984). All of the described resistance genes except *Pg6* and *Pg16* were obtained from cultivated and wild hexaploid oat (Martens, 1978; Martens, 1985). Resistance genes from *Avena sterilis* L. (2n=6x=42) and *Avena barbata* L. (2n=4x=28) have been incorporated into hexaploid oat to provide the oat crop protection against the stem rust fungus.

Over the years, some of the resistance genes were rendered ineffective due to the appearance of new virulent races. It is a continuous battle for oat breeders to bring oat stem rust under control when virulent races continually appear, thus making resistance genes ineffective. For example, *Pg8, Pg13,* and *Pg-a* (a complementary recessive gene complex, Adhikari and McIntosh, 2001) were the genes that were used to provide protection to oat stem rust in Australia. However, *Pg8* and *Pg13* became ineffective when new virulent races appeared (Adhikari, 1996). Also, race TJJ, which is virulent on most cultivated oat grown in Canada, was reported for the first time in western Canada in 1998 (McCallum et al. 2000). This race soon became predominant in western Canada comprising 28, 35, 60, 51, 66, 81, 56, 60, 61, and 56% of the stem rust population found on cultivated oat and 18, 26, 17, 41, 45, 55, 51, 31, 44, and 27% on wild oat in the years 1998 through 2007, respectively (Fetch and Dunsmore, 2004; Fetch personal communication). The avirulence/virulence formula (*Pg6, Pg10, Pg11, Pg12, Pg16, Pg-a / Pg1*, *Pg2*, *Pg3*, *Pg4*, *Pg8*, *Pg9*, *Pg13*, *Pg15*) for race TJJ has been described (McCallum, et al. 2000). Since previously resistant Canadian cultivars rely on *Pg2*, *Pg9,* and *Pg13* for oat stem rust protection, they are susceptible to race TJJ because this race is virulent on *Pg2, Pg9,* and *Pg13*. As stem rust resistance genes are not so commonly available and additional resistance genes are not readily available in hexaploid oats (Harder, 1999), it is necessary to find new sources of resistance.

The potential of resistance genes in lower ploidy oat has been previously reported (Martens, 1985; Martens and Dyck, 1989). More recently, Gold Steinberg et al. (2005) evaluated close to 10,000 accessions from 22 species of *Avenae* and found that 12% and 2% of diploids and tetraploids, respectively, were resistant to race TJJ but found no

resistant hexaploid accessions. To exploit the resistance in the lower ploidy level of *Avena* for cultivar development, the resistance genes have to be transferred into cultivated hexaploid oat. To accomplish this, the first step is to characterize the resistance genes. Hence, this study was initiated with the objective of determining the genetics of inheritance of oat stem rust resistance in seven *A. strigosa* accessions that confer resistance to stem rust race TJJ and to establish whether the genes in these accessions are the same as *Pg6* or alleles of it.

3.3 MATERIALS AND METHODS

3.3.1 Genetic study

The original seeds of the nine *A. strigosa* accessions used in this study were obtained from Dr. Axel Diedrichesen (Plant Gene Resources Canada, Saskatoon, SK, Canada). The accessions were selected from the 2002 stem rust nursery established in field plots at the University of Manitoba (Gold Steinberg et al. 2005). The nine accessions were selected based on their reaction to the fungus when evaluated with race TJJ in the field. Five accessions from Spain (CN21996, CN21997, CN21998, CN22000, and CN22001) had a resistant reaction (R), accession CN55115 from the former USSR had a moderately resistant reaction (MR), accession CN57130 from the USA displayed a resistant to moderately resistant reaction (RMR), accession CN56979 had a susceptible reaction (S), and accession CN56818 (a source of *Pg6*) had a resistant reaction (R). Adult plants were rated in the field using the Roelfs et al. (1992) rating scale.

Genetic studies on the seven accessions (CN21996, CN21997, CN21998, CN22000, CN22001, CN55115, and CN57130) were conducted in the greenhouse and in growth cabinets beginning in 2003. The accessions were germinated weekly in petri-plates and transplanted into soil-less Sunshine Mix #5 (70-80% fine Canadian sphagnum peat moss, fine perlite, dolomitic lime stone, gypsum, wetting agent (Hadashville, MB) in 15 cm x 15 cm x 15 cm fibre pots. Each of the seven resistant accessions was crossed to a susceptible accession (CN56979) that was used as the female parent. All the crosses were done using the conventional crossing method as opposed to the approach method (McDaniel et al. 1967). The F_1 hybrids from the crosses were planted in 15 cm x 15 cm x

15 cm fibre pots and tested for stem rust reaction with race TJJ at the first leaf stage (described below). The seedlings were grown to the adult stage. Some of the F_1 plants were backcrossed to the susceptible accession (female) to produce BC_1F_1 and then BC_1F_2 seeds. The remaining F_1 plants were selfed to produce F_2 progeny lines. F_2 seeds of two F_1 plants for each of the crosses were planted in plastic conetainers (19 cm x 4 cm) to produce F_3 seeds. For disease evaluation, individual F_2 and F_3 seedlings were tested with race TJJ and rated using the 0-4 rating scale (Stakman et al. 1962). Evaluation of F_3 families was done by planting 25 seeds per F_3 family in soil-less mix in 15 cm x 15 cm x 15 cm fibre pots, while evaluation of large numbers of BC_1F_2 seedlings required testing of several pots (25 seeds per pot) from each family.

A half-diallel cross (without reciprocal crosses) among the seven accessions were made (21 crosses) to determine whether the resistance gene(s) in each of the seven accessions were at independent loci. Thirteen to 32 F_1 hybrid seeds were produced for the different crosses, which were grown and tested at the seedling stage with race TJJ, and then were raised to maturity to produce F_2 seeds. F_2 seedlings from the 21 crosses were grown in 15 cm x 15 cm x 15 cm fibre pots and evaluated with race TJJ. At 25 seeds per pot, and depending on the number of seeds available (126 to 437 seeds), several pots per cross were planted to test as many F_2 seedlings as possible.

Pg6 allelism tests were conducted by crossing each of the seven resistant accessions to accession CN56818 (a source of *Pg6*). The resulting F_1 seedlings were evaluated with race TJJ and raised to maturity to produce F_2 seeds. F_2 populations of the seven crosses were tested again at the seedling stage with race TJJ by planting the seeds in several large fibre trays (30 cm x 25 cm x 7 cm) with 40-50 seeds per tray. Several

trays were used to test 124 to 489 seedlings from the seven crosses. This test was necessary to determine whether or not the resistance gene in the seven accessions was at the *Pg6* locus or at a separate locus.

3.3.2 Stem rust inoculation in the greenhouse

Seeds were treated with Gibberellic Acid (GA_3) to break dormancy before they were planted for evaluation at the seedling stage. The treatment helped to obtain uniform germination, which is needed for seedling tests. To treat the seeds with GA_3, seeds were put in envelopes and then, in a laminar flow hood, the envelopes were soaked in a beaker with GA_3 in acetone (one gram of GA_3 in four litres of acetone) for 20 minutes. After the treatment, excess GA_3 solution was poured back into the container and the envelopes were left in the laminar flow hood for the acetone to evaporate (J. Chong personal communication).

Oat stem rust race TJJ is a virulent race on most oat cultivars currently grown in western Canada. It is the most prevalent race in the stem rust population in western Canada (Fetch, 2005). A purified isolate of race TJJ was obtained from the Cereal Research Centre (CRC) oat stem rust collection in Winnipeg and increased on the susceptible oat cultivar, 'AC Assiniboia' in an isolated greenhouse space in the fall of 2003. 'AC Assiniboia' was used as host to increase the rust because it is fully susceptible to race TJJ and could also be used as a biological screening method for rust race purity as opposed to increasing it on a universal susceptible cultivar like 'Rodney 0' where all the known races infect it. Urediniospores from 'AC Assiniboia' were collected, vacuum-dried for about two hours, and stored in sealed glass ampules in an ultra low freezer at -

80°C. For subsequent plant inoculation, a glass ampule was removed from the freezer and spores were heat-shocked by immersion in a water bath at 45°C for 10 minutes. Ampules were opened and viability of spores was always checked by germinating them on 2% agar in a petri plate. Four milligrams of urediniospores were weighed and transferred into a 00-size gelatin capsule. Bayol® oil (Esso Canada, Toronto) was added (0.7 ml) to the capsule, and a fine mist of oil-spore suspension was sprayed onto plants using a micro-inoculator (G-R manufacturing, Manhattan, KS) pressurized by a pump at 20 kPa (Browder, 1971).

The oat stem rust race TJJ was used to test parental materials as well as all the populations and all the families that were produced. When relatively few numbers of plant materials were tested, inoculated seedlings were incubated in a dew chamber (Percival Model 160-D, Boone, Iowa) overnight at an incubation temperature of 18°C - 20°C. Seedlings were removed from the dew chamber the following day and placed on a greenhouse bench under supplemental high-pressure sodium lighting (250 μmol m^{-2} s^{-1}) for at least two hours to complete the infection process. Seedlings were tented with a thin plastic sheet to ensure slow-drying of leaves but avoid overheating. Alternatively, inoculation of a large number of seedlings was done on a greenhouse bench with subsequent incubation on the bench using a plastic tent over the seedlings. High relative humidity was maintained by pumping a fine mist of water into the tent overnight using ultrasonic humidifiers. Humidifiers were removed the following day and lights were turned on for at least two hours for the infection process to be completed. The plastic tent was opened from the sides to avoid overheating. Plastic tents were subsequently removed and seedlings were left in the greenhouse or moved to growth cabinets where

they were maintained at temperature of 20°C/18°C (day/night) with 16h/8h (day/night) light regime until rust reactions were rated.

3.3.3 Disease rating and statistical analyses

Disease reactions were rated by examining the infection types (ITs) 12-14 days after incubation using the 0-4 rating scale as previously described. Seedlings displaying infection types of 0;, 1, and 2 and their modifications (;1^-, 11^+, 2^-...etc) were considered resistant, while those with ITs of 3 or 4 were considered susceptible (Stakman et al. 1962). Chi-square analyses for all populations and families were performed to test the goodness of fit of observed ratios to the theoretical expected genetic ratios.

3.3.4 Field inoculation

The seven *A. strigosa* accessions that displayed a high level of resistance to race TJJ in 2002, the susceptible accession (CN56979), the accession with *Pg*6 (CN56818), and the single-gene differential set lines (*A. sativa*) were tested again in the field in 2003. The *A. sativa* cultivar 'AC Assiniboia' was planted as a spreader row as described by Gold Steinberg, et al. (2005). The test entries were planted in two replicates of a single one-meter row per entry. The differential set with single gene lines (*Pg1, Pg2, Pg3, Pg4, Pg8, Pg9, Pg10, Pg13, Pg15, Pg16*), the *Pg-a* complex, and also oat cultivars 'Kyto', 'Alpha', 'Fidler', 'Rodney 0', 'Triple Crown', 'AC Assiniboia', and 'Sun II' were planted in a field nursery at the University of Manitoba. The spreader rows were inoculated with race TJJ (0.6g/l oil) at the flag leaf growth stage, GS 40, (Zadoks et al., 1974) using a Microfit Herbaflex sprayer (Micron, Herefordshire, UK) at a rate of 2 ml

m^{-1} of row on a calm evening when overnight dew formation was expected. Because of unfavourable conditions for dew formation in the following five nights after the first inoculation, a second inoculation was applied five days later. In the second inoculation, both the spreader rows and all test entries were inoculated. Three weeks post inoculation when seeds on the plants were at the milk stage (GS 75), the entries were rated for disease severity (percent stem infection) and stem rust reaction (R=resistant, MR=moderately resistant, MS=moderately susceptible, and S=susceptible) using the Roelfs et al. (1992) rating scale. The entries were rated for a second time two weeks after the first rating.

3.3.5 Temperature study

Three temperature regimes (12˚C/10˚C, 20˚C/18˚C, and 29˚C/27˚C, day/night), were selected to test for temperature sensitivity of the resistance gene(s) in the seven accessions. One seed per plastic conetainer (19 cm x 4 cm) and ten conetainers per accession were planted for the seven resistant accessions (CN21996, CN21997, CN21998, CN22000, CN22001, CN55115, and CN57130), the susceptible accession (CN56979), and the accession possessing *Pg6* (CN56818). Eight cones of 'AC Assiniboia' were also planted as a susceptible hexaploid check. Seedlings were grown in the greenhouse (20˚C +/-4˚C) with 16h/8h (day/night) light regime until they were ready for inoculation. Inoculation and incubation processes using race TJJ were performed as described previously and infected plants were subsequently moved into growth cabinet at the desired temperature regime. Rating of plants incubated at 12˚C/10˚C and at 18˚C /20˚C (day/night) was done 14 days after incubation, while for those incubated at

29°C/27°C (day/night) rating was done 12 days after incubation since faster rust development was observed on the susceptible checks.

3.3.6 Multi-pathotype allelism test

Multiple alleles at the same locus in rust resistance genes are well known (Roelfs, 1988). Multi-pathotype allelism tests were conducted to investigate whether the same is true with the diploid oat lines in this study. Five seeds per genotype from the seven resistant accessions (CN21996, CN21997, CN21998, CN22000, CN22001, CN55115, and CN57130); the susceptible accession (CN56979), and the accession with *Pg6* gene (CN56818) were planted in a large fibre tray (30 cm x 25 cm x 7 cm). To test purity of the races, oat stem rust differential sets with single gene lines (*Pg1, Pg2, Pg3, Pg4, Pg8, Pg9, Pg10, Pg12, Pg13, Pg15,* and *Pg16*), and the *Pg-a* complex were also planted in the same tray. Along with the *A. strigosa* accessions and the single gene differential lines, 'AC Assiniboia' was also included as a susceptible hexaploid check. A total of 23 trays were planted and each tray was tested with a different race of stem rust. The races used in this study were BDJ, BLD, CLD, DBD, FDJ, HDD, JBD, NGB, NGD, RDD, RGB, RGD, RJJ, SGB, TDD, TDF, TDJ, TGB, TGD, TGL, TJD, TJG, and TJJ (Fetch and Jin, 2007). These races are virulent on some genes and avirulent on others (Table 1). Inoculation and incubation were performed as described previously. After incubation, the seedlings were maintained in a growth cabinet at a temperature of 20°C/18°C (day/night) with a light regime of 16h/8h (day/night). A second confirmation test was done in a greenhouse at a temperature regime of 20°C/16°C+/-4°C. Seedling reaction was rated 14 days after inoculation using the Stakman et al. (1962) rating scale.

Table 1. Effective/ineffective *Pg* genes for 23 oat stem rust races, *Puccinia graminis* Pers. f. sp. *avenae* Eriks. and E. Henn.

Race	Effective/ineffective *Pg* genes
BDJ (NA3)	1,2,3,4,8,16,a/9,13,15
BLD (NA1)	1,2,3,4,8,9,13,16,a/15
CLD (NA70)	1,2,3,8,9,13,16,a/4,15
DBD (NA5)	1,2,4,8,9,13,16,a/3,15
FDJ (NA8)	1,2,8,16,a/3,4,9,13,15
HDD (NA9)	1,3,8,13,16,a/2,4,9,15
JBD (NA10)	1,4,8,9,13,16,a/2,3,15
NGB (NA16)	2,4,9,13,15,16,a/1,3,8
NGD (NA18)	2,4,9,13,16,a/1,3,8,15
RDD (NA20)	3,8,13,16,a/1,2,4,9,15
RGB (NA21)	3,9,13,15,16,a/1,2,4,8
RGD (NA77)	3,9,13,16,a/1,2,4,8,15
RJJ (NA75)	3,16,a/1,2,4,8,9,13,15
SGB (NA23)	4,9,13,15,16,a/1,2,3,8
TDD (NA25)	8,13,16,a/1,2,3,4,9,15
TDF (NA55)	8,13,a/1,2,3,4,9,15,16
TDJ (NA26)	8,16,a/1,2,3,4,9,13,15
TGB (NA27)	9,13,15,16,a/1,2,3,4,8
TGD (NA29)	9,13,16,a/1,2,3,4,8,15
TGL (NA28)	9,13,15,16/1,2,3,4,8,a
TJD (NA30)	13,16,a/1,2,3,4,8,9,15
TJG (NA76)	15,16,a/1,2,3,4,8,9,13
TJJ (NA67)	16,a/1,2,3,4,8,9,13,15

3.4 RESULTS

Plants grown in the greenhouses and growth cabinets as well as in the field were healthy, vigorous, and rust-free prior to inoculation. Inoculation for indoor seedling tests always resulted in uniform infection. When tested with race TJJ in the greenhouse, the seven resistant accessions (CN21996, CN21997, CN21998, CN22000, CN22001, CN55115, CN57130) and the accession with *Pg6* (CN56818), displayed low ITs (;, ;1-, ;11+, or 12), while the susceptible accession (CN56979) had IT of 34 or 4 .

In the field test, although rust spores were viable and germination was over 90%, success of field inoculation depended mostly on if there was an overnight dew formation and whether or not the temperature was not too cold (<12˚C) at night. The result of re-evaluation of the seven accessions with race TJJ in the field in 2003 showed that the resistant accessions from the previous year's test remained resistant (1R, 2R, or 5R). The susceptible accession (CN56979) and the hexaploid check 'AC Assiniboia' were susceptible with a severity of 50S (Table 2). This field test result is in agreement with the 2002 field test result (Gold Steinberg et al. 2005) and also with greenhouse and growth cabinet seedling tests.

Table 2. Stem rust reactions of *Avena strigosa* accessions and 'AC Assiniboia' in greenhouse and field experiments using race TJJ.

			Reactions to race TJJ	
Accession	**Alternate No.**	**Origin**	**Seedling(GH)[a]**	**Adult(Field)[b]**
CN21996	CAV 2839	Spain	1-	1R
CN21997	CAV 2840	Spain	;1-	1R
CN21998	CAV 2841	Spain	1	1R
CN22000	CAV 2843	Spain	1- 1	2R
CN22001	CAV 2844	Spain	;1-	5R
CN55115	Clav 5057	USSR	1	1R
CN57130	Clav 7280	USA	;	5R
CN56979	Clav 7122	Canada	34	50S
CN56818 (*Pg6*)	Clav 6956	Canada	;	1R
'AC Assiniboia'	OT275	Canada	34	50S

[a] seedling rating in the greenhouse using a 0-4 infection type scale (Stakman et al. 1962).

[b] field rating in 2003 using percent severity infection and infection response (resistant, susceptible) Roelfs et al. 1992).

3.4.1 F_2 populations and F_3 families

Seedling reactions for F_1 hybrids and chi-square (χ^2) analyses of F_2 populations produced by crossing each one of the seven resistant accessions (CN21996, CN21997, CN21998, CN22000, CN22001, CN55115, and CN57130) to the susceptible accession CN56979 (female parent) is shown in Table 3.

Table 3. Chi-square analyses of F_2 populations tested with race TJJ at the seedling stage.

		F_2 seedlings					
Cross	**F_1 ITs**	**Resistant**	**Susceptible**	**Total**	**Model**	**χ^2**	**P-value**
CN56979/CN21996	1, 12	109	43	152	3: 1	0.877	0.35
CN56979/CN21997	1, 11+	105	46	151	3: 1	2.404	0.12
CN56979/CN21998	11+, 12+	121	31	152	3: 1	1.719	0.19
CN56979/CN22000	1-, 11+	110	41	151	3: 1	0.373	0.54
CN56979/CN22001	1, 11+	119	32	151	3: 1	1.168	0.28
CN56979/CN55115	1, 11+	119	40	159	3: 1	0.002	0.96
CN56979/CN57130	;1-, ;1	113	39	152	3: 1	0.035	0.85

The total number of F_2 progeny lines tested from two individual F_1 seeds for the seven crosses that were tested with race TJJ at the seedling stage ranged from 151 to 159 plants. The F_2 seedlings evaluated segregated into two distinct phenotypic classes: a resistant class with rust reactions similar to the resistant parent (IT=;1- or 1 or 11^+) and a susceptible class with reactions similar to the susceptible parent (IT=34 or 4). The segregating F_2 populations in the seven crosses had a χ^2 value from 0.002 (lowest) with p-value of 0.96 for CN56979/CN55115 to 2.404 (highest) with p-value of 0.12 for CN56979/CN21997. The rest of the χ^2 and the p-values fall between the high and low values indicated above. The observed resistant to susceptible genetic ratios in all seven crosses fit the expected ratio for three resistant to one susceptible model. This is indicative of each of the accession possessing a single gene conferring resistance to race TJJ. It was also noted that the resistant seedlings from CN56979/CN57130 had lower infections types (;, ;1-, or ;1) compared to the infection types observed in the other crosses (1, 11+, or 12). The phenotypic response of the resistant progeny lines of this cross including the parental accession (CN57130) was very similar to that of the accession CN56818 (a source of *Pg6*).

Chi-square analyses of F_3 families for the seven crosses tested with race TJJ at the seedling stage are presented in Table 4. The total number of F_3 families tested in the seven crosses range from 143 to 159 families. The numbers of F_3 families for CN56979/CN21996, CN56979/CN21997, CN56979/CN22001, and CN56979/CN57130 were slightly smaller than their respective F_2 plant numbers because some F_2 plants did not survive to maturity to produce seeds, and others produced an inadequate amount of

seed for F_3 testing and thus they were removed from the analyses, hence a lower number of F_3 progeny lines.

Table 4. Chi-square analyses of F_3 families tested with race TJJ at the seedling stage.

Cross	F_3 Families				Model	χ^2	P-value
	Resistant	Seg.	Susceptible	Total			
CN56979/CN21996	38	71	42	151	1:2:1	0.748	0.69
CN56979/CN21997	26	74	46	146	1:2:1	5.507	0.06
CN56979/CN21998	37	84	31	152	1:2:1	2.158	0.34
CN56979/CN22000	29	81	41	151	1:2:1	2.709	0.26
CN56979/CN22001	32	82	32	146	1:2:1	2.219	0.33
CN56979/CN55115	42	77	40	159	1:2:1	0.208	0.90
CN56979/CN57130	41	63	39	143	1:2:1	2.077	0.35

As shown in Table 4, all F_3 families segregated into three classes with a good fit to the expected 1:2:1 ratio of homozygous resistant, segregating, and homozygous susceptible, respectively. The F_3 result is supporting the result in F_2 confirming that each accession possesses a single gene. The segregating F_3 families in five crosses (CN56979/CN21996, CN56979/CN21997, CN56979/CN21998, CN56979/CN55115, and CN56979/CN57130) fit a three resistant to one susceptible ratio, which indicates that resistance is dominant in the five accessions. The segregating F_3 families in CN56979/CN22000 and CN56979/CN22001, however, did not have the expected fit for a single dominant gene (data not shown).

3.4.2 Backcrosses

The F_1 seeds produced from crosses of each of the seven accessions to the susceptible accession (female) were planted and grown to the adult stage. The plants were then backcrossed to the susceptible accession to produce BC_1F_1 progeny lines. These plants were allowed to self to produce BC_1F_2 seeds. The BC_1F_1 seeds produced from the seven crosses, ranged from five to 11. They were planted and the seedlings were tested with race TJJ. These BC_1F_1 seedlings segregated into resistant and susceptible categories (Table 5). When advanced to the next generation and tested with race TJJ, progenies of the resistant BC_1F_1 plants segregated into two classes, resistant and susceptible seedlings, as expected. All of the susceptible BC_1F_1 plants produced only homozygous susceptible seedlings at the BC_1F_2, which was what was expected.

Chi-square analyses for the BC_1F_1 seedlings were not conducted because of the small amount of seeds produced and thus insufficient sample size. The chi-square analyses of each of the segregating BC_1F_2 populations from the seven crosses fitted the expected ratio of three resistant to one susceptible model (Table 5).

Table 5. Chi-square analyses of backcross populations tested with race TJJ at the seedling stage.

	BC_1F_1 seedlings		**BC_1F_2 plants from resistant BC1F1 plants**					
Cross	**Res**	**Sus**	**Res**	**Sus.**	**Total**	**Model**	**χ^2**	**P-value**
CN56979//CN56979/CN21996	2	6	413	150	563	3: 1	0.811	0.37
CN56979//CN56979/CN21997	1	4	215	58	273	3: 1	2.053	0.15
CN56979//CN56979/CN21998	3	2	642	231	873	3: 1	0.993	0.32
CN56979//CN56979/CN22000	7	3	681	216	897	3: 1	0.405	0.53
CN56979//CN56979/CN22001	4	1	385	138	523	3: 1	0.536	0.46
CN56979//CN56979/CN55115	2	3	315	124	439	3: 1	2.467	0.12
CN56979//CN56979/CN57130	6	5	726	247	973	3: 1	0.077	0.78

The lowest χ^2 value (0.077) with P-value of 0.78 was for CN56979//CN56979/CN57130 and the highest χ^2 value (2.467) with p-value of 0.12 was for CN56979//CN56979/ CN55115. This result is an additional confirmation that a single dominant gene is conferring resistance in each of the seven accessions and supports the F_2 and F_3 results.

3.4.3 Intercrosses among the seven accessions

The result of F_2 populations from the intercrosses among the seven resistant *A. strigosa* accessions (CN21996, CN21997, CN21998, CN22000, CN22001, CN55115, and CN57130) when tested with race TJJ at the first leaf stage is shown in Table 6. All of the F_1 seedlings that produced the F_2 progenies in the 21 intercrosses were resistant to race TJJ (data not shown). The size of F_2 populations tested ranged from 126 to 437 seedlings, with most near 300. All seedlings from all crosses were highly resistant and displayed very low infection types (; or ;1 with few of them ;11+) when tested with race TJJ, indicating that the resistance in the seven accessions is at the same locus.

3.4.4 Allelism to Pg6

The seven resistant *A. strigosa* accessions were each crossed to *A. strigosa* accession CN56818 (a source of *Pg6*), a gene that confers resistance to race TJJ. The F_1 hybrids were planted and grown to maturity to produce F_2 populations. Both the F_1 hybrids and F_2 populations were tested with race TJJ at the seedling stage and the F_2 result is shown in Table 7.

Table 6. F_2 populations of intercrosses between seven *A. strigosa* accessions tested with race TJJ at the seedling stage.

	F_2 seedlings		
Cross	**Resistant**	**Susceptible**	**Total**
CN21996/CN21997	437	0	437
CN21996/CN21998	298	0	298
CN21996/CN22000	247	0	247
CN21996/CN22001	255	0	255
CN21996/CN55115	282	0	282
CN21996/CN57130	292	0	292
CN21998/CN21997	235	0	235
CN22000/CN21997	297	0	297
CN21997/CN22001	295	0	295
CN21997/CN55115	126	0	126
CN21997/CN57130	293	0	293
CN21998/CN22000	300	0	300
CN21998/CN22001	292	0	292
CN21998/CN55115	294	0	294
CN21998/CN57130	299	0	299
CN22001/CN22000	300	0	300
CN22000/CN55115	298	0	298
CN22000/CN57130	294	0	294
CN22001/CN55115	298	0	298
CN22001/CN57130	299	0	299
CN55115/CN57130	374	0	374

Table 7. Allelism test of F_2 populations from the seven *A. strigosa* accessions crossed to *Pg6* (CN56818) and tested with race TJJ at the seedling stage.

	F_2 seedlings		
Cross	**Resistant**	**Susceptible**	**Total**
CN21996/CN56818 (*Pg6*)	270	1*	271
CN21997/CN56818 (*Pg6*)	489	0	489
CN21998/CN56818 (*Pg6*)	294	1*	295
CN22000/CN56818 (*Pg6*)	291	1*	292
CN22001/CN56818 (*Pg6*)	279	2*	281
CN55115/CN56818 (*Pg6*)	616	0	616
CN57130/CN56818 (*Pg6*)	124	0	124

* rated as susceptible but subsequent progeny tests (Table 7) determined they were resistant

The number of F_2 seedlings tested in the seven crosses ranged from 124 (CN57130/CN56818) to 616 (CN55115/CN56818), (Table 7). In three crosses (CN21997/CN56818, CN55115/CN56818, and CN57130/CN56818), there were no susceptible plants from a total of 489, 616, and 124 seedlings, respectively. This suggests that the resistance gene in these accessions is at the *Pg6* locus. There was one susceptible plant each in the three crosses (CN21996/CN56818, CN21998/CN56818, and CN22000/CN56818) from a total of 271, 295, and 292 seedlings, respectively, and two susceptible plants from 281 seedlings in CN22001/CN56818. All of the susceptible seedlings (except one from CN22001/CN56818 which died after it was transplanted), were grown to maturity and their F_3 progenies were tested with race TJJ at the seedling stage. For each of the four susceptible individual F_2 plants, F_3 families ranging from 121 to 125 were tested. In each of the progeny lines, very low infection types (; or ;1^- or ;1 or ;11^+) were displayed with no susceptible seedlings recovered (Table 8). This result suggests that the F_2 seedlings were misclassified and the resistance gene in the accessions also is at the same locus with *Pg6*.

Table 8. F_3 seedlings from four F_2 plants originally rated as susceptible when tested with race TJJ.

	F_3 progeny		
Cross	**Resistant**	**Susceptible**	**Total**
CN21996/CN56818	125	0	125
CN21998/CN56818	121	0	121
CN22000/CN56818	123	0	123
CN22001/CN56818	125	0	125

3.4.5 Temperature study

The temperature at which the accessions were incubated appeared to have little effect on expression of resistance (Table 9).

Table 9. Rust reaction of nine *A. strigosa* accessions and 'AC Assiniboia' to race TJJ at three temperature regimes.

	Incubation teperature		
Accession	**12°C/10°C (day/night)**	**20°C/18°C (day/night)**	**29°C/27°C (day/night)**
CN21996	;1^-	1^-	11^+
CN21997	;1^-	; 1^-	;1^-
CN21998	;1	1	1
CN22000	;	1^-	;1^-
CN22001	;	; 1^-	;1^-
CN55115	;1^-	1	;1^-
CN57130	0	;	;1^-
CN56979	3	34	34
CN56818 (*Pg6*)	0	;	;1^-
'AC Assiniboia'	34	34	4

Seedlings with ITs of 1 and 2 were classified as resistant while with 3 and 4 were classified as susceptible

Resistant accessions expressed low infection types, while the susceptible CN56979 and the hexaploid 'AC Assiniboia' displayed high infection types, across the range of incubation temperatures (12°C to 29°C). There were slight increase in pustule size for some accessions at higher temperature settings, for example, infection types for CN21996 were ;1^- to ;1 at 12°C while they were 11^+ to 12 at 29°C. The only striking difference found was that CN57130 and CN56818 (*Pg6*) were immune (no visible reaction) at the low temperature setting of 12°C/10°C (day/night), compared to the hypersensitive reaction (;1^-) at the high temperature setting 29°C/27°C (day/night). Also, the susceptible accession CN56979 had smaller pustule size (IT=3) at the lower

temperature regime compared to the bigger pustule size (IT=34) at the higher temperature regime. The same was true for the hexaploid check 'AC Assiniboia', with IT=34 at the lower temperature but slightly bigger pustules (IT=4) at the higher incubation temperature.

3.4.6 Multi-pathotype allelism test

The seven resistant accessions, the susceptible accession, and the accession possessing *Pg6* (CN56818) were tested with 23 races of stem rust. Purity of the races was confirmed by rating reactions of the differential lines (DL) planted in the same tray with the resistant and susceptible accessions (Table 10). The result of multi-pathotype allelism tests with the 23 races indicated that the accession that was used as the female parent in the genetic study (CN56979) was susceptible to all the 23 races. All diploid accessions were susceptible to races BLD and CLD. Accessions CN57130 and CN56818 (*Pg6*) also displayed the similar low infection types (;, ;1^{-}, ;1 or ;11^{+}) for the remaining 21 races. Pustule sizes on CN21997 to some races (BDJ, HDD, RGB, RGD, TDD, and TJD) were slightly bigger than that of the other two accessions (CN57130 and CN56818).

Among the other five diploid entries, there appeared to be four patterns of reaction that could be differentiated from what was observed for the CN21997, CN57130, and CN56818 lines. CN21996 was the only diploid line that was clearly susceptible to races JBD, SBG, and TJG. CN22001 was susceptible to races races BDJ, HDD, and TGL. CN55115 was the only one susceptible to races RDD, RGB, TGF, RGD, and to TGL. CN21998 and CN22000 reacted similarly across the 23 races, and could be differentiated from all other diploid entries based on their pattern of disease response.

Table 10. Seedling reactions of nine *Avena strigosa* accessions, 12 single gene differential lines, and the *Pg-a* complex to 23 races of *Puccinia graminis* f. sp. *avenae*

	Race																						
Genotype	BLD NA 1	BDJ NA 3	DBD NA 5	FDJ NA 8	HDD NA 9	JBD NA 10	NGB NA 16	NGD NA 18	RDD NA 20	RGB NA 21	SGB NA 23	TDD NA 25	TDJ NA 26	TGB NA 27	TGL NA 28	TGD NA 29	TJD NA 30	TDF NA 55	TJJ NA 67	CLD NA 70	RJJ NA 75	TJG NA 76	RGD NA 77
CN 21996	4[a]	21	1	;1	11+	34	11+	1	1+	1	34	34	12+	11+	;1-	11+	12	;1	;1+	34	;1+	4	;2
CN 21997	4	11+	;1-	1	11+	1	;1-	;1-	;1	11+	;1	12+	1	;1-	;1-	1	11+	;1	;1-	4	;1-	;1	;1
CN 21998	4	21	;2+	23	12+	12-	23-	11+	23	2+	;3	3	3	;1	12+	12	11+	12-	11+	34	2+3-	;1+	;21
CN 22000	4	12+	12+	;2	12	12+	2+	1	12+	1+2	;12+	3	3	11+	21	12	12	11+	;1	34	12+	12	12+
CN 22001	4	3-	1	;1	3-	1	12+	12+	12+	13	;1	34	3	;1	4	11+	12	;2	11+	4	;1	11+	12+
CN 55115	4	12+	11+	12	12+	12	2	11+	3-	3-	1	34	34	;2	34	11+	12+	3	1	4	;1	2+	3-
CN 57130	4	;1-	;	;1-	;1	;	;	;1-	;1-	;	;	;1+	;1	;1	;1	;1-	;1	;	;1	4	;1	;	;1-
CN 56979	4	4	4	34	34	4	4	34	4	34	34	4	4	4	34	4	4	34	4	34	34	4	4
Pg1	11+	2	1	11+	12	1	4	4	4	4	4	4	4	4	4	4	4	4	4	12	4	4	4
Pg2	1	1	1	;1-	4	4	1-	12	4	4	4	4	4	4	4	4	4	4	4	1	4	4	4
Pg3	2+	4/1	4	13	12	4	4	4	2+	13	4	4	4	4	4	4	4	4	4	4	12+	4	12
Pg4	1	1	1-	4	34	1-	1	1	4	4	1	4	4	4	4	4	4	4	4	4	4	4	4
Pg6	4	;1-	;	;1-	;1	;1-	;	;	;1	;	;1-	;1+	;1+	;1	;1	;1-	;1	;1-	;1+	4	;1	;1-	;1
Pg8	12	12	1	1	12	1	4	4	11+	4	4	1	12	4	4	4	4	11+	4	1	4	4	34
Pg9	4	4	12+	4	4	1	2+	12	4	1	12+	4	4	12+	23	12[+]	4	4	4	4	4	4	12+
Pg10	23	23	12	12	12	12	2	12	12+	12	12	12	23	23	12	23	12+	12	12	12	12	12	12
Pg12	11+N	123	1-N	;1-N	1N	1-N	11+N	1N	;1N	1-N	11+	11+	11+	12	4	12	11+N	1N	11+N	1-N	;1N	1-N	1-N
Pg13	1	4	1	4	1	1-	1	1	1	1	1	1	4	1	1	1	12+	1	4	1-	4	4	1
Pg15	4	4	4	4	4	4	12	4	4	1	12	4	4	12	12	4	4	4	4	4	4	1	4
Pg16	1	1	1-	1-	1	1	1-	1	1	1-	1	1-	1	1	1-	1	1-	4	1	1-	12	1-	1
Pg-a	;N	;1-N	;N	;N	;N	;N	;N	;N	;N	;N	;N	;N	;N	;N/4	4	;N	;N	;N	;N	;N	;N	;1N	;N

[a] Infection types (ITs) are based on a 0-4 scale as defined by Stakman et al. (1962).

3.5 DISCUSSION

3.5.1 F_2 populations and F_3 families

When F_2 populations from the seven crosses were tested with race TJJ at the seedling stage, they segregated into two classes (resistant and susceptible) and fit a 3:1 (resistant to susceptible) segregation ratio. The result is indicative of a single dominant gene conditioning resistance to race TJJ in each accession. The F_3 families derived from F_2 populations segregated into resistant, segregating, and susceptible classes and fit a 1:2:1 (homozygous resistant: segregating: homozygous susceptible) ratio, supporting the results obtained in the F_2 analyses for a single gene conditioning resistance to race TJJ. For example, progeny testing of F_3 families from CN56979/CN57130 were found to be 41 resistant, 63 segregating (1130 resistant: 313 susceptible plants), and 39 susceptible families supporting the single gene model (χ^2=2.077, P-value=0.35) postulated from F_2 data. Similar results were obtained for the other four crosses in which individual plants in segregating F_3 families were counted and found to fit the 3:1 (resistant to susceptible) ratio. This confirmed that the resistance gene is not only a single gene but also a dominant gene. However, in two crosses (CN56979/CN22000 and CN56979/CN22001), the segregating families did not fit the expected 3:1 (resistant to susceptible) ratio. This might be due to the presence of a modifier gene in these backgrounds which could influence the effect of the other gene as suggested by Dyck (1966). The presence of a modifier gene usually results in a higher infection type, thereby increasing the number of susceptible plants and lack of fit to the expected ratio. It could also be due to background effect as only accession CN57130 had black seed color similar to that of the seeds of

accession CN56818 (a source of *Pg6*), indicating that accession CN57130 may be from a different source compared to the other accessions.

In the backcross tests, there were two phenotypic classes, resistant and susceptible seedlings. The susceptible BC_1F_1 plants produced only susceptible seedlings at the BC_1F_2, whereas the resistant BC_1F_1 plants produced both resistant and susceptible seedlings as expected. The segregating BC_1F_2 families in all seven crosses fitted a three resistant to one susceptible ratio, supporting the results obtained in the F_2 and F_3 experiments. This is additional evidence that a single dominant gene is conferring resistance to race TJJ in each of the seven *A. strigosa* accessions.

In evaluating seedlings from the 21 intercrosses among the seven resistant accessions, all seedlings in all crosses displayed a high level of resistance (ITs of ; or ;1 or ;11+). There were no susceptible seedlings in any of the crosses. This result indicates that the gene conferring resistance to race TJJ in the seven accessions is at the same locus. Since about 300 seedlings were evaluated per F_2 population (except for CN21997/CN55115 where 126 seedlings were tested), it is possible that there are two very tightly linked genes. More progeny need to be tested to confirm if they are independent or are alleles. For example, over 6,400 progeny lines need to be tested to find one susceptible plant if the two genes were 2.5 cM apart. Furthermore, no susceptible seedlings were found in three F_2 populations of crosses to CN56818 (*Pg6*), indicating that the resistance in CN21997, CN55115, and CN57130 is closely linked or allelic to *Pg6*. Seedlings from the other four F_2 crosses (CN21996/CN56818, CN21998/CN56818, CN22000/CN56818, and CN22001/CN56818) rated as susceptible must have been misclassified, because none of the F_3 seedlings (121 to 125 seedlings per

plant) from the four plants were susceptible when tested with race TJJ. Therefore, it is concluded that resistance in all seven diploid lines is allelic to *Pg6* or very tightly linked.

Two of the accessions (CN55115 and CN57130) were listed as originating from the former USSR and the USA, respectively, but it is known that these two countries are not centres of origin of oat (Vavilov, 1951). It is quite likely that the two accessions might have originated from the same area as the others. The idea that the resistant *A. strigosa* accessions possess resistance gene *Pg6* or alleles of *Pg6* is not surprising, because stem rust resistance genes are rare (Martens and Dyck, 1989) and several stem and crown rust resistance genes were obtained from North Africa and the Middle East (Martens et al. 1980). As reported by Martens (1985), since most of the oat stem rust resistance genes are from either the western Black sea (*Pg1, Pg2, Pg9, Pg15,* and possibly *Pg11*) or North African (*Pg4, Pg8, Pg13,* and possibly *Pg3*) regions, it is possible that the *A. strigosa* accessions would possess the same gene. Based on the similarity of seedling rust reactions of several *A. strigosa* accessions tested with six oat stem rust races NA1, NA8, NA16, NA55, NA28, and NA67, Gold Steinberg et al. (2005) suggested that the accessions may possess the same resistance gene.

3.5.2 Temperature study

Temperature plays an important role in stem rust development, the optimal incubation temperature being 15˚C to 20˚C (Martens et al. 1968). High temperature is known to be a factor in making some resistance genes ineffective. For example, McKenzie et al. (1968) reported that oat variety 'Jostrain', which is resistant at temperatures 15˚C and 20˚C, was completely susceptible at 25˚C and 30˚C when tested

with the same race. Another example is that the resistance in *Pg*12 is not expressed at temperatures 25°C and above (Martens et al. 1968; Fetch, 2006). In agreement with Adhikari et al. (2000), Fetch (2006) reported the ineffectiveness of *Pg-a* at a temperature of 26°C and above. Similarly, *Pg*13 was reported to be thermolabile (Martens, 1985 and Fetch, 2006). Genes like *Pg*1, *Pg*2, *Pg*6, and *Pg10* are temperature insensitive, while *Pg*9, *Pg*8, *Pg15*, *Pg*16, *Pg*4, and *Pg*3 (in increasing order) are temperature sensitive (Martens, 1985 and Fetch, 2006). In the current study and consistent with the result reported by Fetch (2006) for *Pg6*, the resistance in the *A. strigosa* accessions was effective against race TJJ over a range of temperature from 12°C to 29°C. The infection types in both low 12°C/10°C (day/night) and high 29°C/27°C (day/night) temperatures were very low (;, or ;1^- or ;11^+) for all of the accessions. The rating of rust reaction at 14 days post inoculation might have not been long enough for optimal rust development at an incubation temperature of 12°C/10°C (day/night). Had it been done few days later (18 to 20 dpi), it might have resulted in bigger pustule size in the susceptible accession and also some pustules with low infection types might have resulted in the CN57130 and CN56818 (*Pg6*) accessions. Temperature sensitivity of the seven accessions also was not observed even in hot summer days where greenhouse temperatures of 30°C and above were not uncommon. The resistance in the diploid *A. strigosa* lines is highly resistant to oat stem rust races in western Canada except for BLD and CLD.

3.5.3 Multi-pathotype allelism tests

Since the three accessions had identical pattern of low response to all but races BLD and CLD, they likely possess the same resistance allele. It is possible that they could be different, but would require further extensive testing with more pathotypes, if available. Since the gene in CN56818 was previously designated as *Pg6* it is proposed to name the allele in accessions CN21997, CN57130, and CN56818 as *Pga.*

Multiple alleles for resistance are not common, but have been described in a number of rust pathotypes including wheat (Loegering and Harmon, 1969) and flax (Flor, 1955). In this study, there clearly were differential patterns of infection among the other five diploid lines when inoculated with 23 pathotypes of oat stem rust, indicating that they likely have different alleles for resistance. As in the previous test, extensive testing of additional progeny would be needed to ascertain if they are allelic or independent (very tightly linked). In the meantime, it is proposed to name the alleles as *Pg6b* (CN21996), *Pg6c* (CN21998 and CN22000), *Pg6d* (CN22001), and *Pg6e* (CN55115) based on their differential pattern of infection to 23 races in the multi-pathotype study. Additional testing of more F_2 progeny of crosses to CN56818, and of the lines with additional races of *P. graminis* f. sp. *avenae*, is planned to try and resolve the independence or allelic relationship among these diploid accessions.

3. 6 CONCLUSIONS

Based on the genetic analyses of F_2 populations and F_3 families, a single dominant oat stem rust resistance gene was identified in the seven *A. strigosa* accessions studied. This conclusion was supported by the results from the BC_1F_2 populations. The F_2 results from the intercrosses among the seven accessions and the allelism tests where each of the seven accessions was crossed to CN56818 (a source of *Pg6*) indicated that the resistance gene in all accessions is at the same locus. The multi-pathotype test using 23 races indicated that there likely are five alleles in the seven accessions, which are proposed as alleles *Pg6a*, *Pg6b*, *Pg6c*, *Pg6d,* and *Pg6e*.

The dominant gene *Pg6* in the diploid species of *A. strigosa* is a very useful source of resistance against the virulent race TJJ. Dyck (1966) suggested that efforts should be made to transfer the dominant gene in *A. strigosa* conditioning resistance to a wide range of races into cultivated oat. Since oat stem rust resistance genes are rare and since no additional resistance genes are available in hexaploid oat (Harder, 1999), making use of the resistance gene in *A. strigosa* as a source of oat stem rust resistance for future oat cultivar development is very essential. However, transferring this resistance into cultivated oat is a challenge. This difficulty is due to low transmission of resistance through pollen (McKenzie et al. 1970; Brown et al. 1986), or due to cross-incompatibility and sterility in F_1 hybrids in interploidy crosses (Rajhathy and Thomas, 1974). Efforts should be made to incorporate the resistance into the background of cultivated oat. Also, it is a very useful gene because it is temperature insensitive. In the temperature study component of this project, *Pg6* and its alleles were stable at 29°C. The multi-pathotype

test also showed that the gene is displaying a very low infection type to a wide range of races. Temperature insensitivity and resistance to a range of races makes the gene a very good candidate to be transferred into cultivated oat.

4.0 TRANSFER OF RESISTANCE GENE TO RACE TJJ (NA67) FROM *AVENA STRIGOSA* SCHREB. TO *AVENA SATIVA* L. ('SUN II').

4.1 ABSTRACT

Stem rust (*Puccinia graminis* Pers. f. sp. *avenae* Eriks. and E. Henn.) is a devastating disease of oat (*Avena sativa* L.). Genetic resistance is the most effective and economical method of controlling the disease. Most oat cultivars currently registered in Canada are susceptible to oat stem race TJJ (NA67). Recent efforts identified resistance to race TJJ (NA67) in diploid *Avena strigosa* Schreb. accessions. The objective of this study was to transfer the resistance from the diploid *A. strigosa* into the hexaploid *A. sativa* background. Crosses were made between five *A. strigosa* accessions (CN21996, CN21997, CN22000, CN22001, and CN57130) and five hexaploid cultivars ('Drummond', 'Triple Crown', 'AC Medallion', 'Hokonui', and 'Sun II') and 16 F_1 interspecific hybrids (2n=3x+1x=28) were recovered. An embryo rescue procedure was used and nine of 11 F_1 hybrids treated with colchicine produced seed. Plants from colchicine-treated CN21997/'Sun II' and CN57130/'Sun II' populations were cytogenetically confirmed to be octaploids, and backcrossed to 'Sun II', and resulting BC_1F_1 plants were selfed to produce BC_1F_2 seeds. BC_1F_2 seedlings were tested with race TJJ (NA67) and resistant progeny lines were identified. Phenotypic data of BC_1F_2 progeny showed that diploid resistance was transferred from CN21997 and CN57130 into 'Sun II'. Cytological work indicated that chromosome numbers of selected BC_1F_2 progeny lines were from 43-47, compared to the hexaploid chromosome number of 42 for

A. sativa. The resistance expressed in BC_1F_4 progeny lines from 'Sun II'//CN21997/'Sun II' and also in BC_1F_3 from 'Sun II'//CN57130/'Sun II' is from the addition of an *A strigosa* chromosome carrying the resistance gene into 'Sun II' derivatives as shown by the 42+ chromosomes in the resistant progeny lines. The resistant progeny lines were also crossed to another octaploid ('Sun II'/*Avena longiglumis* Dur. [Cw57]) to induce chromosome pairing between *A. strigosa* and 'Sun II' chromosomes. Several backcrosses will be required to stabilize the diploid resistance into a hexaploid 'Sun II' background, which then could be used in oat cultivar development.

4.2 INTRODUCTION

Oat stem rust, caused by the fungus *Puccinia graminis* Pers. f. sp. *avenae* Eriks. and E. Henn., is a major disease that threatens oat (*Avena sativa* L.) production world wide. Though the disease can be controlled by the use of fungicides, genetic resistance is the preferred and economically sound method. To use genetic resistance to control the disease and secure oat production, effective resistance genes need to be identified and incorporated into adapted oat cultivars.

In contrast to the other cereal rusts, virulence in *Puccinia graminis* f. sp. *avenae* was relatively stable until 1940, dynamic from 1940-1960, then followed by stability from 1960-1990 (Harder, 1994). New stem rust races that are virulent on cultivated oat appear periodically (Green and McKenzie 1967). Resistant cultivars may become susceptible because of change in virulence in the pathogen when a new race appears. For example, race 94, (race TJD [NA30]) with virulence on all resistance genes in cultivated oat suddenly appeared in 1965 (Rothman 1986). McCallum et al. (2000) reported the appearance of the highly virulent race TJJ (NA67) in 1998. It is now the most prevalent race in western Canada and is virulent on most currently grown Canadian cultivars.

In an attempt to find additional sources of oat stem rust resistance, there have been several efforts searching for new resistance genes in oat. Over 4,500 hexaploid entries were evaluated, but no new oat stem resistance genes were found (McKenzie et al. 1971). Rines et al. (1980) evaluated 1,600 accessions of wild oats (*Avena fatua* L.) and did not find a single accession resistant to race 94 (TJD). Gold Steinberg et al. (2005) evaluated almost 10,000 accessions of diploid, tetraploid, and hexaploid oat species with

race TJJ (NA67) and no resistance was found in nearly 7,000 hexaploid accessions. Gold Steinberg et al. (2005) also reported that 98.8% of all the accessions evaluated were susceptible to the virulent oat stem rust race TJJ (NA67). It was also reported that no more oat stem rust resistance genes are available in hexaploid oat (Harder, 1999).

The genetic base of North American oat cultivars is very narrow. As reported by Frey (1986), only seven European cultivars were primary contributors to North American germplasm. Canadian cultivars have ten parental lines (Fu et al. 2004). Since there is lack of resistance genes in hexaploid oat to the highly virulent oat stem rust race TJJ (NA67), it is crucial to find resistance from sources of related weedy oat species and use them to protect cultivated oat. In the past, wild diploid, tetraploid, and hexaploid oat species have been very useful sources of stem rust resistance. Resistance genes from *Avena sterilis* L. have been identified and incorporated into cultivated oat (McKenzie et al. 1970; Harder et al. 1971; Martens et al. 1980). Gold Steinberg et al. (2005) identified a number of diploid and tetraploid accessions that were resistant to race TJJ (NA67).

The availability of oat stem rust resistance in lower ploidy oat has been investigated by several researchers. Since there is lack of resistance in cultivated and wild hexaploid oat, the transfer of resistance from related wild species of lower ploidy level into cultivated hexaploid oat has been suggested by several investigators (Dyck, 1966; McKenzie et al. 1971; Aung et al. 1977). Therefore, to overcome the shortage of oat stem rust resistance genes in common oat, the transfer of resistance from diploid and tetraploid species into cultivated hexaploid oat should be considered very seriously (Dyck 1966; McKenzie et al. 1971). However, the actual transfer has been a challenge. This was because of the lack of seed set or very poor seed set from interspecific crosses. The

lack of seed set could be due to reduced efficiency in the transmission of gametes that result in cross-incompatibility and sterility in F_1 hybrids (McKenzie et al. 1970; Rajhathy and Thomas 1974; Brown et al. 1986; Leggett and Thomas, 1995). Despite these challenges, efforts were made to transfer resistance from lower ploidy into cultivated oat. Zillinsky and Derick (1960); Sadanaga and Simons (1960 and 1967); Dyck and Zillinsky, 1963; Aung et al. (1996); and Rines et al. (2007) reported that crown rust resistance had been transferred from diploid into hexaploid oat. Mildew resistance (Aung et al. 1977) and oat stem rust resistance (Brown et al. 1986) were successfully transferred from a tetraploid into a hexaploid background through the use of disomic addition lines. Rothman (1984) reported that oat stem rust resistance gene *Pg6* from diploid *Avena strigosa* Schreb. had been transferred into a hexaploid background, resulting in the resistant *A. sativa* cultivar 'Delredsa'. However, studies recently conducted on 'Delredsa' at the Cereal Research Centre in Winnipeg, did not support Rothman's report (Fetch, personal communication). As reported by Forsberg and Shands (1969) from their study on the breeding behaviour of 6x-amphiploid x *Avena sativa* F_1 hybrids, bridging of the diploid and hexaploid genomes is possible.

This study was initiated from a consideration of the shortage of oat stem rust resistance genes in cultivated and wild hexaploid oat and an understanding of the challenges of transferring resistance from lower ploidy oat into hexaploid background. The objective of this study was to transfer oat stem rust resistance to race TJJ (NA67), the highly virulent and most prevalent race in western Canada, from *A. strigosa* (diploid) into an adapted hexaploid oat (*A. sativa*).

4.3 MATERIALS AND METHODS

The oat cultivated worldwide are the hexaploids, *A. sativa* and *A. byzantina.* However, *A. strigosa* is cultivated to a limited extent in Wales, Scotland, Poland, and South America (Rajhathy and Thomas, 1974; Thomas, 1992). This species has the 'A_s' genome while the cultivated hexaploid oat has the 'A', 'C', and 'D' genomes. In the crosses made in this study between *A. stigosa* and *A. sativa*, it was hoped that the 'A_s' chromosomes from *A. strigosa* would pair with the 'A' chromosomes of *A. sativa.*

4.3.1 Avena strigosa by hexaploid crosses

Seven *A. strigosa* accessions (CN21996, CN21997, CN21998, CN22000, CN22001, CN55115, and CN57130) that displayed a high level of resistance to oat stem rust race TJJ (NA67), and twelve susceptible *A. sativa* oat cultivars ('Rodney 0', 'AC Medallion', 'Vista', 'Hifi', 'Sun II', 'Triple Crown', 'Flamingsgold' 'Tulunskij 86/5', 'Drummond', 'Echinida/'Drummond'', 'Charlton', and 'Hokonui') were selected for use in this study. The selected resistant *A. strigosa* materials were from Spain, the former USSR, and the USA (Gold Steinberg et al. 2005). The *A. sativa* oat cultivars were from three oat-growing geographical areas (North America, Europe, and New Zealand) and had different plant height and heading dates. The selected materials were planted for crossing in 2003.

Seeds were germinated in petri dishes weekly and as soon as shoots and root started growing (GS 07, Zadoks et al. 1974) they were transplanted into soil-less sunshine mix #5 (70-80% fine Canadian sphagnum peat moss, fine perlite, dolomitic lime stone,

gypsum, wetting agent) (Hadishville, MB) in 15cm x 15cm x 15cm fibre pots. The seedlings were grown in a greenhouse up to the time of inoculation at the first leaf stage. In the greenhouse, seedlings were grown at 20˚C/18˚C (day/night) +/-4˚C and 16h photoperiod, supplemented by high pressure sodium lighting (250 μmol m^{-2} s^{-1}).

4.3.2 Stem rust inoculum – race TJJ (NA67)

A pure sample of the highly virulent oat stem rust race TJJ (NA67) was obtained from the Cereal Research Centre at Winnipeg, Manitoba, Canada rust collections. The race was increased on the susceptible host 'AC Assiniboia' as described in chapter I. Inoculations and incubations were also performed as described earlier and rust reactions were scored 14 days after inoculation using a 0-4 scale where 0, 1, and 2 were considered as resistant, and 3 and 4 were considered susceptible (Stakman et al. 1962).

After the seedlings were inoculated, they were moved to a growth cabinet under photoperiod and temperature regimes of 16h/8h and 18˚C/16˚C (day/night), respectively. Three weeks after inoculation, the temperature of the growth cabinet was raised to 20˚C/18˚C (day/night) for the rest of the growing period. Seedlings were fertilized with 15-30-15 (N-P-K) two weeks after planting and then with 20-20-20 (N-P-K) weekly. Iron and manganese micronutrients were added once (⅛ tsp/litre) in the form of iron chelate and manganese chelate (13%) with 68.1% ethylene diamine tetraacetate chelating agent. Depending on availability of growth cabinet space, the plants to be used as males and females in crossing were separated into two growth cabinets when plants were at the boot stage (GS 40, Zadoks et al. 1974). The light regime in the growth cabinet with the male parents was set in such a way that the plants received six hours of light before lights

in the cabinets with the female parents. Keeping male and female plants in separate growth cabinets with staggered light hours helped to improve the success of seed set in previous hexaploid by hexaploid cultivated oat crosses. This procedure was adapted in interploidy crosses whenever growth cabinet space was available.

4.3.3 Crosses

Crosses and reciprocal crosses between diploid and hexaploid lines were made. In interploidy crosses between diploid and hexaploid lines, it is reported that the resulting F_1 plants (if there is seed set) are sterile because gametes can not be formed with a balanced assortment of chromosomes (Rajhathy and Thomas, 1974). Therefore, a method of overcoming this sterility was required to produce fertile seeds in these interploidy crosses. Two approaches were considered to generate fertile seeds.

The first method was adaptation of an embryo rescue technique being used in the doubled haploid laboratory at the Cereal Research Centre (CRC) in Winnipeg to produce wheat haploid plants from wheat x maize crosses (D. Jones, personal communication). In the endeavour to transfer resistance from *A. strigosa* into a hexaploid background, the embryo rescue technique and the general crossing scheme is shown in Figure 1. In this approach, the strategy was to make crosses and reciprocal crosses between the diploid *A. strigosa* accessions and *A. sativa* cultivars. The resulting embryo is rescued before it is aborted and grown in a nutrient media (Appendix 1). The F_1 amphiploid plants (2n=3x+1x=28) produced would be sterile, thus they were treated with colchicine to produce octaploid (2n=2x=56) sectors which were fertile and produced seeds. Plants grown from the octaploid seeds were grown to the adult stage and backcrossed to their

respective hexaploid parents. Reciprocal crosses were also made to produce BC_1F_1seeds. The BC_1F_1 seeds produced were planted and the resulting plants were selfed to produce BC_1F_2 populations and subsequently BC_1F_3 and BC_1F_4 progeny lines. Throughout the interploidy crosses in this study, the embryo rescue protocol (described in the embryo rescue section) was adopted to produce fertile seeds. In addition to this method, once F_1 (2n=3x+1x=28) plants were produced, another attempt to produce fertile seeds was made by crossing the sterile F_1 tetraploid plants as female parents and pollinating them with hexaploid pollen.

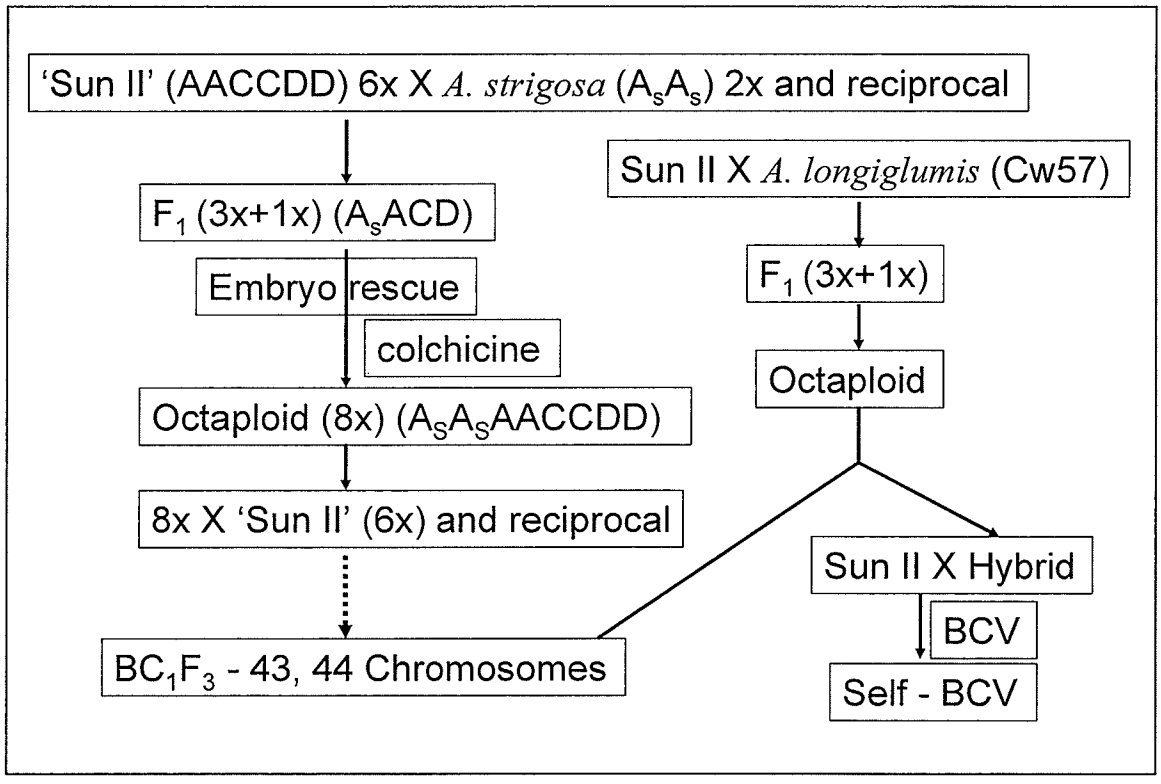


Figure 1. Crossing scheme between diploid and hexaploid oat to transfer stem rust resistance from *A. strigosa* to *A. sativa*.

The second strategy to produce fertile seeds from the interploidy crosses was through the use of autotetraploids. This approach was successfully implemented to overcome incompatibility between diploid and hexaploid species by Zillinsky and Derick (1960). In this alternate approach, the objective was to produce autotetraploids of *A. strigosa* and then cross the autotetraploids to hexaploids. Therefore, autotetraploids from three *A. strigosa* accessions (CN 21997, CN 22000, and CN 22001) were produced by treating 20 seedlings from each of the accession with colchicine. The plants were grown to maturity and the autoteraploid seeds were harvested and saved. These autotetraploid seeds were not used for further work in this study because the first method (embryo rescue) was successful in producing fertile seeds from the interploidy crosses.

All of the seedlings produced in the different populations were tested with race TJJ (NA67) at the first leaf stage and the infection types (IT) were rated as described in chapter 3.0. Seedling rust reactions of the parental materials to race TJJ (NA67) are shown in Table 11.

Table 11. Seedling reactions of diploid *A. strigosa* and hexaploid oat cultivars to oat stem rust race TJJ (NA67).

***Avena strigosa* (Diploid)**			***Avena sativa* (Hexaploid)**		
Accession	**Reaction To TJJ (NA67)[a]**	**Origin**	**Cultivar**	**Reaction to TJJ (NA67)[a]**	**Origin**
CN21996	1-	Spain	'Rodney 0'	34 to 4	North America
CN21997	;1-	Spain	'AC Medallion'	34 to 4	North America
CN21998	1	Spain	'Vista'	34	North America
CN22000	1- to 1	Spain	'HiFi'	34 to 4	North America
CN21001	;1-	Spain	'Sun II'	34 to 4	North America
CN55115	1	Former USSR	'Triple Crown'	34	Europe
CN57130	;	USA	'Flamingsgold'	34	Europe
			'Tulunskij 86/5'	34 to 4	Europe
			'Drummond'	34 to 4	New Zealand
			'Echinida'/'Drummond'	34 to 4	New Zealand
			'Charlton'	34 to 4	New Zealand
			'Hokonui'	34 to 4	New Zealand

[a] Rated with the 1 - 4 rust rating scale (Stakman et al. 1962).

4.3.4 Avena longiglumis by 'Sun II' crosses

The genotype Cw57 (CN21411) of the diploid species *Avena longiglumis* Dur. is known to induce bivalent chromosome pairing when crossed to hexaploid *A. sativa* (Thomas, 1992). *Avena longiglumis* has a winter growing habit, therefore, the seeds were vernalized for three to five weeks by keeping germinated seeds in a refrigerator (4°C). Vernalized seeds were planted weekly in soil-less Sunshine Mix #5 in 15cm x 15cm x 15cm fibre pots. The hexaploid cultivar 'Sun II' was also planted weekly along with Cw57. The plants were grown in a growth cabinet and at the flowering stage, crosses and reciprocal crosses were made between Cw57 and 'Sun II'. Since it was expected that the F_1 hybrids would be sterile, the embryo rescue technique (described below) was used to produce fertile octaploid seeds.

4.3.5 The embryo rescue technique

The protocol that is used at the doubled haploid laboratory at the Cereal Research Centre (CRC) in Winnipeg was adopted. The technique included initiating embryos in interploidy crosses, rescuing the embryos, growing them on nutrient medium (Appendix 1) to producing F_1 hybrid or amphiploid plants, produce chromosome-doubled sectors on seedlings by colchicine treatment, and finally produce seeds from these fertile plants. The procedure began by pollinating emasculated florets with the male parent of interest and treating pollinated florets with growth hormones on the second day after pollination. The florets were sprayed with 100mg/L of aqueous 2,4-dichlorophenoxyacetic acid (2,4-D) or with 100mg/L Gibberellic Acid (GA_3) until growth hormone ran off the florets. Treatments were applied for two consecutive days after pollination. Twenty minutes

after each application of growth hormones, crossing bags were replaced onto the panicles. Some panicles were left untreated to observe whether or not there would be seed set without hormonal treatment. Sixteen days after pollination, caryopses were removed from each floret and were put into the barrel of a 50 ml syringe with sterile distilled water. The syringe allowed easy exchange of solutions during subsequent surface sterilizations. In a laminar flow hood, the caryopses were surface sterilized with 10% sodium hypochlorite solution (Javex bleach) for two minutes, followed by a 40 second treatment with 70% ethanol. The caryopses were then washed with sterile water twice. Embryos were then carefully excised with the help of a dissecting microscope in the flow hood. If the embryos were left on the plant they would be aborted due lack of endosperm development (Laurie and Bennett 1988). When embryos were found they were placed in glass vials with nutrient medium. Embryos were subsequently subjected to three days of cold treatment in a refrigerator (4°C), followed by a three-day dark treatment at room temperature (≈20°C). The vials were then placed under continuous light until haploid plantlets started growing. When the growing haploid seedlings developed a shoot and well developed roots, they were transplanted into soil-less Sunshine Mix #5 in 7 cm x 7 cm plastic pots, grown to 3-4 leaf stage, and treated with colchicine.

Interspecific F_1 seedlings were treated with 0.2% aqueous colchicine N*(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl) acetamide: SIGMA C-3915, or $C_{22}H_{25}NO_6$) at the 3-4 leaf stage in order to produce chromosome doubled sectors. For this treatment F_1 seedlings were up-rooted, roots were washed, and then trimmed to about one centimetre. Damaged leaves, if any, were removed and leaves were clipped to 5-8cm for easier handling. If seedlings were past the 3-4 leaf stage and

secondary tillers were produced, the secondary tillers were separated at the crown where one half was treated with colchicine while the other half remained untreated. Trimmed F_1 seedlings were aligned at the crown and the roots were then immersed completely in 0.2% aqueous colchicine in a beaker. Colchicine treatment was for two and half hours, followed by washing of the roots with running tap water for three hours. The seedlings were then transplanted into soil-less Sunshine Mix #5 in 7cm x 7cm plastic pots. To maintain high humidity, each transplanted seedling was covered with transparent plastic pop bottles whose bottoms were removed. The bottles were removed three to four days after transplanting, and the colchicine-treated plants were grown to maturity to produce seeds.

In the interploidy crosses, some crosses were more successful than others in producing seeds. Those that produced seedlings through colchicine treatment and cytologically confirmed to be octaploids (2n=8x=56) were grown to maturity to produce C_1 seeds. Octaploid C_1 seeds were planted, grown to the adult stage, and backcrossed to the hexaploid cultivar 'Sun II' to produce BC_1F_1 seeds. BC_1F_1 seeds were individually planted in conetainers (19cm x 4cm) and tested with race TJJ (NA67) at the seedling stage. After rating their rust reaction, the seedlings were transplanted into 15 cm x 15 cm x 15 cm fibre pots and grown to maturity to produce BC_1F_2 seeds. BC_1F_2 seeds were planted (25 seeds per pot) in 15cm x 15cm x 15cm fibre pots. Seedlings displaying a high level of resistance to race TJJ (NA67) (IT = ;1- or ;1 or 1) were selected and grown to maturity to produce BC_1F_3 seeds. Forty to fifty seeds from the selected resistant BC_1F_3 plants were planted in large fibre flats (30cm X 25cm x 7cm). In each flat, five seeds of the susceptible *A. strigosa* (CN56979) along with five seeds of 'AC Assiniboia' were

planted. The seedlings were tested with race TJJ (NA67) at the first leaf stage and resistant seedlings were identified, selected, and transplanted into 13cm x 13cm clay pots. When sufficient amount of new root growth took place, root tip samples were taken from each of the selected resistant plants and the plants were grown to maturity to produce BC_1F_4. Cytological evaluation for selected BC_1F_1, BC_1F_2, and BC_1F_3 seedlings was done (as described below) and chromosomes were counted.

4.3.6 Chromosome counting

Healthy root tips of about one centimetre long were collected from seedlings of interest when they were at the 3-5 leaf stage. If root tips were collected from transplanted seedlings, it was done when new root growth appeared after they recovered from transplanting shock. The root tips collected were put into labelled vials filled with chilled distilled water, placed in a tray with ice, and kept in a refrigerator (4°C) for 24 hours. Root tips were then fixed by transferring them into a corresponding set of labelled vials containing a 95% ethanol and glacial acetic acid solution at a 3:1 ratio, respectively. Five days later, the samples were hydrolized in 1N HCl for seven minutes in a water bath preheated to 60°C (Appendix 2). Root tips were then transferred into a small glass dish with Feulgen stain, where they were left to stain until the root tips turned purple (20 minutes).

Root tips were taken out of the glass dish one at a time and were placed on a paper towel, where excess Feulgen stain was blotted off. A root tip was then put on a clean glass slide and the root cap was sliced off using a sharp razor blade. A very thin transverse section of the meristematic root tip was cut with a razor blade and a drop of

acetocarmine was added on to it. A cover slip was placed on the specimen and then the sample was gently squashed by tapping the cover slip with the rubber end of a pencil to get a good chromosome spread. Extreme care was taken to avoid getting air trapped under the cover slip, which would prevent chromosome spread and make counting difficult. Excess acetocarmine stain was blotted off from the slide and then it was warmed on a hot plate for about five seconds. To get a good seal of the cover slip, pressure was applied on the cover slip using the thumb. The specimen was then examined under a phase contrast microscope and chromosomes were counted. Chromosomes of at least five cells per specimen (depending on availability of well-spread countable chromosomes) and 2-3 root tips per seedling were counted to establish the number of chromosomes for each sample examined. Chromosome numbers of parental materials, F_1 octaploid, and selected BC_1F_2 and BC_1F_3 plants were counted.

4.5 RESULTS

4.5.1 Avena strigosa by hexaploid crosses

In the interploidy crosses and reciprocal crosses between five *A. strigosa* accessions and five hexaploids, a total of 2153 florets were pollinated (Table 12). From

Table 12. F_1 and colchicine doubled F_1 plants producing seeds from interploidy crosses between diploid (*A. strigosa*) and hexaploid (*A. sativa*) oat.

		Number of		
Cross	**Pollinated Florets**	**Embryos**	**F_1 plants**	**F_1 plants with seeds**
'AC Medallion'/CN21997	36	3	0	0
CN21997/''AC Medallion'	44	19	2	1
'AC Medallion'/CN22001	19	0	0	0
CN22001/'AC Medallion'	94	41	0	0
'AC Medallion'/CN57130	91	0	0	0
'Sun II'/CN21996	23	0	0	0
'Sun II'/CN21997	27	1	0	0
CN21997/'Sun II'	25	9	1	1
'Sun II'/CN22000	49	2	2	0
CN22000/'Sun II'	19	12	2	2
'Sun II'/CN22001	59	2	0	0
CN22001/'Sun II'	65	26	1	1
'Sun II'/CN57130	802	0	0	0
CN57130/'Sun II'	82	18	2	2
'Drummond'/CN21997	31	3	0	0
CN21997/''Drummond'	62	23	0	0
'Triple Crown'/CN21997	73	7	0	0
CN21997/'Triple Crown'	73	11	0	0
'Triple Crown'/CN22000	15	2	0	0
CN22000/'Triple Crown'	79	27	1	0
CN22000/'AC Medallion'	97	41	2	1
CN22000/'Drummond'	71	30	2	1
CN22000/'Hokonui'	32	17	1	0
CN22001/'Drummond'	14	8	0	0
CN22001/'Triple Crown'	115	19	0	0
CN22001/'Hokonui'	56	25	0	0
Total	**2153**	**346**	**16**	**9**

All CN lines were diploid *A. strigosa* lines and the remaining lines were hexaploid *A. sativa*

these pollinations, 346 embryos (16.1%) resulted in 16 F_1 amphiploid plants (0.74%) were produced. Of the 16 F_1 plants 11 of them (0.51%) received colchicine treatment of which nine resulted in plants with fertile sectors. All seeds produced in this study were from these nine plants (Table 13). The F_1 plants that did not receive colchicine treatment and two of the F_1 plants (CN22000/'Hokonui' and CN21997/Medallion) that were treated with colchicine did not produce seeds.

Table 13. Seeds produced from colchicine-treated and non-treated F_1 plants from *A. strigosa* by *A. sativa* crosses.

Cross	Colchicine Treatment	Seeds
CN22000/'Drummond'	No	0
CN22000/'Triple Crown'	No	0
CN22000/'AC Medallion'	No	0
CN22000/'Drummond'	Yes	Many
CN22001/'Sun II'*	Yes	Many
CN21997/'Sun II'*	Yes	56
CN22000/'Sun II'	Yes	6
CN22000/'Sun II'*	Yes	Many
CN22000/'AC Medallion'*	Yes	12
'Sun II'/CN22000	No	0
'Sun II'/CN22000	No	0
CN22000/'Hokonui'	Yes	0
CN21997/'AC Medallion'	Yes	0
CN21997/'AC Medallion'	Yes	5
CN57130/'Sun II'	Yes	Many
CN57130/'Sun II'	Yes	1

* Seedlings were divided at the crown and one half was treated with colchicine while the other half was left untreated.

In a different method to produce fertile seeds from interspecific crosses, the sterile F_1 haploid plants were used as female parents and were pollinated with pollen from hexaploid pollen donors. However, this method was not very successful and thus was an inefficient method of producing fertile BC_1F_1 seeds because of 526 pollinated florets from two crosses only three seeds were produced (data not presented). The three seeds were not used for further work in this study since the embryo rescue method of hybrid seed production was found to be a successful method in these crosses. The three seeds from the two crosses were saved for future use.

As the F_1 plants from the interspecific crosses were grown to maturity, the panicles of colchicine-treated plants that produced fertile seeds were easily distinguishable from panicles of untreated plants that did not produce seeds. Plump and tan florets from colchicine-treated plants were easy to notice because of the weight of the florets they were pointing downwards, while most florets of the sterile panicles from non-colchicined F_1 plants were pointing upwards or sideways because the whitish-beige florets were empty (Figure 2).

Seeds of the diploid *A. strigosa* were thin and long compared to those of the hexaploid 'Sun II', while seeds of the hybrid octaploid were much bigger and plumper than seeds of 'Sun II' (Figure 3). Seedlings from the presumed octaploid seeds were also more vigorous than both parental seedlings. Cytological evaluation of root tip samples from the C_1 hybrid seedlings confirmed that chromosomes were doubled (49-56) compared to haploids with only 28 chromosomes.

Figure 2. Panicles from a non-colchicined (A) and colchicine-treated (B) plants.

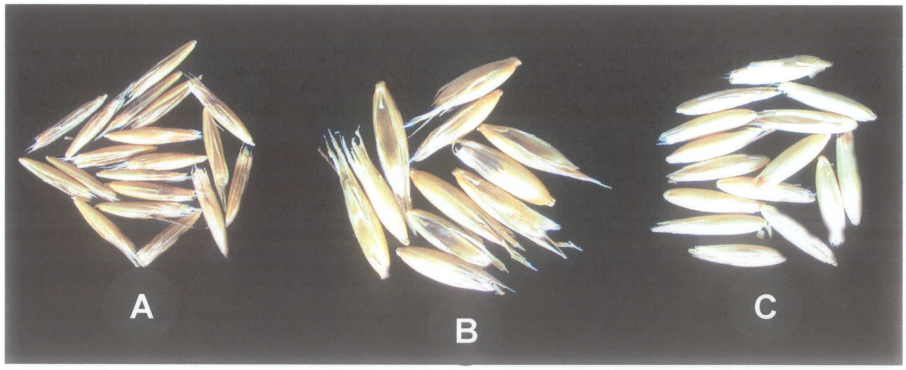


Figure 3. Seeds from the diploid *A. strigosa* (CN21997) (A), octaploid from CN21997/'Sun II' (B), and hexaploid *A. sativa* ('Sun II') (C).

Several F_1 hybrid amphiploids were produced from four *A. strigosa* accessions (CN21997, CN22000, CN22001, and CN57130) crossed to five cultivars of *A. sativa* ('AC Medallion', 'Sun II', 'Triple Crown', 'Drummond', and 'Hokonui'). However, it is only the crosses from CN22000/'Drummond', CN22001/'Sun II', CN21997/'Sun II', CN22000/'Sun II', CN22000/'AC Medallion', CN21997/'AC Medallion', and CN57130/'Sun II' that produced octaploid seeds (Table 13). From these F_1 octaploids, only CN21997/'Sun II' and CN57130/'Sun II' backcrossed to 'Sun II' successfully produced BC_1F_1 seeds and even then the seed set was low, 68 seeds were produced from 287 pollinations (Table 14). The octaploid plants from CN21997/'Sun II' that were

Table 14. BC_1F_1 seeds from octaploids backcrossed to 'Sun II' as well as from reciprocal crosses.

Backcrosses	Pollinated florets	Seeds
'Sun II' X DH# 50 ('Sun II'//CN21997/'Sun II')	26	11
'Sun II' X DH# 57 ('Sun II'//CN21997/'Sun II')	24	7
'Sun II' X DH# 43 ('Sun II'//CN21997/'Sun II')	49	1
'Sun II' X DH# 51 ('Sun II'//CN21997/'Sun II')	21	7
Sub total	**120**	**26**
DH# 53 X 'Sun II' (CN21997/'Sun II'//'Sun II')	21	1
DH# 55 X 'Sun II' (CN21997/'Sun II'//'Sun II')	22	1
DH# 45 X 'Sun II' (CN21997/'Sun II'//'Sun II')	33	16
DH# 47 X 'Sun II' (CN21997/'Sun II'//'Sun II')	32	8
DH# 48 X 'Sun II' (CN21997/'Sun II'//'Sun II')	22	4
DH# 62 X 'Sun II' (CN21997/'Sun II'//'Sun II')	9	3
DH# 62 X 'Sun II' (CN21997/'Sun II'//'Sun II')	15	3
Sub total	**154**	**36**
Total	**274**	**62**
DH# 80 X 'Sun II' (CN57130/'Sun II'//'Sun II')	13	6
Grand Total	**287**	**68**

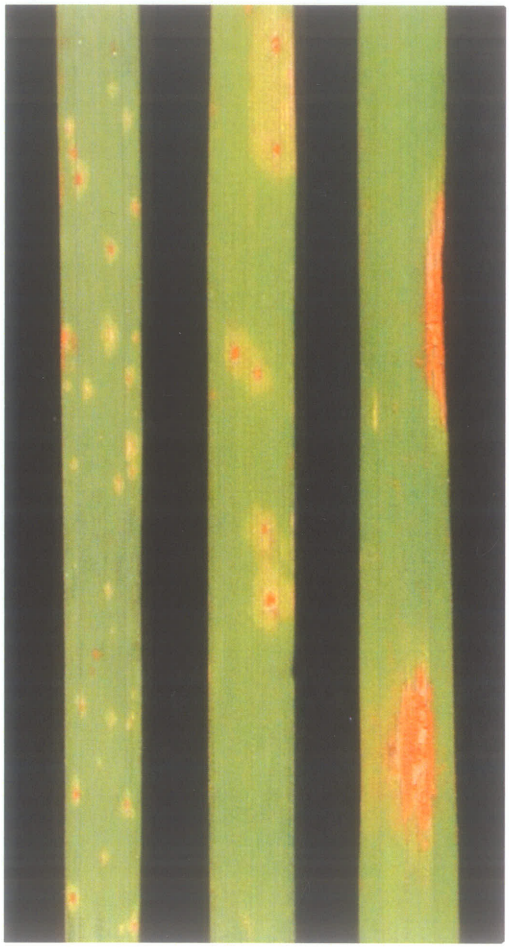
DH = doubled amphiploid

backcrossed to 'Sun II' in 11 different crosses produced 62 BC_1F_1 seeds in total. Twenty six of the BC_1F_1 seeds were obtained when the hexaploid 'Sun II' was used as the female parent, and 36 seeds were produced when octaploid plants (CN21997/'Sun II') were used as the female parent.

When BC_1F_1 seeds from CN21997/'Sun II'//'Sun II' were germinated on petri-plates, only 35% (22/62) germinated. Surprisingly, all seeds that germinated were produced from the crosses when the doubled F_1 plants (CN21997/'Sun II') were used as the female parent. This is probably due to pollen transmission deficiency in the octaploids when used as pollen donors similar to what was reported by McKenzie et al. (1970) and Brown et al. (1986) for low transmission of resistance through pollen. When the 22 BC_1F_1 seedlings were tested with race TJJ (NA67) at the first leaf stage, infection types were higher (IT = 2, 2+, 23) compared to the rust reaction (IT = ; or ;1 or 11+) of the resistant *A. strigosa* parent (CN21997). Of the 22 BC_1F_1 plants tested, 10 were selected for lower infection type (IT = 2 or 2+) and were raised to maturity to produce BC_1F_2 seed. Subsequently, a total of 75 pots with 25 BC_1F_2 seeds per pot were planted and tested with race TJJ (NA67) at the seedling stage. Highly resistant BC_1F_2 seedlings displaying a comparable resistance (IT = ; to 11+) to that of the *A. strigosa* parent (CN21997) (Figure 4) were selected.

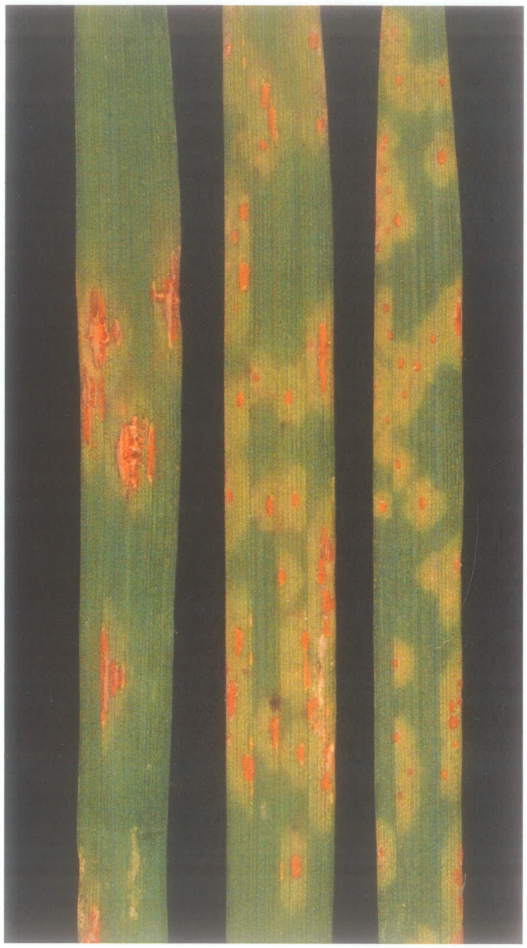
Based on the phenotypic rust reactions at the seedling stage and also based on chromosome counts, 44 highly resistant (IT = ;1- to 11+) BC_1F_2 seedlings were selected and grown to maturity to produce the next generation. BC_1F_3 seedlings from the selected 44 BC_1F_2 plants were grown in 30cm x 25cm x 7cm fibre flats and tested at the seedling stage with race TJJ (NA67). In the BC_1F_3 progeny lines, some seedlings displaying a

high level of resistance and also others with intermediate phenotypic reactions were obtained (Figure 5). A total of 101 highly resistant (IT = ;1- to 11+) BC_1F_3 seedlings were selected, transplanted, and raised to maturity. Although pustule sizes were slightly bigger than the parental material and CN56818 (*Pg6*), it is clear that the resistance in the selected BC_1F_3 seedlings was from the resistant *A. strigosa* parent. The rust reaction of



Pg6 **R** **S**

Figure 4



S **I** **R**

Figure 5

Figure 4. Reactions of CN56818 (*Pg6*), resistant (R), and susceptible (S) BC_1F_2 plants (CN21997/'Sun II'/'Sun II') to race TJJ (NA67).

Figure 5. Reactions of susceptible (S), intermediate (I), and resistant (R) BC_1F_3 plants (CN21997/'Sun II'/'Sun II') to race TJJ (NA67).

the susceptible seedlings (IT = 34 or 4) was similar to that of the susceptible diploid accession (CN56979). Cytological evaluation showed that chromosome counts of the selected BC_1F_2 progeny were from 43-47 (Figure 6), and that of the selected resistant BC_1F_3 seedlings showed a reduced number of chromosomes (42- 45). In 2007, selected BC_1F_4 seeds from CN21997/'Sun II'//'Sun II' were planted out in the field and evaluated with race TJJ (NA67). Several resistant individual plants were selected and BC_1F_5 seeds were harvested. The BC_1F_5 plants are currently being evaluated in the field.

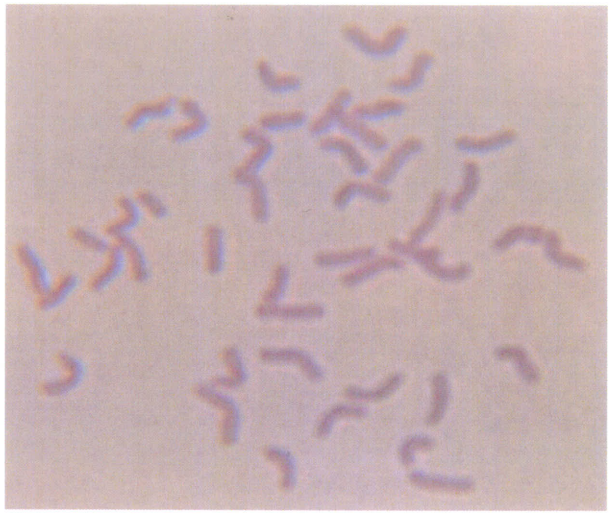


Figure 6. Chromosomes (44) of a resistant BC_1F_2 seedling from CN21997/'Sun II'//'Sun II'.

The other backcross from CN57130/'Sun II'//'Sun II' produced only six viable seeds. As was found in the previous backcross, viable seeds were produced only when the doubled F_1 plants (CN57130/'Sun II') were used as the female parent. It is possibly because of pollen transmission deficiency when octaploids were used pollen donors. Also, it was noted that phenotypic reactions of the resistant BC_1F_1 progeny lines from

CN57130/'Sun II'//'Sun II' had higher infection types (IT = 2, 2+3) than reaction types of the *A. strigosa* parent CN57130 (IT = ; to ;1). The six BC_1F_1 plants were grown to maturity in the greenhouse and BC_1F_2 seeds were harvested. For each of the six BC_1F_2 plants, about 45 seeds per flat were planted in five fibre flats (30cm x 25cm x 7cm) and the seedlings were evaluated with race TJJ (NA67) at the first leaf stage. Seedlings with a high level of resistance (;1$^-$, ;1 or 1$^-$) were identified, transplanted, and grown to maturity to produce BC_1F_3 seeds. Fifteen BC_1F_3 plants were grown to maturity and crossed with the F_1 hybrid from *A. longiglumis* (Cw57)/'Sun II. Some of the BC_1F_3 plants were selfed and BC_1F_4 seeds were produced. BC_1F_2, and BC_1F_3 progeny lines that were not tested in the greenhouse and the BC_1F_4 seeds that were produced have been planted and are being evaluated in the field.

4.5.2 Avena longiglumis (Cw57) *by 'Sun II' cross*

In the interploidy cross between the diploid *A. longiglumis* genotype Cw57 and 'Sun II', 646 florets were pollinated which resulted in 114 embryos (17.7%). Out of the 114 embryos, only 10 F_1 seedlings (1.55%), which were subsequently treated with colchicine, produced fertile octaploid (2n=8x=56) plants. In this interploidy cross, all F_1 plants were produced when Cw57 was used as the female parent and 'Sun II' as the pollen donor. Several reciprocal crosses were attempted using 'Sun II' as the female parent but were unsuccessful, similar to previous results with octaploid by 'Sun II' crosses.

4.6 DISCUSSION

4.6.1 Avena strigosa by hexaploid crosses

In the past, several investigators (Dyck 1966; McKenzie et al. 1971; Rajhathy and Thomas 1974) described the difficulty of transferring resistance from lower ploidy *Avena* species into *A. sativa*. This was once again confirmed in this study because only 16 F_1 plants (0.74%), of which nine produced doubled sectors after colchicine treatment were obtained from 2153 pollinated florets. Production of F_1 plants was made possible because of the application of growth regulators on pollinated florets that helped in the prevention of ovule degeneration and stimulated embryo development (Sidhu et al. 2006). Sectors of F_1 plants produced fertile seeds because colchicine treatment doubled the chromosome number by preventing spindle formation at metaphase (Fankhauser and Humphrey 1952; Taylor 1965).

Amphiploid F_1 plants that were not treated with colchicine were sterile, as expected. The attempt to produce fertile seeds from crosses of amphiploid F_1 plants to hexaploid 'Sun II', hoping that the *A. strigosa* chromosomes would pair with 'Sun II' chromosomes, did not result in production of large number of seeds. In fact, it was only three seeds that were produced. The expectation that a reasonable number of fertile seeds would be produced from the interspecific crosses between *A. strigosa* and 'Sun II' was not unreasonable, because both *A. strigosa* and 'Sun II' have the 'A' genome, though the 'A' genome of *A. strigosa* (A_s) is a bit different. According to Rajhathy and Thomas (1974) the 'A_s' genome in *A. strigosa* and the 'A' genome of *A. sativa* are related. Thomas (1992), citing Nishiyama (1929) reported that genome 'A_s' has some affinity

with one of the genomes of *A. sativa* and indicated that there is a considerable amount of pairing between *A. strigosa* and *A. sativa*, but only partial homology. Partial homology seems to be plausible, because Rajhathy and Morrison (1959) found seven pairs of chromosomes that matched the *A. strigosa* chromosomes in karyotype comparison.

Several investigators (Gauthier and McGinnis 1968; Rajhathy and Thomas 1972; Rajhathy and Thomas 1974) reported that regular meiotic behaviour in hexaploid oat is genetically controlled, similar to the *Ph* gene on chromosome 5B in wheat (Riley and Chapman 1958; Riley 1960). It was reported that oat chromosomes paired preferentially with their homologous partners (Swami and Thomas 1966; Thomas and Rajhathy 1967; Thomas 1970). The preferential nature of chromosome pairing and the genetic control of diploid-like behaviour in oat were also reported by Hutchinson et al. (1983). It was shown (Thomas 1992) that the mechanism of controlling chromosome pairing in *A. sativa* was by restricting alien chromosome pairing with *A. sativa*. However, a genotype of *A. longiglumis* (Cw57) apparently suppresses the genetic control of regular behavior of bivalent chromosome pairing in hexaploid oat and increased the frequency of chromosome pairing between an alien chromosome and its homoeologue from cultivated oat (Rajhathy and Thomas 1972; Thomas et al. 1980; Aung et al. 1996). The suppression of bivalent chromosome pairing in *A. sativa* and the induction of alien chromosome pairing with *A. sativa* is controlled by a single gene (Thomas and Bhatti, 1975). However, Leggett (1977) suggested that more than one chromosome may be involved in the diploid-like chromosome pairing.

In this study, although both parents have the 'A' genome, chromosomes between the diploid *A. strigosa* and the hexaploid 'Sun II' did not pair readily because of the genetic control of chromosome pairing in oat. Thus, the expected seed set by direct crossing of the diploid by hexaploid was poor. Only three seeds were produced from 526 pollination attempts.

Seedlings treated with colchicine at the right stage will produce plants with double the number of chromosomes. From the 16 F_1 amphiploid seedlings 11 were treated with colchicine of which nine were fertile and produced seeds. One colchicine-treated F_1 plant died shortly after the chemical treatment. A certain percent of death of colchicine-treated seedlings is expected because of the harsh treatment and physiological complications (Zamani et al. 2000). One other colchicine-treated F_1 plant grew to full maturity but did not produce seeds. This too should not be a surprise because not all colchicine-treated F_1 plants were expected to produce fertile seeds. In fact, Sidhu et al. (2006) reported 72.4% to 81.3% of chromosome doubling in the crosses that was studied. This is mainly because colchicine treatment only works on dividing cells. It is expected that there will be several cells at different stages of cell division, but it appeared that the dividing cells did not respond to the colchicine treatment with the particular haploid plant. Cells that were not at the appropriate cell division stage were not expected to have their chromosomes doubled, and thus were not expected to produce seeds. In any case, obtaining embryos and then F_1 plants is the major challenge in producing fertile doubled F_1 plants in interspecific crosses. The major constraint in producing amphiploids is growing the embryos rescued on nutrient media. Thus, it is important to work on improving the medium for future work.

In the 1980s, rice, wheat, and barley cultivars were already released by making use of the doubled haploid technique (Han and Shui 1980; DeBuyser et al. 1987; Ho and Jones 1980). In oat, however, the first reports of haploid oat consisted of only six haploids of which five were reported to be of spontaneous origin recovered from twin embryo seeds or from crosses involving aneuploids (Leggett, 1977). The sixth was recovered from anther culture where over 65,000 anthers were cultured (Rines, 1983). Oat haploid production from anther culture was a very inefficient method. Therefore, Rines and Dahleen (1990) used maize pollen and recovered 14 haploid oat plants from 3,300 emasculated florets pollinated with maize pollen. As in wheat x maize pollination where maize chromosomes were eliminated during early embryonic cell divisions (Laurie and Bennett 1986), the same was found in oat x maize pollination (Rines and Dahleen, 1990). The recovery of oat embryos at a very low frequency was due to lack of endosperm development as was observed in wheat (Laurie and Bennett 1988). In wheat, about 20% of embryo development was observed at an early stage, but because of lack of endosperm development only one in 2,440 (0.04%) embryos was large enough to be rescued (Laurie and Bennett 1988).

The difficulty of getting doubled F_1 plants and then seeds in oat was mainly due to lack of chromosome pairing between the diploid *A. strigosa* and the hexaploid *A. sativa* cultivar 'Sun II'. Unless there was chromosome pairing between the two species, the probability of recombination was very low, which makes transferring the resistance from *A. strigosa* and stabilizing it into the 'Sun II' *A. sativa* background a very difficult task. While transferring crown rust resistance from diploid into hexaploid oat, Marshall and Myers (1961) reported the difficulty in the interspecific crosses to be due to meiotic

instability. Brown et al. (1986) and Sharma and Forsberg (1977) explained the difficulty of transferring stem rust resistance from tetraploid into hexaploid oat to be due to a very low rate of alien chromosome transmission through pollen. This is perhaps true in the in the interspecific crosses conducted in this study between *A. strigosa* and *A. sativa.* Also, McKenzie et al. (1971) had observed the disappearance of resistance in interploidy crosses and attributed this to the presence of a suppressor gene in hexaploid oat (*A. sativa*). The concept of a suppressor gene was suggested earlier, and conclusive evidence of suppression of crown rust resistance genes were reported by several investigators (Simons et al. 1978; Wilson and McMullen 1997; Chong and Aung 1996, 1998; and Rines et al. 2007).

The objective of this study was to introgress the resistance gene from *A. strigosa* into an adapted hexaploid 'Sun II' background because introgressed resistance is more stable than the addition lines. Therefore, chromosome pairing between the diploid and the hexaploid parents was essential. If the resistance gene was not introgressed and yet was expressed, it was probably due to chromosome addition (McKenzie et al. 1971). Aung and Thomas (1976) and Thomas (1992) indicated that rust resistance transferred from lower ploidy was due to chromosome addition or substitution, and not due to recombination. This is because chromosome pairing between homoeologous chromosomes of *A. strigosa* and *A. sativa* ('Sun II') were restricted due to the genetic control of chromosome pairing in *A. sativa.* In fact, Leggett and Thomas (1995) reported that recombination between homoeologous partners is rare in *Avenae* because of lack of chromosome homology and control of chromosome pairing. Resistance from addition or substitution lines would not be of immediate use, because the resistance could be lost

since the chromosome with the resistance gene from *A. strigosa* would not pair with its homoeologous chromosome in the hexaploid. Also, transferring whole chromosomes through addition or substitution lines has additional disadvantages of carrying undesirable characteristics from diploid into hexaploid oat. It was also reported that the foreign chromosome is responsible for partial sterility (Dyck and Zillinsky 1963; Thomas 1992). The degree of incorporation of resistance genes from a diploid to a hexaploid chromosome complement is variable. For example, F_1 hybrids involving some *Pc* genes have regular meiosis while others could have irregular meiosis (Dyck and Zillinsky 1963). The desirable resistance transfer is one that incorporates the smallest diploid chromosome segment possible into a hexaploid background.

In this study, cytogenetic analyses showed that F_1 hybrids from *A. strigosa*/*A. sativa* ('Sun II') were octaploids. This means that part of the *A. strigosa* genome has become part of the genome of the hybrid octaploid. In agreement with Leggett (1986), chromosome numbers of the hybrid plants gradually decreased from 49-56 in F_1 to 44-47 in BC_1F_2 to 43-44 in BC_1F_3.

Evaluation of F_1 hybrids with race TJJ (NA67) displayed an intermediate reaction (IT=2, 2+, 23) and improved in subsequent generations. In agreement with the report of Rines et al. (2007), expression of resistance in selected progeny lines improved (IT = ;1-, or ;1 or 1) in subsequent generations (BC_1F_2 and BC_1F_3) compared to the resistance expressed in the octaploid F_1. This could be due to more and more dosage of 'A_s' from *A. strigosa* in subsequent generations when BC_1F_1 were selfed to produce BC_1F_2 and subsequently BC_1F_3. The resistance expressed in the BC_1F_2 and BC_1F_3 seedlings in the *strigosa*/'Sun II' populations must have come from the resistant *A. strigosa* accessions

(CN21997 and CN57130), indicating that resistance was transferred from *A. strigosa* into 'Sun II'. This was as expected if the resistance was transferred because the hexaploid parent 'Sun II' was susceptible to race TJJ (NA67). This resistance from *A. strigosa* is conferred by the single dominant gene as characterized in the inheritance component of this project. If resistance was not transferred from *A. strigosa* into 'Sun II', the octaploids and subsequent progeny lines would all have been susceptible.

4.6.2 Avena longiglumis by hexaploid crosses

The height of the diploid *Avena longiglumis* (Cw57) plants was a problem in conducting Cw57/'Sun II' inter-ploidy crosses in a growth cabinet. The plants were very tall and panicles were very close to the light bank in growth cabinets. This situation compounded the sterility problem and increased the difficulty of obtaining hybrid seeds from this interploidy cross. This was mainly because pollinated florets of the primary tillers were too close to the light source and probably overheated inside the crossing bags. Due to this unfavorable microenvironment, no embryos were initiated to be rescued, and thus no haploid plants were produced. Thus, it was very difficult to obtain fertile seeds from pollinated florets of the primary tillers. Therefore, most primary tillers were removed and only secondary and tertiary tillers were used in the crosses when Cw57 was the female parent.

Fertile F_1 octaploid plants were produced from genotype Cw57 of *A. longiglumis* and the hexaploid crossed to 'Sun II' using the embryo rescue technique. Ten doubled F_1 plants (1.55%) were obtained when the diploid Cw57 was used as the female parent and the hexaploid 'Sun II' was the pollen donor. No embryos and thus no F_1 seedlings were

produced when Cw57 was the pollen donor. It was also noted that under the growth cabinet setting of 16h/8h (day/night) light period and a temperature regime of 20˚C/18˚C (day/night), Cw57 was not a very good pollen donor despite having big anthers while 'Sun II' was an excellent pollen donor. In addition to interspecific cross-incompatibility, the poor pollen donation of Cw57 might have contributed to the poor seed set when Cw57 was used as the male parent. On the other hand, the excellent pollen donating ability of the hexaploid 'Sun II' might have contributed to the success of producing fertile seeds. In fact, it was because of this prolific pollen production and fertility that the octaploid *A. longiglumis* (Cw57)/'Sun II' was able to out yield the hexaploid parent ('Sun II') by 350 Kg/ha (Fritz and Sorrells, 1986).

In the past, limited success has been reported in transferring resistance from lower ploidy into hexaploid oat. Thomas et al. (1980) reported the transfer of powdery mildew resistance from *A. barbata* to *A. sativa* through the use of the genotype Cw57 of *A. longiglumis*. As previously reported, Cw57 increases homoeologous chromosome pairing in crosses with hexaploid oat and can be used in transferring alien genes as was done with *Aegilops speltoides* in wheat (Riley et al. 1968). In oat, gene transfer was the result of a crossover between *A. barbata* and *A. sativa* chromosomes (Thomas et al, 1980). Crown rust resistance gene *Pc94* from *A. strigosa* was transferred into a hexaploid oat using Cw57 to stabilize the resistance in the hexaploid background (Aung et al. 1996). In contrast, Rines et al. (2007) reported stable introgression of an *A. strigosa* rust resistance into 42 chromosome backcross derivatives of *A. strigosa* x *A. sativa* crosses. The opportunity for alien segment chromosome introgression may depend on the location of the desired gene whether or not the particular 'A' chromosome in which it

is located shows any pairing with its 'A' chromosome counterpart or homoeolog, as there is some limited pairing observed in *A. strigosa/A. sativa* F_1 hybrids (Rajhathy and Thomas (1974) and the amount may vary depending on the genotypes involved (Fritz and Sorrells, 1986). In any case, as previous investigators indicated, the use of Cw57 likely is crucial to stabilize the resistance gene from *A. strigosa* into the hexaploid 'Sun II' background. That was why F_1 hybrids from Cw57/'Sun II' were produced. These F_1 hybrids were crossed to selected BC_1F_3 plants from CN57130/'Sun II'//'Sun II' and F_1 hybrid were produced. The selected BC_1F_3 plants were highly resistant to race TJJ (NA67) with 43-44 chromosomes. Several F_1 seeds were produced and will be tested with race TJJ (NA67) and cytological assessment will also be performed by taking root tip samples as previously described.

4.7 CONCLUSION

This study confirmed reports by previous investigators that it was difficult to produce fertile oat seeds from diploid by hexaploid interspecific crosses of oats. The result of this study also showed that in interspecific crosses seeds were produced when the hexaploid *A. sativa* was used a pollen donor. This is likely because of pollen transmission deficiency when diploids or octaploids are used as pollen donors. Introgression of resistance genes from *A. strigosa* into hexaploid oat was difficult because of low frequency of chromosome pairing in *A. strigosa*/'Sun II' (CN21997/'Sun II' and CN57130/'Sun II'). Nevertheless, through the use of an embryo rescue technique, fertile octaploid plants were produced from crosses between two resistant accessions of *A. strigosa* (CN21997 and CN57130) and the hexaploid 'Sun II' (*A. sativa*). The rust reaction of the octaploids to race TJJ (NA67) was intermediate type (IT = 2, 2+, 23). By backcrossing to the hexaploid 'Sun II', BC_1F_1 and BC_1F_2 seedlings with high level of resistance (IT = ;1-, or ;1 or 1) to race TJJ (NA67), plants with 44 and 45 chromosomes were identified. In the following generation (BC_1F_3), seedlings with resistance comparable to *Pg6* (IT = ;1- or ;1 or 1) and with 43 or 44 chromosomes were identified.

The results of this study clearly showed that oat stem rust resistance from *A. strigosa* had been successfully transferred into a near-hexaploid background. This resistance was expressed by low infection types both in the BC_1F_2 and BC_1F_3 progeny lines. To stabilize the resistance transferred into the hexaploid background, the highly resistant BC_1F_3 plants were grown to maturity and BC_1F_4 progeny lines were produced for the *A. strigosa*/'Sun II' population (CN21997/'Sun II'//'Sun II'). The resistant lines

were tested in the field and BC_1F_5 seeds from the highly resistant BC_1F_4 plants were harvested. The BC_1F_5 generation is currently being evaluated in the field. The second population from CN57130/'Sun II'//'Sun II' is at the BC_1F_4 stage. Highly resistant BC_1F_3 progeny lines from CN57130/'Sun II'//'Sun II', BC_1F_3 with 43-44 chromosomes were crossed to the F_1 octaploid that was produced from the hexaploid 'Sun II' by the diploid *A. longiglumis* (Cw57). F_1 hybrid seeds were obtained from Cw57/'Sun II'///CN57130/'Sun II'//'Sun II'. These hybrid seeds will be backcrossed to 'Sun II' four to five times and finally the fifth backcross will be selfed which by then the resistance from *A. strigosa* hopefully would have stabilized in the hexaploid 'Sun II' background. A few BC_1F_4 lines are being evaluated in the field. As Rajhathy and Thomas (1972) reported, Cw57 is used to induce chromosome pairing between the 'A_s' genome of *A. strigosa* and the 'A' genome of *A. sativa* by suppressing bivalent homologous chromosome pairing in 'Sun II'. Lines with resistance to race TJJ (NA67) introgressed into 'Sun II' and stabilized in the hexaploid background will be extremely useful in oat breeding programs for cultivar development.

5.0 IDENTIFICATION OF AN AFLP MOLECULAR MARKER FOR OAT STEM RUST RESISTANCE IN *AVENA STRIGOSA.*

5.1 ABSTRACT

Oat stem rust is an important disease that can effectively be controlled with host genetic resistance. Resistance to the highly virulent and prevalent oat stem rust race TJJ (NA67) was identified in the diploid species *Avena strigosa* Schreb. The objective of this study was to identify molecular markers associated with this resistance using AFLP, a DNA finger-printing technique based on PCR amplification of restriction fragments utilizing genomic DNA. Two F_2 populations were developed by crossing resistant *A. strigosa* accessions CN22000 and CN57130 to a susceptible accession (CN56979). Bulked segregant analysis (BSA) was performed with 256 *Pst*I+*Mse*I primer pair combinations, using DNA bulked from 10 resistant and 10 susceptible F_2 progeny lines. For each of the two populations, candidate markers identified in BSA were tested with 94 F_2 progeny lines that were selected based on the phenotypic reactions of their respective F_3 families to race TJJ (NA67). PCR products were resolved by capillary electrophoresis using an ABI3100. The genetic map produced for the F_2 population (CN56979/CN57130) displayed 12 AFLP markers covering a total distance of 94.7 cM flanking the resistance gene at LOD 2.8. The AFLP markers *P-acg/M-cga-370* and *P-aag/M-ctc-140* flanked the resistance gene at 10.9 cM and 36.2 cM, respectively. No useful AFLP marker was identified for the second F_2 population (CN56979/CN22000). The marker identified in F_2 populations from CN56979/CN57130 may be useful in marker assisted selection

(MAS) for breeders. However, the genetic map needs to be more saturated to identify more closely linked markers.

5.2 INTRODUCTION

Conventional plant breeding requires intensive utilization of resources. Each year, breeders advance several thousand progeny lines to subsequent generations to obtain a few promising lines possessing traits of interest. Any tool that helps to minimize intense utilization of resources and yet achieve similar results is desirable. One such tool is biotechnology, in which molecular markers are used to identify alleles associated with desired traits. A molecular marker is a particular DNA sequence in an organism that is associated with the trait of interest. A DNA marker is very useful in early generation selection so that a minimum number of progeny lines would be advanced to subsequent generations.

DNA markers are useful for several different purposes. In conventional plant breeding, selecting for several traits simultaneously is difficult, thus the use of molecular markers would enhance the selection process. Markers could be used for recurrent parent recovery and they are also advantageous in accelerating the transfer of a desirable gene. For example, in conventional plant breeding 75% of the recurrent parent alleles would be fixed in a BC_2F_1 population, whereas in molecular breeding it is possible to identify BC_2F_1 individuals with 88-91% fixed recurrent parent alleles (Somers, 2004; Somers et al. 2005).

Different kinds of molecular markers have previously been utilized to identify traits of interest. Storage proteins have been used as molecular markers to identify stem rust resistance alleles in wheat (Howes 1986; Dyck et al. 1987; Dhaliwal et al. 1988). In oat using amplified fragment length polymorphism (AFLP), Hoffman et al. (1990)

reported the possibility of determining associations between molecular markers and quantitative trait loci using recombinant inbred lines (RILs). Also, Howes et al. (1992) reported an oat endosperm protein marker for stem rust resistance gene *Pg13* resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Penner et al. (1993a) reported the first molecular marker linked to stem rust resistance gene (*Pg3*) using the random amplified polymorphic DNA (RAPD) finger-printing technique. It was also reported that *Pg9* is linked to avenin as resolved by acid-PAGE (Chong et al. 1994). Since the early reports that showed linkage of molecular markers to resistance genes, other markers linked to a single gene or quantitative trait loci (QTLs) involved in resistance have been identified. For example, QTL markers for flowering time in oat (Locatelli et al. 2006) and for resistance to stem rust (O'Donoughue et al. 1996; Cheng et al. 2002), crown rust (Penner et al. 1993b; Rooney et al. 1994; Bush et al. 1994; Bush and Wise, 1998; Zhu and Kaeppler, 2003; Chong et al. 2004; Wight et al. 2004; Chen et al. 2006), and BYDV (Zhu et al. 2003) have been identified.

Molecular markers are useful in identifying several genes that could be pyramided together into a common background which otherwise is difficult to achieve in conventional plant breeding. It is even more difficult to pyramid several rust resistance genes in one background by conventional rust testing due to lack of races with appropriate virulence combination. Overcoming similar problems and through the use of molecular markers, major resistance genes against rice blast (*Pyricularia grisea*) were pyramided into a rice cultivar (Tabien et al, 2000; Hittalmani et al, 2000). Singh et al. (2001) also successfully pyramided three bacterial-blight (*Xanthomonas orysae*) resistance genes in rice.

There are several DNA finger-printing techniques available to identify molecular markers. The selection of a specific DNA finger-printing technique depends on factors such as mode of inheritance (co-dominant/dominant), informativeness (how much information is obtained), the speed at which results are obtained, cost, reliability, and reproducibility. Mueller and Wolfenbarger (1999) suggested that the choice depends on the project, finances, the genetic resolution required, and the technical support available. In this regard, one of the finger-printing techniques available is amplified fragment length polymorphism (AFLP), where each fragment represents a unique genetic locus. The information obtained using AFLP, its reproducibility, ease of use, and cost efficiency is as good as or superior to other molecular markers (Mueller and Wolfenbarger, 1999). An AFLP marker segregates in a Mendelian fashion and large numbers of polymorphisms can be generated. However, as a dominant marker, it cannot differentiate homozygous loci from heterozygous loci. An AFLP finger-printing technique does not involve radioisotopes and it does not require prior knowledge of sequencing and/or cloning, because the adapter sequences flanking genomic DNA sequences serve as primer binding sites. Although partially degraded DNA could be used for an AFLP finger-printing, the use of high quality genomic DNA is essential for optimum results.

The AFLP DNA finger-printing technique is based on polymerase chain reaction (PCR), which is amplification of restriction fragments utilizing genomic DNA (Vos et al. 1995). Although the application of PCR-based finger-printing is limited due to sensitivity to reaction conditions, DNA quality, and PCR temperature profiles; the AFLP technique is robust and reliable. It combines the reliability of restriction fragment length polymorphism (RFLP) and the strength of sensitive PCR amplification together with the

high resolution of polyacrylamide gel electrophoresis. It also employs stringent reaction conditions for primer annealing (Vos et al. 1995). As a result, AFLP can detect a single nucleotide change and differentiate several amplified DNA fragments without prior knowledge of the sequence (Yu and Wise, 2000). The objective of this study was to identify molecular markers associated with oat stem rust resistance to race TJJ (NA67) in the diploid species *Avena strigosa* Schreb. using the AFLP finger-printing technique.

5.3 MATERIALS AND METHODS

5.3.1 Plant materials, rust phenotyping, and DNA extraction

Two F_2 populations were produced by crossing the resistant *A. strigosa* accessions CN22000 and CN57130 to a susceptible *A. strigosa* accession (CN56979). The two F_2 populations were tested with stem rust race TJJ (NA67) at the seedling stage and rated to obtain phenotypic rust reaction data for individual F_2 plants. The F_2 plants were grown to maturity to produce F_3 seeds. Seedlings of F_3 families were again tested with race TJJ (NA67) at the first leaf stage, where homozygous resistant, segregating, and homozygous susceptible families were identified. Since AFLP markers are dominant markers, obtaining the phenotypic information of the F_3 is required to classify the families into the three classes (homozygous resistant, segregating, and homozygous susceptible) which is important for subsequent steps in identifying molecular markers. The F_3 data is also important to confirm the rust reaction of the individual F_2 plants.

Two pieces of leaf tissue (about 2 cm each) were collected from the youngest leaves of four-week old F_2 plants and put in 1.2 ml collection tubes containing 2 mm beads (200μl). When a plate of 96 samples was collected, the plate was put in -80°C freezer. A second sample of two or three leaf pieces (4-5 cm) were collected in perforated envelopes and the envelopes were immediately immersed into liquid nitrogen (-196°C). Samples were lyophilized over two nights in a vacuum freeze dryer and then stored in -80°C.

Lyophilized leaf issues in collection tubes were ground to a fine powder by shaking the samples for ten minutes with a desk top paint shaker (Retsch MM 301, Fisher Scientific). Genomic DNA was extracted using a Qiagen DNeasy 96 plant kit (Qiagen Inc., Mississauga, Ontario, Canada) according to manufacturer's instructions, with a minor modification (the second elution was performed with 50 µl of AE buffer instead of 100 µl). A heavy duty centrifuge (Sigma 4-15C, C-FRID-021) was used during the DNA extraction process. Genomic DNA concentration was determined by using Hoechst 33258 stain and quantified with Ascent software using a Microtiter Plate Fluorometer (DYNEX Technology, Thermo Labsystems, Helsinki, Finland). Stock DNA was diluted to a working DNA concentration (25 ng/µl) by adding 0.1X TE (pH 7.5) buffer. PCR was performed using a Thermal Cycler, PTC-200 (DYAD DISCIPLE, MJ Research Incorporated).

5.3.2 Digestion, ligation, and amplification

Genomic DNA digestion, ligation, amplification, and gel analysis was performed as described by Vos et al. (1995). Ten µl of template genomic DNA (25 ng/µl) was simultaneously digested with two restriction endonucleases (the rare-cutter enzyme *Pst*I with 6-bp recognition site [CTGCAG] and the frequent-cutter *Mse*I with 4-bp recognition site [TTAA]) at 37°C for two hours, and then incubated for 15 minutes at 70°C. Cohesive ends of genomic DNA fragments were ligated to *Pst*I and *Mse*I adapter pairs (Table 15) by adding 24 µl of adapter/ligation solution together with 1 µl of T4 DNA ligase per sample and incubating the mixture at room temperature for two hours.

Preamplification, the first step of amplification, of adapter-ligated DNA fragments was performed using one selective base-pair primer, nucleotide 'A' for the *Pst*I primer, and nucleotide 'C' for the *Mse*I primer. A mixture of 40 µl pre-amp mix (1M Tris-HCl, 3M KCl, 1M $MgCl_2$, and water), 5 µl of 10X PCR buffer, 0.05 µl of 20 units/µl themostable *taq* DNA polymerase, and 0.95 µl of water was added to 5 µl of 1:10 diluted and ligated genomic DNA. The mixture was amplified for 20 cycles at 94°C for 30s (denaturing), 56°C for 60s (annealing), and 72°C for 60s (extension).

Table 15. *Pst*I and *Mse*I primers' and adapters' sequences.

Adapter or primer	Core sequence
*Pst*I adapter (Pa1.1)	5'-GACTGCGTAGGTGCA-3'
*Pst*I adapter (Pa1.2)	5'-CCTACGCAGTCTACGAG-3'
*Mse*I adapter (Ma1.1)	5'-GACGATGAGTCCTGAG-3'
*Mse*I adapter (Ma1.2)	5'-TACTCAGGACTCAT-3'
*Pst*I preamplification primer	5'-GACTGCGTAGGTGCAGA-3'
*Mse*I preamplification primer	5'-GATGAGTCCTGAGTAAC-3'
*Pst*I selective primer	5'-GACTGCGTAGGTGCAGANN-3'
*Mse*I selective primer	5'-GATGAGTCCTGAGTAACNN-3'

In selective amplification, *Pst*I and *Mse*I primer pairs with three selective bases (Table 16) were used to amplify the diluted and pre-amplified PCR products that were used as a template At this stage, the *Pst*I primer used was ^{32}P-γ-ATP labelled *Pst*I. A mixture of 1 µl of 10X PCR buffer, 1.5 µl of 25mM $Mgcl_2$, 0.8 µl of 10mM dNTP, 0.03µl of *taq* DNA polymerase, 0.4 µl of *Pst*I (1pmol/µl) primer, and 0.25 µl of *Mse*I (10pmol/µl) primer with 1 to 3 selective nucleotides was prepared and added to 4 µl of

1:50 diluted pre-amplified DNA. The mixture was amplified for 13 cycles at 94°C for 30s, 65°C for 30s, and 72°C for 60s by lowering annealing temperature by 0.7°C each cycle followed by 23 cycles at 94°C for 30s, 56°C for 30s, and 72°C for 60s.

Table 16. Selective three base-pair *Pst*I and *Mse*I primers used for screening.

*Pst*I core sequence selective bases	*Mse*I core sequence selective bases
AAA	CAA
AAC	CAC
AAG	CAG
AAT	CAT
ACA	CCA
ACC	CCC
ACG	CCG
ACT	CCT
AGA	CGA
AGC	CGC
AGG	CGG
AGT	CGT
ATA	CTA
ATC	CTC
ATG	CTG
ATT	CTT

5.3.3 Bulked Segregant Analysis (BSA) and gel analysis

PCR products from three different colors of fluorescent labelled primer tags (blue, green, and yellow for FAM, HEX, and NED, respectively) were pooled together (2.5 µl FAM, 3.5 µl HEX and 2.5 µl NED). From the pooled PCR product, 1.6 µl was transferred into a new plate to which 5 µl of HiDi Formamide: Gene Scan Rox 500 (1000:40) was added. Samples were centrifuged and denatured at 95°C for 10 minutes

and immediately put in ice to chill. Analyses were performed using an ABI 3100 Genetic Analyser (Applied Biosystems, Foster City CA) as recommended by the manufacturer. Determination of the banding pattern in the automated genetic analyser was performed by capillary electrophoresis that uses dye-labelled primers during selective amplification (Hayashi et al. 2005). Amplified fragment sizes were estimated with Genescan-LIZ software version 3.7, and data was converted into gel images using a Genographer version 1.6.

Bulk segregant analysis (BSA) requires a segregating population for the trait of interest. Following procedures described by Michelmore et al. (1991), BSA was performed with 256 *Pst*I and *Mse*I primer pair combinations on the two parents and the two bulks. To prepare the bulks, equal amount of DNA from 10 homozygous resistant F_2 plants were combined to make the resistant bulk. The susceptible bulk was made by combining equal amount of DNA from 10 homozygous susceptible F_2 plants. Core reagents to perform BSA were prepared using restriction enzymes *PstI* and *Mse*I (New England Biolabs Ltd. Ontario, Canada). Restriction digests of genomic DNA, *Pst*I and *Mse*I adapter-ligation solution, ligation of adapters, pre-amplification reactions, selective amplification, and resolving PCR products by capillary electrophoresis were performed as described above. After an ABI analyses and when candidate markers were identified, the markers were tested with the two parents, the two bulks, and 10 resistant and 10 susceptible individual F_2 plants. Putative markers were confirmed by using a mapping population of 94 selected F_2 progeny lines that were selected based on the phenotypic reactions of their respective F_3 families to oat stem rust race TJJ (NA67). A genetic map was also produced using JoinMap (version 4.0).

5.4 RESULTS

5.4.1 Bulked Segregant Analysis

Parental materials as well as selected F_2 plants from CN56979/CN57130 that made up the resistant and susceptible bulks were screened for AFLP markers. When screened with 256 *Pst*I+*Mse*I primer pairs, polymorphism was observed between the resistant and susceptible parents as well as between the resistant and susceptible bulks. On average, each primer pair produced 40 fragments for the parents with 11 polymorphic bands and 50 fragments for the bulks with 15 polymorphic bands. A total of 145 polymorphic bands (97 associated with the resistant parent and the resistant bulk; and 48 associated with the susceptible parent and the susceptible bulk) were identified. Fifty three *Pst*I+*Mse*I primer pairs that included all 145 fragments were tested with 24 samples (resistant and susceptible parents, resistant and the susceptible bulks, and 10 resistant and 10 susceptible F_2 individuals). The 10 resistant and 10 susceptible F_2 individuals were selected for homozygosity based on the rust reactions of their respective F_3 families. Some of the polymorphic bands present in the parents and in the bulks when screened with the 53 *Pst*I+*Mse*I primer pairs in BSA were absent when tested with the 24 samples. Twenty *Pst*I+*Mse*I primer pairs produced 32 markers associated with resistance or susceptibility by amplifying different fragments.

The *P-acg+M-cga-370* primer pair resulted in a very strong band at 370 base-pairs (bp) in the resistant parent and the resistant bulk. The band was absent in the susceptible parent and the susceptible bulk and was present in the resistant parent,

resistant bulk, and in nine of the 10 resistant F_2 individuals (Figure 7). The band was not present in the susceptible F_2 individuals except one.

The F_2 mapping population from CN56979/CN57130 was analyzed after BSA analysis was performed (Figure 8). When *P-acg+M-cga-370* primer pair was tested with the mapping population of 94 F_2 individuals (24 homozygous resistant, 46 segregating, and 24 homozygous susceptible, based on their F_3 rust reactions), the band at 370-bp was present in 71 F_2 individuals and the resistant parent (lane 97). This band was absent in 22 F_2 individuals and the susceptible parent (lane 96). The sample in lane 76, which is a heterozygous resistant line (based on the F_3 rust reaction to race TJJ [NA67]), failed to generate fragments and as a result it was excluded from the analysis.

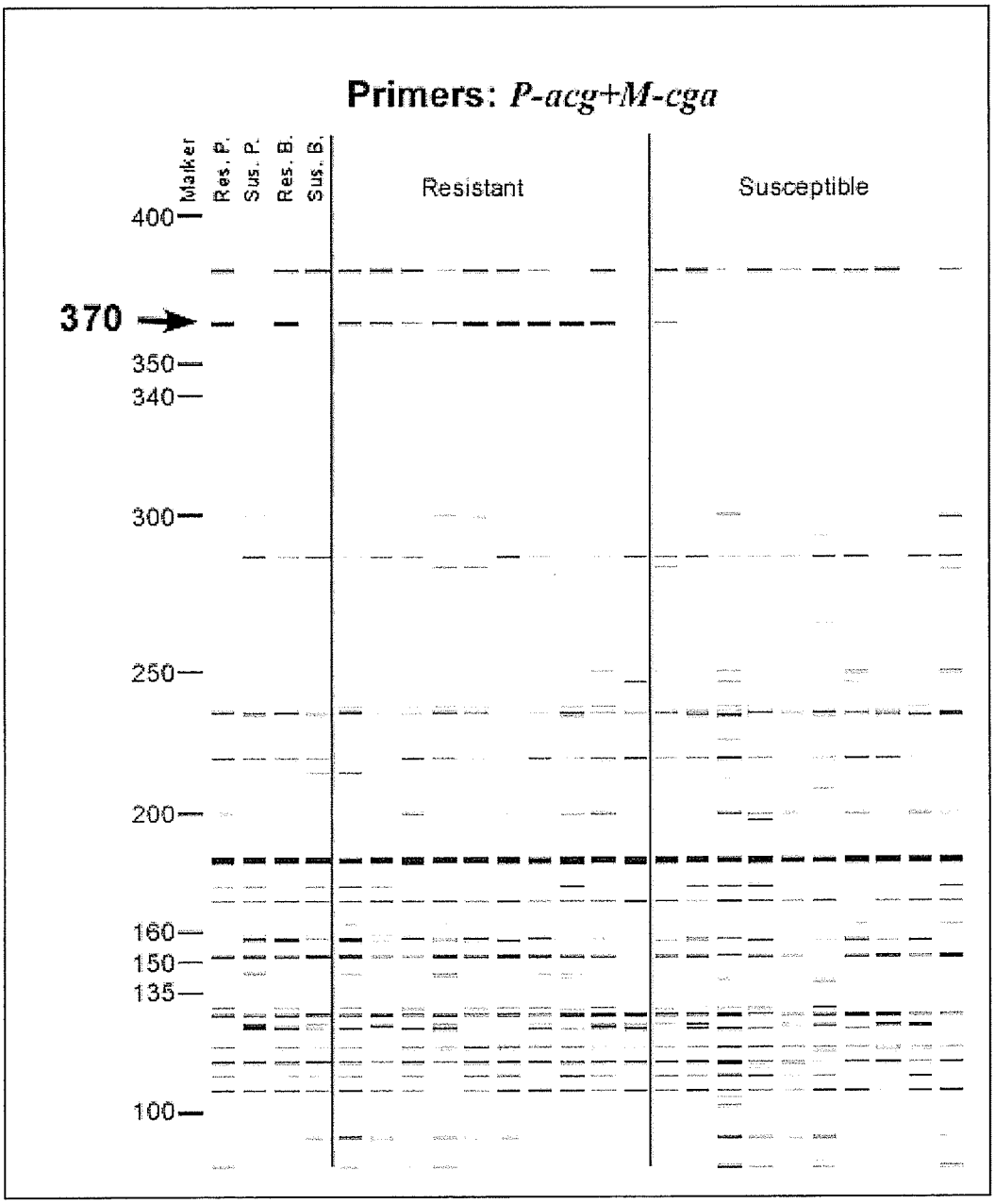


Figure 7. ABI gel analysis of segregating F2 population from CN56979/CN57130. Lane one (Marker) – ladder, lane 2 (Res. P.) – resistant parent, lane 3 (Sus. P.) – susceptible parent, lane 4 (Res. B.) – resistant bulk, lane 5 (Sus. B.) – susceptible bulk, lanes 6-15 resistant F_2 individuals, and lanes 16-25 susceptible F_2 individuals. An AFLP marker at 370-bp identified with *P-acg+M-cga* primer pair combination.

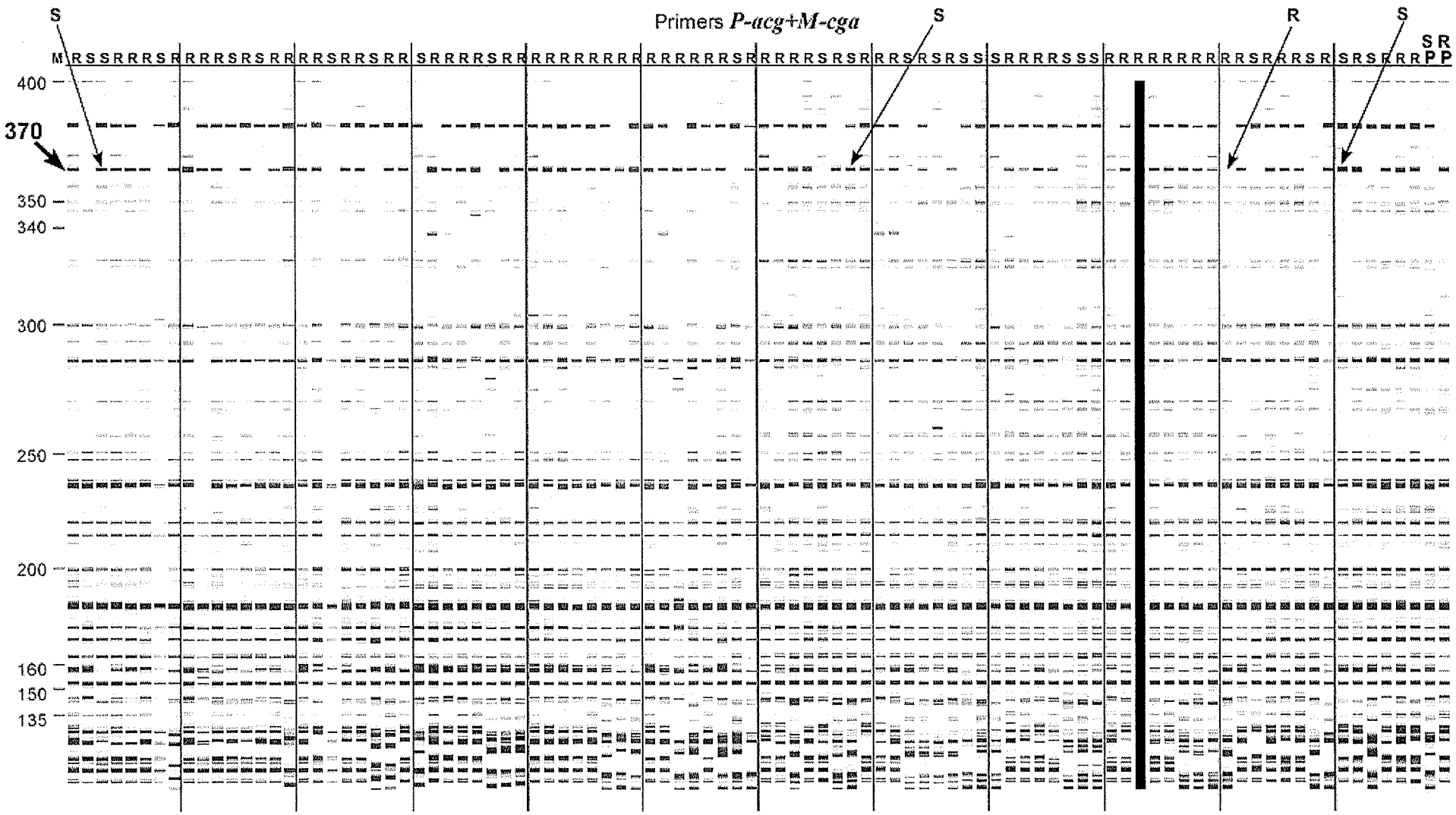


Figure 8. Gel analysis for F_2 population from CN56979/CN57130. Lane one (M) is the ladder, lanes 2-95 are resistant (R) and susceptible (S) F_2 individuals, lanes 96 (SP) and 97 (RP) are the susceptible and resistant parents, respectively. An AFLP marker at 370-bp identified with *P-acg+M-cga* primer pair combination.

A genetic map was produced for the F_2 population from CN56979/CN57130 using JoinMap (version 4.0). The genetic map displayed 12 AFLP markers flanking the resistance gene (*Sr_57130*), four of them on one side of the resistance gene and the rest on the other side (Figure 9). The closest marker (*P-acg+M-cga-370*) identified was 10.9 centi-Morgan (cM) from the resistance gene. The second closest marker (*P-acc+M-cgg-230*) was 16.8 cM away. Both markers are on the same side of the resistance gene (*Sr_57130*). The marker *P-aag+M-ctc-140* is on the other side of the resistance gene and 36.2 cM away. Two clusters of markers that were 2-3 cM apart from each other were found on both sides, but further away from the resistance gene. The rest of the markers on both sides of the resistance gene (*Sr_57130*) were further away from it with genetic distances of 6-7 cM between them (Figure 9).

Bulk segregant analysis was performed for a second F_2 population (CN56979/CN22000). This population was also screened for AFLP markers using the 256 *Pst*I+*Mse*I primer pair combinations. Fifty seven markers with 51 *Pst*I+*Mse*I primer pairs showed polymorphism between the resistant and the susceptible parents and also between the resistant and the susceptible bulks from 10 resistant and 10 susceptible F_2 individuals. These primer pairs were tested with the mapping population of 94 F_2 individuals (24 homozygous resistant, 46 segregating, and 24 homozygous susceptible progeny lines). However, no linked AFLP marker was identified in this population. The marker (*P-acg+M-cga-370)* identified in the first mapping population (CN56979/CN57130) was absent in both the resistant and the susceptible F_2 individuals in this population.

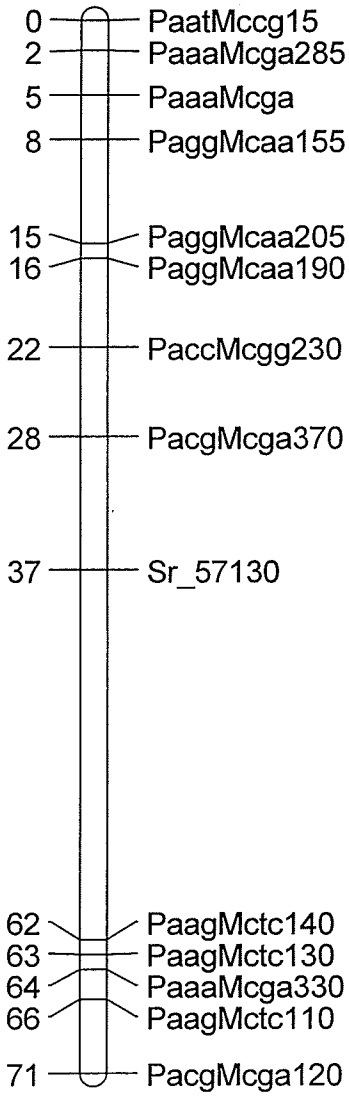


Figure 9. Genetic map of F_2 population from CN56979/CN57130

5.5 DISCUSSION

Avena strigosa is a good source of disease resistance to both stem and crown rust (Zillinsky and Derick, 1960; Dyck and Zillinsky, 1963; Dyck, 1966; McKenzie et al. 1971; Aung et al. 1977). Despite the difficulty of transferring the resistance from a diploid to a hexaploid background, crown rust resistance genes have been successfully transferred from *A. strigosa* into cultivated oat (Zillinsky and Derick 1960; Sadanaga and Simons 1960; Aung et al. 1996; Rines et al. 2007). Rothman (1984) also reported that oat stem rust resistance gene *Pg6* was transferred from diploid *A. strigosa* into a hexaploid background, resulting in the resistant cultivar 'Delredsa'. In conventional plant breeding, it is not only difficult and laborious to transfer resistance from a diploid species into a hexaploid, it is also difficult to know whether or not the resistance gene has been incorporated into the hexaploid background. In addition, even if the resistance gene is incorporated, by the time a resistant cultivar is released, the fungus might have already mutated and a new pathotype with increased virulence may have developed. This is particularly true with crown rust, where the average life span of a resistance gene is about five years. Thus, selection at early generation would help not only to reduce the materials to be advanced to the next generation but also to shorten the time required to release a resistant variety. In this regard, molecular markers can play a significant role in marker assisted selection (MAS). Therefore, it is important to identify molecular markers associated with resistance genes. An efficient method of identifying molecular markers is required, and one such method that helps increase the efficiency of identifying molecular marker is bulked segregant analysis (BSA).

An AFLP marker associated with stem rust resistance gene to race TJJ (NA67) was identified using BSA for the first F_2 population (CN56979/CN57130). This marker was present and expressed as a strong band at 370-bp both in the homozygous resistant and heterozygous individuals, except for one F_2 individual when 10 resistant and 10 susceptible plants were tested along with the parents. The marker, if indeed associated with the resistance gene, will be expected to be expressed both in homozygous and heterozygous individuals because AFLP is a dominant marker and does not differentiate homozygotes from heterozygotes. In the mapping population of the 94 selected F_2 individuals, the marker was also present as a strong band in the resistant parent and resistant F_2 progeny lines except in one line (lane 82 in Figure 8). The band was absent in the susceptible parent and susceptible F_2 individuals, except for three F_2 plants (lanes 4, 56, and 90 in Figure 8) where the band was present. Although highly unlikely, technical error could not be ruled out as was reported by Chong et al. (2004) even with a very tightly linked marker (0.9 cM to 3.4 cM) associated with crown rust resistance gene *Pc94,* the marker was amplified in one susceptible F_2 individual that does not possess *Pc94.* In any case the result generally agreed with the phenotypic rust reaction of the 94 F_2 individuals when tested with race TJJ (NA67).

The absence of the band in one resistant F_2 individual and its presence in the three susceptible F_2 individuals is most likely explained by a low association (10.9 cM) between the marker and the resistance gene. It is likely that crossover between the marker and the resistance gene took place, resulting in recombinants. As the marker is fairly a large fragment (370-bp), there might have been mutation, insertion, or deletion of bases within the marker itself resulting in no amplification and thus no band in the one

resistant F_2 individual where the expected band is missing. The same could be said for the three susceptible F_2 individuals where the band was present.

In the second F_2 population (CN56979/CN22000), although CN22000 was resistant to race TJJ (NA67), the AFLP marker (*P-acg+M-cga-370*) identified in the first F_2 population (CN56979/CN57130) that was loosely linked to the resistance gene (10.9 cM) was not observed in this population. This suggests that the DNA sequence of the second resistant parent (CN22000) is different from that of the other resistant *A. strigosa* accession (CN57130). The result in the characterization part (chapter 3.0) showed that a single dominant gene confers resistance to race TJJ (NA67) in both accessions. In the multi-pathotype allelism tests, it was found that the resistance gene in CN57130 is different from the gene in CN22000. It is possible that the differences in DNA sequences of the two resistant accessions could have once again resulted from mutation, insertion, or deletion of bases. This means that there would have been mismatch of the marker and the DNA sequence in the second accession. Therefore, there will be no amplification in the second population using the AFLP marker (*P-acg+M-cga-370*) identified in the first population. In addition, even though the marker linked to the resistance gene was expected to be present in progeny lines that possess the resistance gene, molecular markers are not always present in all backgrounds. Different backgrounds could react differently as reported by Wight et al. (1994), where using RAPD they found that the day-length insensitivity marker found in one backcross was not found in a different background.

Linkage maps have been developed for diploid by diploid crosses using RFLP (O'Donoughue et al. 1992; Rayapati et al. 1994; Van Deynze et al. 1995). AFLP is also a very useful tool in developing genetic maps. Using AFLP, a genetic map for the F_2 population of CN56979/CN57130 was produced (Figure 9). At LOD 2.8, the map showed that the closest marker (*P-acg+M-cga-370*) was 10.9 cM from the resistance gene and two cluster of markers on both sides of the gene. However, at LOD 3.0 there was no linkage between one cluster and the resistance gene. This was detected because AFLP is a very sensitive and highly reproducible finger-printing technique. AFLP is as sensitive as RFLP and RAPD in detecting markers that are closer than 25 cM, and even loci further away from the target area by up to 36.2 cM, but at a lower frequency (Michelmore, 1991).

5.5.1 Bulked Segregant Analysis (BSA)

Selective primer pairs are expected to amplify and produce different fragments of the genomic DNA. In the mapping population of 94 F_2 individuals (CN56979/CN57130), the fragment was amplified in 69 of the 70 resistant F_2 individuals. Also, the band was not present in 21 of the 24 susceptible F_2 individuals. This suggests that the marker, located 10.9 cM from the resistance gene, is associated with the resistant gene in this population.

In identifying AFLP markers associated with stem rust resistance for the F_2 population from CN56979/CN57130, several fragments were produced. This is in agreement with the result of several studies where it was reported that AFLP is the most efficient in generating many markers linked to target genes (Zhu et al. 1998; Xu et al.

1999; and Brandshaw et al. 1998). Since AFLP is suitable for analyses of relatedness (Fu et al. 2004) and parentage and mating frequency (Mueller and Wolfenbarger, 1999), the presence of the unique band at 370-bp in the resistant parent and the resistant bulk and its absence in the susceptible plants indicates relatedness of the F_2 plants where the band was present. The probability of an unrelated locus between bulks from 10 individuals to be polymorphic was very low, 2×10^{-6}, (Michelmore et al. 1991). Hence, the AFLP marker *P-acg+M-cga-370* is highly likely to be associated with the resistance gene.

When linkage analysis was performed on the F_2 population from CN56979/CN57130 using JoinMap, the resulting genetic map at LOD 2.8 showed 12 markers distributed on both sides of the resistance gene. The markers covered a total distance of 94.7 cM, with two clusters on each side of the resistance gene (Figure 9). Since there are gaps between the markers and there is a lack of closely associated markers flanking the resistance gene, additional markers are needed to saturate the genetic map to find more closely linked markers. In this study, the restriction enzymes used were *Mse*I and *Pst*I. Replacing *Mse*I or *Pst*I with *EcoR*I could probably help in identifying additional AFLP markers because *EcoR*I has a different cutting site (GAATTC) from *Mse*I (TTAA) and *Pst*I (CTGCAG). The use of other DNA finger-printing techniques such as microsatellites needs to be considered to identify additional markers, not just to saturate the map but to identify markers that are tightly linked to the resistance gene. In barley, AFLP and RFLP markers complemented each other to saturate the genetic map and fill the gaps (Becker and Vos 1995). The same approach could be applied for this population as well. The merging of markers from different finger-printing techniques

will increase the total map length (Becker and Vos 1995). The AFLP marker identified could be isolated, sequenced, and a probe could be produced.

5.6 CONCLUSION

Two F_2 populations from CN56979/CN22000 and CN56979/CN57130 were analyzed using AFLP. In the F_2 populations from CN56979/CN57130, 12 AFLP markers flanking the resistance gene and covering 94.7 cM were identified at LOD 2.8. The closest AFLP marker (*P-acg+M-cga-370*) was 10.9 cM from the resistance gene. The second closest marker (*P-acc+M-cgg-230*) was on the same side as the first marker, and was 16.8 cM from the resistance gene. The marker on the other side of the resistance gene (*P-aag+M-ctc-140*) was 36.2 cM away. For the F_2 population of CN56979/CN57130, a genetic map that has gaps between the resistance gene and the markers and also between the markers was produced. Although the markers were not closely associated with the resistance gene, the two markers (*P-acg+M-cga-370* and *P-acg+M-ctc-140*) flanked the resistance gene (*Sr_57130*) at LOD 2.8. At LOD 3.0, there is no linkage between the cluster of four markers and the resistance gene. These four markers could be in a different linkage group. Additional work needs to be done to saturate the genetic map and decrease the distance between the resistance gene and the markers. The gaps could be saturated using other restriction enzymes and/or other finger-printing techniques so that more markers closely linked to the resistance gene could be identified. Having access to different molecular markers that are tightly linked to the resistance gene is very useful. The AFLP marker identified in this study (*P-acg+M-cga-370*) may be very useful in identifying lines possessing the resistance from CN57130. In the second population, CN56979/CN22000, no useful marker was identified.

6.0 GENERAL DISCUSSION

In this study, genetic analyses of the data from F_2 populations and F_3 families from the seven *Avena strigosa* accessions crossed to a susceptible accession showed that a single dominant gene confers resistance to race TJJ in all accessions. It is also confirmed with the segregating progeny lines from the BC_1F_2 families, which fit a 3:1 (resistant to susceptible) ratio. The presence of a single resistance gene in the seven accessions is not surprising, because several thousand oat lines of all ploidy levels have been evaluated in the past several years for stem rust resistance and very few resistant lines have been identified. This is also true both for wheat and barley. Although effective resistance genes (alleles of *Pg6*) were identified in the resistant *A. stigosa* accessions, the result of this study clearly shows that diversity in *A. strigosa*, in terms of stem rust resistance, is not available. A possible explanation of this is that five out of the seven accessions originated from Spain. Perhaps more diploid lines collected from different parts of oat growing regions and the centre of diversity need to be evaluated to find a new source of resistance.

In the analyses of the segregating F_3 families in five crosses, the result fit the expected three resistant to one susceptible ratio, but not in the remaining two crosses. The lack of fit to the expected ratio in the crosses could be due to the presence of a modifier gene that might be present in the backgrounds of these two accessions. The presence of a modifier gene influences the actions of the resistance gene, thereby affecting the resistant to susceptible ratio.

In the allelism tests of crosses between the seven accessions, there was no segregation of plants into resistant and susceptible categories when F_2 populations were tested with TJJ. This means that the resistance in each of the seven accessions is at the same locus. Also, analyses of the F_2 populations from allelism tests of the seven accessions with accession CN56818 (a source of *Pg6*) indicated that the resistance in each of the seven accessions was at the *Pg6* locus. In addition, results of multi-pathotype tests with 23 oat stem rust races indicated that there are five alleles of *Pg6* at the *Pg6* locus. To confirm that the genes in the seven accessions were independent, it is required to test a much larger population size to find recombinants and determine the linkage distance. For example, if the genetic distance between two independent genes was 5 cM, 2.5 cM, or 1 cM, then 1,600, 6,400, or 40,000 seedlings, respectively, are required to be tested to find a single susceptible plant. An even larger population size is required if the distance is less than 1 cM. Although it is possible to test larger numbers, it is not practical. Therefore, if the genes are that close they could be treated as one unit for all practical purposes.

The ultimate goal of identifying an effective oat stem rust resistance gene against a virulent race in the diploid *A. strigosa* is to transfer the resistance into an adapted hexaploid background so that it will be used for oat cultivar development. Unlike in wheat, hybrid seeds are not easily produced in oat, even from straight crosses within the same species let alone from interspecific crosses. In the process of transferring resistance from *A. strigosa* into adapted oat 'Sun II', obtaining doubled F_1 amphihaploid plants was a real challenge. This is apparent considering the fact that very few haploid/doubled haploid oat plants were reported in the early 1980s, whereas during the same period,

varieties of wheat, rice, and barley were released using the doubled haploid technique. In this study, the embryo rescue technique was used to help transfer the resistance from two of the resistant *A. strigosa* accessions (CN21997 and CN57130) into a hexaploid *A. sativa* background. However, only 16 amphiploid F_1 plants, which is less than one percent (0.74%) of pollinated florets, were produced. The low success rate from crosses of the seven accessions to 'Sun II' confirms how difficult it is to produce fertile seeds from interspecific crosses. The major problem in generating F_1 amphiploids is the lack of interspecific chromosome pairing to induce embryo production. In the initial interspecific crosses in this study, embryos were produced mainly when the diploid *A. strigosa* was used as the female parent. Cross-incompatibility due to low transmission of gametes was evident when the diploid was used as pollen donor probably due to pollen deficiency. Therefore, hexaploids were used as male donor for the initial crosses. As part of the embryo rescue procedure in interspecific crosses, growth hormones (GA_3 and 2,4-D) were applied to pollinated florets to stimulate ovary enlargement and help the stigma to be more receptive to pollination. Concentration of the hormones was 100 mg/L, which was working well for wheat but not so well for oat. The rate and the number of days of growth hormone application, and also probably applying different growth regulators (dicamba as is used in wheat), might need to be considered to attempt to produce more embryos.

The other hurdle after embryos were produced was regenerating F_1 amphiploids on the nutrient media. The 346 embryos (16.1%) produced in the interspecific crosses resulted only in 16 F_1 amphiploid plants (0.74%). There was a significant reduction from production of embryos to regenerating F_1 amphiploids on a nutrient media. The media

used in this study was B-5 (agar+sucrose+Gamborg*s B-5) media. Obviously, the nutrient media for regenerating rescued embryos is not optimal, and needs to be improved by either changing the proportions of the B-5 components, trying other media such as Murashige and Skoog (MS), or finding a better medium to grow the rescued embryos on.

One interesting observation in this experiment was that octaploids displayed intermediate or susceptible reactions (IT = 2 or 2^+ or 23) when tested with race TJJ. This could be due to the dilution effect, where the resistance gene is on one of the 14 chromosomes in diploids whereas it is on one of the 56 chromosomes in the octaploids. The effect of the resistance gene might have been diluted in the octaploids and thus is intermediate or not displayed. However, it was found that some of the seedlings displayed a high level of resistance (IT = ;1-, or ;1 or 1) after backcrossing the octaploids to the hexaploid 'Sun II' and selfing the resulting BC_1F_1 to produce BC_1F_2 and BC_1F_3 progeny lines. These seedlings were selected and advanced to the BC_1F_5 for CN21997/'Sun II, and to the BC_1F_4 for CN57130/'Sun II'. In all the backcrossed generations, highly resistant lines were identified confirming that the resistance gene from *A. strigosa* is transferred into the 'Sun II' background.

The attempt to produce fertile seeds from crosses between the F_1 amphihaploids and the hexaploid 'Sun II', hoping that the *A. strigosa* chromosomes would pair with 'Sun II' chromosomes, did not result in a large number of fertile seeds (only three seeds were produced). This is probably due to partial chromosome homology between the 'A_s' and 'A' genomes, where pairing occurs at a very low frequency. To induce random homoeologous chromosome pairing, *A. longiglumis* (Cw57), which suppresses preferential paring of homologous chromosomes in *A. sativa*, was used. Chromosome

pairing of the resistant *A. strigosa* accessions with chromosomes of the hexaploid 'Sun II' is critical to transfer the resistance from *A. strigosa* into 'Sun II' and stabilize it in this background.

Resistance could be transferred into hexaploid background as addition or substitution lines. However, it is possible that the resistance could be lost through the process of chromosome decay. In the past, the disappearance of resistance transferred from lower ploidy into adapted oat has been observed and was attributed to the presence of a suppressor gene in the hexaploid. Although it is documented in crown rust that the presence of a resistance gene suppresses the effect of another resistance gene, the disappearance of resistance transferred from a diploid into hexaploid background is most likely due to preferential chromosome pairing. Unless a suppressor gene(s) suppresses the genetically controlled preferential pairing as accomplished through the use of *A. longiglumis* (Cw57), the chromosome carrying the resistance gene from *A. strigosa* could be lost.

In developing oat stem rust resistant cultivars, it is best to deploy the *Pg6* resistance together with other effective oat stem rust resistance genes such as *Pg2*, *Pg9*, and *Pg13*. It might not always be possible to detect the combinations of the resistance genes due to lack of appropriate rust races. That is where the application of the molecular markers becomes very useful. Using an AFLP finger-printing technique with *Mse*I and *Pst*I primer pair combinations, several AFLP markers were identified in one of the populations. Although 12 markers were identified when one of the F_2 populations was analyzed, the resistance gene was not flanked with closely linked markers. There are gaps between the markers and also between the marker and the resistance gene.

Therefore, the genetic map need to be saturated with additional markers. When markers that are tightly linked to the resistance gene are identified, they will be more useful in identifying the resistance with better accuracy. The AFLP marker (*P-acg+M-cga-370*) identified in one of the populations could still be useful to screen for resistance in progeny lines from CN57130. It will help in discarding progeny lines that do not carry the resistance gene without losing too many lines carrying the gene. A more closely linked marker will further enhance the screening process. Identification of the resistant progeny lines through the use of AFLP markers at an early stage, combined with the use of chromosome doubling technique, will certainly help to shorten the time needed to release an oat cultivar with stable stem rust resistance.

7.0 GENERAL CONCLUSIONS

Genetic analyses of F_2 populations and F_3 families showed that a single dominant gene is conferring resistance to oat stem rust race TJJ in the seven *A. strigosa* accessions (CN21996, CN21997, CN21998, CN22000, CN22001, CN55115, and CN57130). Analyses of results from the backcrosses supported the single dominant gene conclusion. Results of intercrosses between the seven accessions and from allelism tests with CN56818 (a source of *Pg6*) also showed that the gene conditioning resistance to race TJJ is at the *Pg6* locus. The resistance gene in the seven accessions is effective at a range of incubation temperature (12°C to 29° C). Multi-pathotype tests using 23 races indicated that there appears to be five alleles of *Pg6*, designated as *Pg6a*, *Pg6b*, and *Pg6c*, *Pg6d*, and *Pg6e* in the seven resistant *A. strigosa* accessions.

Since oat stem rust resistance genes are rare and there appears to be no more resistance genes available in hexaploid oat (McKenzie et al. 1971; Rines et al. 1980; Harder, 1999; Gold Steinberg et al. 2005), sources of oat stem rust resistance from *A. strigosa*, would be very useful in developing oat cultivars against highly virulent stem rust races. However, transferring this resistance into an adapted oat cultivar is a challenge. Introgression of resistance genes from *A. strigosa* into a hexaploid background was difficult because of low frequency of chromosome pairing between the 'A' genomes of *A. strigosa* and 'Sun II' in the interspecific crosses. Although it was difficult to produce fertile seeds from diploid by hexaploid interspecific crosses, it became possible using an embryo rescue technique. A major restriction to the success of

this technique that needs further refinement of the media used to regenerate plants from rescued embryos.

The result of this study showed that oat stem rust resistance from *A. strigosa* had been successfully transferred into a near-hexaploid 'Sun II' background. Seedlings with a high level of resistance with infection types similar to *Pg6* (; or ;1- or ;1) were expressed both in the BC_1F_2 and BC_1F_3 progeny lines in a growth cabinet and in the BC_1F_4 in the field. The transfer of resistance was further confirmed by cytological work, because the resistant progeny lines identified had chromosome counts from 44-47 at the BC_1F_2 generation and 43-44 at the BC_1F_3. While the resistance was transferred, it is not yet stable in a 42 chromosome oat line. Thus, the resistant progeny lines in the BC_1F_2, BC_1F_3, and BC_1F_4 are likely the result of addition lines. Therefore, there is a possibility that the resistance could be lost during cell division, unless it is introgressed in the 'A' genome of 'Sun II'. To stabilize the resistance, the BC_1F_3 progeny lines of CN57130/'Sun II', displaying a high level of resistance (IT=1-, or ;1) to race TJJ, were crossed to an octaploid hybrid produced from *A. longiglumis* (Cw57)/'Sun II'. The F_1 hybrid seeds produced will be planted and seedlings will be grown to maturity to be backcrossed to 'Sun II'. Backcrossing will be done to induce chromosome paring between the 'A_s' genome of *A. strigosa* and the 'A' genome of 'Sun II' (*A. sativa*), using the suppressing effect of Cw57 to the bivalent homologous chromosome pairing in 'Sun II' (Rajhathy and Thomas, 1972). Once the chromosome number in the resistant progeny lines is brought down to 42 through repeated backcrosses, the lines with resistance to TJJ will then be stable in the hexaploid 'Sun II' background.

It would be useful if molecular markers associated with *Pg6* resistance were identified to effectively utilize resources and to save time required in conventional breeding method. Twelve AFLP markers located on both sides of the resistance gene were identified in the F_2 population from CN56979/CN57130. These markers were distributed over 94.7 cM, and the closest AFLP marker (*P-acg+M-cga-370*) to the resistance gene (*Sr_57130*) was 10.9 cM away. A genetic map showing gaps between the markers and also between the resistance gene and the markers was produced. These gaps need to be saturated using restriction enzymes other than the ones used in this study and/or by the use of other finger-printing techniques so that markers that are more closely associated with the resistance gene would be identified. The AFLP marker identified in this study (*P-acg+M-cga-370*) may be useful in oat breeding programs to identify lines possessing resistance from CN57130. There was no useful AFLP marker identified for the second population (CN56979/CN22000).

8.0 SIGNIFICANCE OF THIS STUDY AND FUTURE WORK

The inheritance component of this study showed that there appears to be five alleles of oat stem rust resistance gene *Pg6* (*Pg6a*, *Pg6b*, *Pg6c*, *Pg6d*, and *Pg6e*). This is the first report that multiple alleles at the same locus exist in diploid oat. The transfer of this resistance from *A. strigosa* into *A. sativa* is very significant. It is hoped that the *Pg6* resistance from *A. strigosa* will help in the development of oat stem rust cultivars.

The hexaploid 'Sun II' is susceptible to race TJJ. Highly resistant BC_1F_3 progeny lines from 'Sun II'//CN57130/SunII and BC_1F_5 from 'Sun II'//CN21997/SunII were produced. Hybrids were also produced from the cross between the F_1 octaploid from *A. longiglumis* (Cw57)/'Sun II' and the highly resistant BC_1F_3 progeny lines from 'Sun II'//CN57130/'Sun II'. This step is essential because Cw57 induces chromosome pairing between the 'A_s' genome of *A. strigosa* and the 'A' genome of *A. sativa.* Since the resistance transferred from the two diploid *A. strigosa* accessions (CN21997 and CN57130) into the *A. sativa* is not stable, further work is required to stabilize this resistance in the 'Sun II' background. The F_1 hybrid produced from *A. longiglumis* (Cw57)/'Sun II' and the highly resistant BC_1F_3 progeny lines from CN57130/'Sun II'// 'Sun II' needs to be backcrossed to 'Sun II' at least five times. After the fifth backcross, the progeny could be selfed to result in a stable line where the resistance from *A. strigosa* (CN57130) is incorporated into 'Sun II' background.

The closest AFLP marker (*P-acg+M-cga-370*) identified in this study was 10.9 cM away from the resistance gene. There are gaps between the AFLP markers and the resistance gene. Saturating the genetic map with additional markers is required to identify flanking molecular marker(s) that are closely linked to the resistance gene.

9.0 LITERATURE CITED

Adhikari, K.N. 1996. Genetic studies of stem rust resistance in oat and triticale. PhD thesis, University of Sydney, Australia.

Adhikari, K.N., and McIntosh, R.A. 2001. Identification of stem rust and leaf rust resistance genes in Amagalon oats. Aust. J. Agric. Res. 52: 1011-1016.

Adhikari, K.N., McIntosh, R.A., Oates, J.D. 2000. Distribution and temperature sensitivities of genes for stem rust resistance in Australian oat cultivars and selected germplasm. Aust. J. Agric. Res. 51: 75-83.

Aggarwal, R.K., Brar, D.S., Nandi, S., Huang, N., and Khush, G.S. 1999. Phylogenetic relationships among *Oryza* species revealed by AFLP markers. Theor. Appl. Genet. 98: 1320-1328.

Agrios, G.N. 1988. Diseases caused by Basidiomycetes. In: Plant pathology. 3rd ed. Academic Press Inc. San Diego. pp. 451-474.

Anderson, J.W., and Gustafson, N.J. 1988. Hypocholesterolemic effects of oat and bean products. Am. J. Clin. Nutr. 48: 749-53.

Anderson, J.W., Story, L., Sieling, B., Chen, W-J.L., Petro, M.S., and Story, J. 1984. Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. Am. J. Clin. Nutr. 40: 1146-55.

Anikster, Y. The Formae speciales. *In* The cereal rusts. Vol. I. Bushnell, W.R. and Roelfs, A.P. (eds). Academic Press Inc. Orlando. FL. 1984. pp. 115-130.

Aung, T., and Thomas, H. 1976. Transfer of mildew resistance from the wild oat *Avena barbata* into the cultivated oat. Nature 260: 603.

Aung, T., Thomas, H., and Jones, I.T. 1977. The transfer of the gene for mildew resistance from *avena barbata* (4x) into the cultivated oat *A. sativa* by an induced translocation. Euphytica 26: 623-632.

Aung, T., Chong, J., and Leggett, M. 1996. The transfer of crown rust resistance gene *Pc94* from a wild diploid to cultivated hexaploid oat. *In* Proceedings of the 9th European and Mediterranean Cereal Rusts and Powdery Mildews conference. 2-6 Sept. 1996, Kema, G.J.J., Niks, R.E., and Daamen, R.A. (eds). Lunteren, Netherlands. European and Mediterranean Cereal Rust Foundation. Wageningen. pp. 167-171.

Baker, J.H.A., Matthes, M., Arnold, G.M., Edwards, K.J., Åhman, I., Larsson, S., and Karp, A. 1999. Characterisation of genetic diversity in potential biomass willows (*Salix* spp.) by RAPD and AFLP analyses. Genome 42: 173-183.

Baum, B.R. Oats: Wild and cultivated. A momograph of the genus *Avena* L. (Poaceae). Biosystmatics Research Institute. Canada Department of Agriculture, Research Branch, Ottawa, Ontario, Canada. Monograph No.14. 1977. pp. 463.

Becker, J., and Vos, P., 1995. Combined mapping of AFLP and RFLP markers in barley. Mol. Gen. Genet. 249: 65-73.

Beismann, H., Barker, J.H.A., Karp, A., and Speck, T. 1997. AFLP analysis sheds light on distribution of two *Salix* species and their hybrid along a natural gradient. Molecular Ecology 6: 989-993.

Brandshaw, J.E., Hackett, C.A., Meyer, R.C., Milbourne, D., McNichol, J.W., Philips, M.S., and Waugh, R. 1998. Identification of AFLP and SSR marker associated with quantitative resistance to *Globodera pallida* (Stone) in tetraploid potato (*Solanum tuberosum* sub sp. *tuberosum*) with a view to marker-assisted selection. Theor. Appl. Genet. 97: 202-210.

Browder, L.E. 1971. Pathogenic specialization in cereal rust fungi, especially *Puccinia recondite* f. sp. *tritici:* concepts, methods of study, and application. Technical Bulletin No. 1432. Agric. Res. Serv. USDA in cooperation with Kansas Agric. Exper. Stan. pp. 1-51.

Brown, M.C., and Shands, H.L. 1957. Pollen tube growth, fertilization and early development in *Avena sativa*. Agron. J. 9: 286-288.

Brown, P.D. 1984. The transfer of oat stem rust resistance gene *Pg*16 from tetraploid *Avena barbata* Pott. to hexaploid *Avena sativa* L. A Ph.D Thesis. University of Wisconsin, Madison.

Brown, P.D., Forsberg, R.A., McKenzie, R.I.H., and Martens, J.W. 1986. The use of disomic alien addition lines in the transfer of oat stem rust resistance to hexaploid oats. *In* Proceedings of the second international oats Conference. Lawes, D.A. and Thomas, H. (eds). Martinus Nijhof Publishers, Dordrecht, Netherlands. pp.16-20.

Bush, A.L., and Wise, R.P. 1998. High-resolution mapping adjacent to the *Pc71* crown rust resistance locus in hexaploid oat. Mol. Breed. 4: 13-21.

Bush, A.L., Wise, R.P., Rayapati, P.J., and Lee, M. 1994. Restriction fragment length polymorphisms liked to genes for resistance to crown rust (*Puccinia coronata*) in near-isogenic lines of hexaploid oat (*Avena sativa*). Genome 37: 823-831.

Chen, G., Chong, J., Gray, M., Prashar, S., and Procunier, J.D. 2006. Identification of single-nucleotide polymorphisms linked to resistance gene *Pc68* to crown rust in cultivated oat. Can. J. Plant Pathol. 28: 214-222.

Cheng, D.W., Armstrong K.C., Tinker, N., Wight, C.P., He, S., Lybaert, A., Fedak, G., and Molnar, S.J. 2002. Genetic and physical mapping of *LrK10*-like receptor kinase sequences in hexaploid oat (*Avena sativa* L.). Genome 45: 100-109.

Chong, J., and Aung, T. 1996. Interaction of the crown rust resistance gene *Pc94* with several *Pc* genes. *In* Proceedings of the 9th European and Mediterranean Cereal Rusts and Powdery Mildews conference. 2-6 Sept. 1996, Kema, G.J.J., Niks, R.E., and Daamen, R.A. (eds). Lunteren, Netherlands. European and Mediterranean Cereal Rust Foundation. Wageningen. pp.172-173.

Chong, J., and Aung, T. 1998. Suppression of crown rust resistance gene *Pc94.* Oat Newsl. vol. 44.

Chong, J., Howes, N.K., Brown, P.D., and Harder, D.E. 1994. Identification of the stem rust resistance gene *Pg9* and its association with crown rust resistance and endosperm proteins in 'Dumont' oat. Genome 37: 440-447.

Chong, J., Reimer, E., Somers, D., Aung, T., and Penner G.A. 2004. Development of Sequence-Characterized Amplified Region (SCAR) markers for resistance gene *Pc94* to crown rust in oat. Can. J. Plant Pathol. 26: 89-96.

Coffman, F.A. (ed.). Oats and oat improvement. American Society of Agronomy, Madison, WI. 1961. pp 463.

Craigie, J.H. 1927. Discovery of the function of the pycnia of the rust fungi. Nature 120: 765-67.

Craigie, J.H. 1940. The origin of physiologic races of rust fungi through hybridization. In: The Genetics of Pathogenic Organisms. Lancaster: Science Press. 12: 66-72.

Curtis, B.C., and Croy, L.I. 1958. The approach method of making crosses in small grains. Agron. J. 50: 49-51.

DeBuyser, J., Henery, Y., Lonnet, P., Hertzog, R., and Hespel, A. 1987. 'Florin': A doubled haploid wheat variety developed by the anther culture method. Plant Breed. 98: 53-56.

Dhaliwal, A.S., Mares, D.J., Marshall, D.R., and Skerrit, J.H. 1988. Protein composition and pentosan content in relation to dough stickiness of 1B/1R translocation wheats. Cereal Chem. 65: 143-149.

Dyck, P.L. 1966. Inheritance of stem rust resistance and other characteristics in diploid oats, *Avena strigosa.* Can. J. Genet. Cytol. 8: 444-450.

Dyck, P.L., and Zillinsky, F.J. 1963. Inheritance of crown rust resistance transferred from diploid to hexaploid oats. Can. J. Gent. Cytol. 5: 398-407.

Dyck, P.L., Kerber, E.R., and Lukow, O.M. 1987. Chromosome location and linkage of a new genome (Lr33) for reaction to *Puccinia recondita* in common wheat. Genome 29: 463-466.

Fankhauser, G., and Humphrey, R.R. 1952. The rare occurrence of mitosis without spindle apparatus ("colchicines mitosis") producing endopolyploidy in embryos of the Axolotl. Zoology 38: 1073-1082.

FAO statistics 2007. Online citation. http://faostat.fao.org/faostat/form?collection=Production.Crops.Primary&Domain=Production&servlet=1&hasbulk=0&version=ext&language=EN

Fehr, W.R. 1980. Artificial hybridization and self pollination. Amer. Soc. Agron. Crop Sci. Soc. Amer. Iowa State University, Ames.

Fetch, 2003. WGRF Online citation. http://www.westerngrains.com/endow/2001_04.html

Fetch, T.G. 2005. Races of *Puccinia graminis* on wheat, barley, and oat in Canada, in 2002 and 2003. Can. J. Plant Pathol. 27: 572-580.

Fetch, T.G. 2006. Effect of temperature on the expression of seedling resistance to *Puccinia graminis* f. sp. *avenae* in oat. Can. J. Plant Pathol. 28: 558-565.

Fetch, T.G., and Dunsmore, K. 2004. Stem rusts of cereals in Western Canada in 2003. Can. Plant Dis. Surv. 84: 52.

Fetch, T.G., and Jin, Y. 2007. Letter code system of nomenclature for *Puccinia graminis* f. sp. *avenae.* Plant Dis. 91: 763-766.

Findlay, W.M. Oats. Aberdeen University studies No. 137. 1956. pp. 205.

Flor, H.H. 1955. Host-parasite interaction in flax rust – its genetics and other implications. Phytopathol. 45: 680-685.

Forsberg, R.A., and Shands, H.L. 1969. Breeding behaviour of 6x-amphiploid x *Avena sativa* F_1 hybrids. Crop Sci. 9: 67-69.

Frey, K.J. Genetic resources and their use in oat breeding. In Proc. Int. Oats Conf., 2nd. Lawes, D. A. and Thomas, H. (eds.). Martinus Nijhof Publishers, Dordrecht, the Netherlands. 1986. pp. 7-15.

Fritz, S.E., and Sorrells, M.E. . Introgression of diploid wild *Avena* species into *A. sativa.* Proc. Int. Oats Conf., 2nd. Lawes, D. A. and Thomas, H. (eds). Martinus Nijhof Publishers, Dordrecht, the Netherlands. 1986. pp. 21-24.

Fu, Y-B., Kibite, S., and Richards, K.W. 2004. Amplified Fragment Length Polymorphism analysis of 96 Canadian oat cultivars released between 1886 and 2001. Can. J. Plant Sci. 84: 23-30.

Gauthier, F.M., and McGinnis, R.C. 1968. The meiotic behaviour of a nullihaploid plant in *Avena sativa* L. Can. J. Genet. Cytol. 10: 186-189.

Gold Steinberg, J., Mitchell Fetch, J., and Fetch, T.G. Jr. 2005. Evaluation of *Avena* spp. accessions for resistance to oat stem rust. Plant Dis. 89: 521-525.

Greaney, F.J. 1936. Cereal rust losses in western Canada. Sci. Agric. 16: 608-614.

Green, G.J., and McKenzie, R.I.H. 1967. Mendelian and extra chromosomal inheritance of virulence in *Puccinia graminis* f. sp. *avenae*. Can. J. Genet. Cytol. 9: 785-793.

Han, H., and Shui, H. The present status of investigations of plant tissue and cell culture in China. In: Plant cell culture: Results and perspectives. Sala, F. et al. (eds.) Elsevier/North-Holland Biomedical Press, Amsterdam. 1980. pp. 89-104.

Harder, D.E. 1989. Incidence and virulence of *Puccinia graminis* f. sp. a*venae* in Canada in 1988. Can. J. Plant Pathol. 11: 435-438.

Harder, D.E. 1994. Virulence dynamics of *Puccinia graminis* f. sp. *avenae* in Canada , 1921-1993. Phytopathol. 84: 739-746.

Harder, D.E. 1999. Usefulness of gene *Pg10* as a source of stem rust resistance in oat breeding. Phytopathol. 89: 1214-1217.

Harder, D.E., and Haber, S. Oat diseases and pathologic techniques. In: Oat Science and Technology. Marshall H.G, Sorrells M.E (eds). Crop Sci. Soc. Am. Pub. Madison, Wis. 1992. pp. 307-425.

Harder, D.E., Martens, J.W., and McKenzie, R.I.H. 1971. Changes in chlorophyll and carotenoid content in oats associated with the expression of adult plant resistance to stem rust conferred by gene *Pg*11. Can. J. Bot. 49: 1783-1785.

Harder, D.E., Chong, J., Brown, P.D., and Martens, J.W. 1990. Inheritance of resistance to *Puccinia coronata avenae* and *P. graminis avenae* in an accession of *Avena sterilis* from Spain. Genome 33:198-202.

Harder, D.E., Chong, J., and Brown, P.D. 1995. Stem and crown rust resistance in Wisconsin oat selection X1588-2. Crop Sci. 35: 1011-1015.

Hartl. L., Mohler, V., Zeller, F.J., Hsam, S.L.K., and Schweizer, G. 1999. Identification of AFLP markers closely linked to powdery mildew resistance genes *Pm1c* and *Pm4a* in common wheat (*Triticum aestivum* L.). Genome 42: 322-329.

Hayashi, E., Chi, Han-Chang, Boyer, S.K., and Still, D.W. 2005. Fragment Length Polymorphism Protocol for Plant Science on CEQ™ Series Genetic Analysis System. Genetic analysis application information. Beckman Coulter. pp. 1-11.

Helback, H. 1959. Domestication of food plants in the old world. Science, N.Y. 130: 365-372.

Heun, M., Schäfer-Pregl, R., Klawan, D., Castagna, R., Accerbi, M., Borghi, B., and Salamini, F. 1997. Site of einkorn wheat domestication identified by DNA fingerprinting. Science 278: 1312-1314.

Hittalmani, S., Parco, A., Mew, T.V., Ziegler, R.S., Huang, N. 2000. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor. Appl. Genet. 100: 1121-1128.

Ho, K.M., and Jones. G.E. 1980. Mingo barley. Can. J. Plant Sci. 60:279-280.

Hoffman, D.L., Murphy, J.P., and Guttieri, M.J. 1990. The development of a molecular linkage map in oats. Agron. Abst. p. 197.

Hoffman, L.A. World production and use of oats. In: The oat crop. Production and utilization. Welch, R.W. (ed). 1st ed. Chapman & Hall, London. 1995. pp. 34-61.

Holden, J.H.W. Oats. In: Evolution of crop plants. Simmonds (ed). Longman, London. 1976. pp. 86-89.

Howes, N.K. 1986. Linkage between the *Lr10* gene conditioning resistance to leaf rust, two endosperm proteins, and hairy glumes in hexaploid wheat. Can. J. Genet. Cytol. 28: 595-600.

Howes, N.K., Chong, J., and Brown, P.D. 1992. Oat endosperm proteins associated with resistance to stem rust of oats. Genome 35: 120-125.

Hulbert, S.H., and Bennetzen, J.L. 1991. Recombination at the *Rp1* locus of maize. Mol. Gen Genet. 226: 377-382.

Hutchinson, E.S., Hakim-Elahi, A., Miller, R.D., and Allard, R.W. 1983. The genetics of the diploidized tetraploid *Avena barbata*. J. Hered. 74: 325-330.

Hunter, H. 1924. Oats: Their varieties and characteristics, Ernest Benn, London.

Jones, D.G., and Clifford, B.C. 1983. Cereal disease. Their pathology and control. 2nd ed. John Wily and Sons. England.

Johnson, R. 1984. A critical analysis of durable resistance. Ann. Rev. Phytopathol. 22: 309-30.

Kardolus, J.P., van Eck, H.J., and van den Berg, R.G. 1998. The potential of AFLPs in biosystematics: a first application in Solanum taxonomy (*Solanaceae*). Pl. Syst. Evol. 210: 87-103.

Kirilov, A. Fodder oats in Europe. In fodder oats: a world review. Suttie and Reynolds (eds). Food and Agriculture Organization of the United Nations. Plant production and protection series No. 33. 2004. pp.179-195.

Kislev, M.E. 1982. Stem rust of wheat 3300 years old found in Israel. Science. 216:993-994.

Knott, D.R. The wheat rusts – Breeding for resistance. Mono. Theor. Appl. Genet. 12. Springer-Verlag. 1989. pp. 201.

Knox, R.E., Menzies, J.G., Howes, N.K., Clarke, J.M., Aung, T., and Penner, G.A. 2002. Genetic analysis of resistance to loose smut and an associated DNA marker in durum wheat doubled haploids. Can. J. Plant Pathol. 24: 316-322.

Krauss, S.L., and Peakall, R. 1998. An evaluation of the AFLP fingerprinting technique for the analysis of paternity in natural populations of *Persoonia mollis* (Proteaceae). Aust. J. Bot. 46: 533-546.

Laurie, D.A., and Bennett, M.D. 1986. Wheat x Maize hybridization. Can. J. Genet. Cytol. 28: 313-316.

Laurie, D.A., and Bennett, M.D. 1988. The production of haploid wheat plants from wheat x maize crosses. Theor. Appl. Genet. 76: 393-397.

Leggett, J.M. 1977. The meiotic behaviour of aneupolyhaploids of the cultivated oat *Avena sativa* (2n=6x=42). Can. J. Genet. Cytol. 19: 651-656.

Leggett, J.M. Interspecific hybrids in *Avena.* In: World Crops: Production, Utilization, Description. *In* Proceedings of the second International oats Conference. Lawes, D. A. and Thomas, H. (eds). Martinus Nijhof Publishers, Dordrecht, the Netherlands. 1986. pp. 25-28.

Leggett, J., and Thomas, H. Oat evolution and cytogenetics. *In* The oat crop. Production and utilization. R.W. Welch (ed). 1st ed. Chapman & Hall, London. 1995. pp. 120-149.

Leonard, K. 2001. Black stem rust biology and threat to wheat growers. http://www.ars.usda.gov/Main/docs.htm?docid=10755.

Lin, J-J., Kuo, J., Ma, J., Saunders, J. A., Beard, H.S., MacDonald, M.H., Kenworthy, W., Ude, G.N., and Matthews, B.L. 1996. Identification of molecular markers in soybean: comparing RFLP, RAPD and AFLP DNA mapping techniques. Plant Mol. Biol. Report, 14: 156-169.

Locatelli, A.B., Federizzi, L.C., Milach, S.C.K., Wight, C.P., Molnar, S.J., Chapados J.T., and Tinker, N.A. 2006. Loci affecting flowering time in oat under short-day conditions. Genome 49: 1528-1538.

Loegering, W.Q., and Harmon, D.L. 1968. Wheat lines Near-isogenic for reaction to *Puccinia graminis tritici.* Phytopathol. 59: 456-459

Ma, Z.Q., and Lapitan, N.L.V. 1998. A comparison of amplified and restriction fragment length polymorphism in wheat. Cereal Res. Commun. 26: 7-13.

Mackill, D.J., Zhang, Z., Redona, E.D., and Colowit, P.M. 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. Genome. 39: 969-977.

Marshall, H.G., and Myers, W.M. 1961. A cytogenetic study of certain interspecific *Avena* hybrids and the inheritance of resistance in diploid and tetraploid varieties to race of crown rust. Crop Sci. 1: 29-34.

Martens, J.W. 1978. Stem rust of oats in Canada in 1977. Can. Plant Dis. Surv. 58: 51-52.

Martens, J.W. Oat stem rust. In: The Cereal Rusts: Vol II: A. P. Roelfs and W. R. Bushnell (eds). Academic Press Inc. Orlanda FL. 1985. pp. 103-129.

Martens, J.W., and Harder, D.E. 1983. Incidence and virulence of *Puccinia graminis* f. sp. *Avenae* in Canada in 1982. Can. J. Plant Pathol. 5: 189-193.

Martens, J.W., and P.L. Dyck. 1989. Genetics of resistance to rust in cereals from a Canadian perspective. Can. J. Plant Pathol. 11: 78-85.

Martens, J.W., McKenzie, R.I.H., and Fleischmann, G. 1968. The inheritance of resistance to stem and crown rust in Kyto oats. Can. J. Genet. Cytol. 10: 808-812.

Martens, J.W., McKenzie, R.I.H., and Harder, D.H. 1980. Resistance to *Puccinia graminis avenae* and *P. coronata avenae* in the wild and cultivated *Avena* populations of Iran, Iraq, and Turkey. Can. J. Genet. Cytol. 22: 641-649.

Martinelli, J.A. 2004. Oat disease and their control. In fodder oats: a world review. J.M. Suttie and S.G. Reynolds (eds). Food and Agriculture Organization of the United Nations. Plant production and protection series No. 33: 197-214

Maughan, P.J., Saghai Maroof, M.A., and Buss, G.R. 1996. Amplified Length Polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. Theor. and Appl. Genet. 93: 392-401.

McCallum, B.D., Harder, D.E., and Dunsmore, K. 2000. Stem rusts on wheat, barley, and oat in Canada in 1998. Can. J. Plant Pathol. 22: 23-28.

McDaniel, M.E., Kim, H.B., and Hathcock, B.R. 1967. Approach crossing of oats (*Avena* spp.). Crop Sci. 7: 538-540.

McKenzie, R.I.H., and Martens, J.W. 1968. Inheritance in the oat strain C.I.3034 of adult plant resistance to race C 10 of stem rust. Crop Sci. 60: 625-627.

McKenzie, R.I.H., and Martens, J.W. 1974. Breeding for stem rust resistance in oats. Induced mutations for disease resistance in crop plants. International Atomic Energy Agency. Vienna. pp. 45-48.

McKenzie, R.I.H., Martens, J.W., and Rajhathy, T. 1970. Inheritance of oat stem rust resistance in a Tunisian strain of *Avena sterilis*. Can. J. Genet. Cytol. 12: 501-505.

McKenzie, R.I.H., Martens, J.W., and Green, G.J. 1971. "The oat stem rust problem". Mutation breeding for disease resistance. *In* Proceedings Panel Vienna, 1970. IAEA. Vienna. pp. 151-157.

McKenzie, R.I.H., Martens, J. W., Fleischmann, G., and Samborski, D.J. 1968. An association of stem rust and crown rust resistance in Jostrain oats. Can. J. Genet. Cytol. 10: 190-195.

Michelmore, R.W., Paran, I., and Kesseli, R.V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *In* Poc. Natl. Acad. Sci. USA. 88: 9828-9832.

Milne, D.L. and McIntosh, R.A. *Triticum aesitivum* (common wheat). *In* Genetic maps. 5th edn. O'Brien S.J. (ed) Cold Spring Harbor Laboratory Press, Cold Spring Harbor. New York. 1990. pp. 6.16-6.27.

Moore-Colyer, R.J. Oats and oat production in history and pre-history. *In* The oat crop. Production and utilization. Welch, R.W. (ed). 1st ed. Chapman & Hall, London. 1995. pp. 1-33.

Morey, D.D. 1949. The extent and causes of variability in Clinton oats. Agric. Exper. Stn. IOWA State College of Agriculture and Mechanical Arts. Res. Bull. 363. Ames, IOWA. p. 44.

Mueller, U.G., and Wolfenbarger, L.L. 1999. AFLP genotyping and fingerprinting. TREE. 14(10): 389-394.

Murphy, J.P., and Hoffman, L.A. 1992. The origin, history, and production of oat. In: Oat Science and Technology. H.G. Marshall and M.E. Sorrells eds. Am. Soc. Agron. Madison, WI. pp. 1-28.

Nakajima, Y., Oeda, K., and Yamamoto, T. 1998. Characterization of genetic diversity of nuclear and mitochondrial genomes in *Daucus* varities by RAPD and AFLP. Plant cell reports 17: 848-853.

O'Donoughue, L.S., Chong, J., Wight, C.P., Fedak, G., and Molnar, S.J. 1996. Localization of stem rust resistance genes and associated molecular markers in cultivated oat. Phytopathol. 86: 719-727.

O'Donoughue, L.S., Wang, Z., Röder, M., Kneen, B., Leggett, M., Sorrells, M.E., and Tanksley, S.D. 1992. An RFLP-based linkage map of oats based on a cross between two diploid taxa (*Avena atlantica X A. hirtula*). Genome 35: 765-771.

Paran, I.R., Kesseli, R., and Michlemore, R.W. 1991. Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near isogenic lines. Genome 34: 1021-1027.

Pavek, J.J., and Myers, W.M. 1965. Inheritance of seedling reaction to *Puccinia graminis* Pers. f. sp. *avenae* race 13A in crosses of oat strains with four different reactions. Crop Sci. 5: 501-504.

Penner, G.A., Chong, J., Lévesque-Lemay, M., Molnar, S.J., and Fedak, G.. 1993a. Identification of a RAPD marker linked to the oat stem rust gene *Pg3*. Theor. and Appl. Genet. 85: 702-705.

Penner, G.A., Chong, J., Wight, C.P, Molnar, S.J., and Fedak, G. 1993b. Identification of a RAPD marker for the crown rust resistance gene *Pc68* in oats. Genome 36: 818-820.

Person, C. 1959. Gene-for-gene relationship in host-parasite systems. Can. J. Bot. 37: 1101-30.

Person, C., Samborski., D.J., and Rohringer, R. 1962. The gene-for-gene concept. Nature 194: 561-562.

Pomeranz, Y. Industrial uses of oats. In: The oat crop. Production and utilization. R.W. Welch (ed). 1st ed. Chapman & Hall, London. 1995. pp. 480-503.

Poehlman, J.M. Breeding field crops. 2nd ed. Avi Publl. Co., Westport, CT. 1979. p. 724.

Poehlman, J.M., and Sleper, D.A. Breeding field crops. 4th ed. Iowa State University Press. 1995. pp. 494.

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

Rajhathy, T. 1963. A standard karyotype for *A. sativa.* Can. J. Genet. Cytol. 5: 127-132.

Rajhathy, T., and Morrison, J.W. 1959. Chromosome morphology in the genus *Avena.* Can. J. Bot. 37: 331-337.

Rajhathy, T., and Thomas, H. 1972. Genetic control of chromosome pairing in hexaploid oats. Nature New Biol. 239: 217-219.

Rajhathy, T., and Thomas, H. Cytogenetics of Oats. Misc. Publ. 2. Wilkes, A. (ed). The Genetics Society of Canada. Ottawa. Canada. 1974. pp. 1-90.

Ranhotra, G.S. and Gelroth, J.A. Food uses of oats. In: The oat crop. Production and utilization. Welch, R.W. (ed). 1st ed. Chapman & Hall, London. 1995. pp. 409-432.

Rayapati, P.J., Gregory, J.W., Lee, M., and Wise, R.P. 1994. A linkage map of diploid *Avena* based on RFLP loci and a locus conferring resistance to nine isolates of *Puccinia coronata* var. *avenae*. Theor. Appl. Genet. 89: 831-837.

Richter, K., Schondelmair, J., and Jung, C. 1998. Mapping of quantitative trait loci affecting *Drechslera teres* resistance in barley with molecular markers. Theor. Appl. Genet. 97: 1225-1234.

Ridout, C.J., and Donini, P. 1999. Use of AFLP in cereals research. Trends in Plant Science 4: 76-79.

Rieseberg, L.H., Kim, M.J., and Seiler, G.J. 1999. Introgression between the cultivated sunflower and a sympatric wild relative, *Helianthus petiolaris* (Asteraceae). Int. J. Plant Sci. 160: 102-108.

Riley, R. 1960. The diploidization of polyploidy wheat. Heredity. 15: 407-429.

Riley, R., and Chapman, V. 1958. Genetic control of cytologically diploid behaviour in hexaploid wheat. Nature 182: 713-715.

Riley, R., Chapman ,V., and Johnson, R. 1968. Introduction of yellow rust resistance of *Aegilops comosa* into wheat by genetically induced homoeologous recombination. Nature 217: 383-384.

Rines, H.W. 1983. Oat anther culture: Genotype effects on callus initiation and the production of a haploid plant. Crop Sci. 23: 268-272.

Rines, H.W., and Dahleen, L.S. 1990. Haploid oat plants produced by application of maize pollen to emasculated oat florets. Crop Sci. 30: 1073-1078.

Rines, H.W., Porter, H.L., Carson, M.L., and Ochocki, G.E. 2007. Introgression of crown rust resistance from diploid oat *Avena strigosa* into hexaploid cultivated oat *A. sativa* by two methods: direct crosses and through an initial *2x+4x* synthetic hexaploid. Euphytica 158: 67-79.

Rines, H.W., Stuthman, D.D., Briggle, L.E., Youngs, V.L., Jedlinski, H., Smith, D.H., Webster, J.A., and Rothman, P.G. 1980. Collection and evaluation of *Avena fatua* for use in oat improvement. Crop Sci. 20: 63-68.

Roelfs, A.P. 1978. Estimated losses caused by rust in small grain cereals in the United States, 1918-1976. Misc. Publ. –U.S. Dep. Agric. 1363, 1-85.

Roelfs, A.P. Race specificity and methods of study. *In* The cereal rusts. Vol. I. Bushnell, W.R. and Roelfs, A.P. (eds). Academic Press Inc. Orlando. FL. 1984. pp. 131-164.

Roelfs, A.P. Wheat and rye stem rust. In: The Cereal Rusts: Vol II: Roelfs, A. P. and Bushnell, W. R. (eds). Academic Press Inc. Orlanda FL. 1985. pp. 3-37.

Roelfs, A.P. 1988. Genetic control of phenotypes in wheat stem rust. Ann. Rev. Phytopathol. 26: 351-67.

Roelfs, A.P., Singh, R.P., and Saari, E.E. 1992. Rust Diseases of wheat: Concepts and Methods of Disease Management. CIMMYT, Mexico.

Rooney, W.L., Rines, H., and Phillips, R.L. 1994. Identification of RFLP markers liked to crown rust resistance genes *Pc91* and *Pc92* in oat. Crop Sci. 34: 940-944.

Rosenquist, C.E. 1927. An improved method of producing F_1 hybrid seeds of wheat and barley. J. Amr. Soc. Agron. 19: 968-971.

Rothman, P.G. 1984. Registration of four stem rust and crown rust resistant oat germplasm lines. Crop Sci. 24: 1217-1218

Rothman, P.G. Adequate rust resistance in oats. In: Proc. Int. Oats Conf., 2nd. Lawes, D. A. and Thomas, H. (eds). Martinus Nijhof Publishers. Dordrecht, the Netherlands. 1986. pp. 72-76.

Russell, J.R., Weber, J.C., Booth, A., Powell, W., Sotelo-Montes, C., and Dawson, I.K. 1999. Genetic variation of *Calycophyllum spruceanum* in the Peruvian Amazon Basin, revealed by Amplified fragment length polymorphism (AFLP) analysis. Molecular Ecology 8: 199-204.

Sadanaga, K., and Simons M. D. 1960. Transfer of crown rust resistance of diploid and tetraploid species to hexaploid oats. Agron. J. 52: 285-288.

Sadanaga, K., and Simons, M.D. 1967. Inheritance of crown rust resistance transferred to hexaploid oats from diploid C.I. 3815. Crop Sci. 7: 39-40.

Sadasivaiah, R.S., and Rajhathy, T. 1968. Genome relationships in *4x Avena*. Can. J. Genet. Cytol. 10: 655-659.

Schrickel, D.J. World oats use and marketing. In: Proceedings of the second international oats Conference. D. A. Lawes and H. Thomas (eds). Martinus Nijhof Publishers, Dordrecht, the Netherlands. 1986. pp. 197-199.

Sears, E.R. 1956. The transfer of leaf-rust resistance from *Aegilops umbellulata* to wheat. Brookheaven Symposia in Biology 9. pp. 1-22.

Sharma, D.C., and Forsberg, R.A. 1977. Spontaneous and induced interspecific gene transfer for crown rust resistance in *Avena*. Crop Sci. 17: 855-860.

Sharp, E.L., Schmitt, C.G., Staley, J.M., and Kingsolver, C.H. 1958. Some critical factors involved in establishment of *Puccinia graminis* var. *tritici*. Phytopathol. 48: 469-474.

Sidhu, P.K., Howes, N.K., Aung, T., Zwer, P.K., and Davies, P.A. 2006. Factors affecting oat haploid production following oat x maize hybridization. Plant Breed.125: 243-247.

Simons, M.D., Martens, J.W., McKenzie, R.I.H., Nishiyama, I., Sadanaga, K., Sebesta, J., and Thomas, H. 1978. Oats: a standardized system of nomenclature for genes and chromosomes and catalog of genes governing characters. U.S. Government Printing Office, Washington, DC. USDA Agric. Handb. pp. 408.

Singh, S., Sidhu, J.S., Huang, N., Vikal, Y., Li, Z., Brar, D.S., Dhaliwal, H.S., and Klush, G.S. 2001. Pyramiding three bacterial blight resistance genes (*xa5, xa13, and Xa21*) using marker-assisted selection into rice cultivar PR106. Theor. Appl. Genet. 102: 1011-1015.

Small, E. New Crops for Canadian agriculture. In: Perspectives on New Crops and New Uses. Janick, J. (ed). ASHS Press, Alexandria, VA. 1999. pp. 15-52.

Somers, D.J. Molecular markers systems and their evaluation for cereal genetics. In: Cereal Genomics. Gupta, P.K. and Varshney, R.K. (eds.). Kluwer Academic Publishers. Netherlands. 2004. pp. 19-34.

Somers, D.J., Thomas, J., DePauw, R., Fox, S., Humphreys, G., and Fedak, G. 2005. Assembling complex genotypes to resist *Fusarium* in wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 111: 1623-1631.

Spielmeyer, W., Green, A.G., Bittisnich, D., Mendham, N., and Lagudah, E.S. 1998. Identification of quantitative trait loci contributing to Fusarium wilt resistance on an AFLP linkage map of flax (*Linum usitatissimum*) . Theor. Appl. Genet. 97: 633-641.

Stakman, E.C., Levine, M.N., and Bailey, D.L. 1923. Biologic forms of *Puccinia graminis* on varieties of *Avena* spp. J. Agric. Res. 24: 1013-1018

Stakman, E.C., Stewart, D.M., and Loegering, W.Q. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. U.S. Dept. Agric. Agric. Res. Serv. Publ. E-617. pp. 1-53.

Steffenson, B.J. 1992. Analysis of durable resistance to stem rust in barley. Euphytica 63: 153-167.

Stevens, E.J., Armstrong, K.W., Bezar, H.J., Griffin W.B., and Hampton, J.G. Fodder oats: an overview. *In* fodder oats: a world review. J.M. Suttie and S.G. Reynolds (eds). Food and Agriculture Organization of the United Nations. Plant production and protection series No. 33. 2004. pp. 11-18.

Stuthman, D.D. Oat breeding and genetics. In: The oat crop. Production and utilization. Welch, R.W. (ed). 1st ed. Chapman & Hall, London. 1995. pp. 150-176.

Suttie, J. M., and Reynolds, S.G (eds). Background to fodder oats worldwide. In: Fodder oats: a world over view. Food and Agriculture Organization of the United Nations. Plant production and protection series No. 33. 2004. pp. 1-10.

Swain, J.F., Rouse, I.L., Curley, C.B., and Sacks, F.M. 1990. Comparison of the effects of oat bran and low-fiber wheat on serum lipoprotein levels and blood pressure. N. Eng. J. Med. 322: 147-152.

Swami, U.B.S., and Thomas, H. 1966. Studies on the relationship of *Avena sativa* (6x) and *Avena hirtula* (2x). Can. J. Genet. Cytol. 8: 51-56.

Tabien, R.E., Li, A., Paterson, A.H., Marchetti, M.A., Stansel, J.W., and Pinson, S.R.M. 2000. Mapping four major rice blast resistance genes from 'Lemon" and Teqing" and evaluation of their combinatorial effect for field resistance. Theor. Appl. Genet. 101: 1215-1225

Tayler, E.W., 1965. The mechanism of colchicine inhibition of mitosis. I. kinetics of inhibition and the binding of H^3-colchicine. J. Cell Biol. 25: 145-160.

Thomas, H. 1970. Chromosome relationships between cultivated oat *Avena sativa* (6x) and *A. ventiricosa* L. Can. J. Genet. Cytol. 12: 36-43.

Thomas, H. Cytogenetics of *Avenea* in: Oat Science and Technology. Marshall, H.G., and Sorrells, M.E. (eds). American Society of Agronomy and Crop Science Society of America. Madison, Wisconsin. USA. 1992. pp. 473-507.

Thomas, H., and Bhatti, I. M. 1975. Notes on the cytogenetic structure of the cultivated oat *Avena sativa.* Euphytica 29: 149-157.

Thomas, H., and Rajhathy, T. 1967. Chromosome relationships between *Avena sativa* (6x) and *Avena pilosa* (2x). Can. J. Genet. Cytol. 9: 154-162.

Thomas, H., Powell, W. and Aung, T. 1980. Interfering with regular meiotic behaviour in *Avena sativa* as a method of incorporating the gene for mildew resistance from *A. barbata.* Euphytica 29: 635-640.

Toojinda, T., Baird, E., Booth, A., Broers, L., Hayes, P., Powell, W., Thomas, W., Vivar, H., and Young, G. 1998. Introgression of quantitative trait loci (QTLs) determining stripe rust resistance in barley: an example of marker-assisted line development. Theor. Appl. Genet. 96: 123-131.

Van Deynze, A.E., Nelson, J.C., O'Donoughue, L.S., Ahn, S.N., Siripoonwiwat, W., Harrington, S.E., Yglesias, E.S., Braga, D.P., McCouch, S.R., and Sorrells, M.E. 1995. Comparative mapping in grasses. Oat relationships. Mol. Gen. Genet. 249: 349-356.

Vavilov, N.I. 1951. The origin, variation, immunity and breeding of cultivated plants. In: Selected writings, of N.I. Vavilov. Transl. by K. Stass Chaster, Chronica Bot. 12 (1/6). p. 364.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T.V.D., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23(21): 4407-4414

Wahl, I., Anikster, Y., Manisterski, J., and Segal, A. *In* The cereal rusts. Vol. I. Bushnell, W.R. and Roelfs, A.P. (eds). Academic Press Inc. Orlando. FL. 1984. pp. 39-78.

Welch, R.W. Oats in human nutrition and health. In: The oat crop. Production and utilization. Welch, R.W. (ed). 1st ed. Chapman & Hall, London. 1995. pp. 433-479.

Wight, C.P., O'Donoughue, L.S., Chong, J., Tinker, N.A., and Molnar, S.J. 2004. Discovery, localization, and sequence characterization of molecular markers for the crown rust resistance genes *PC38, PC39* and *Pc48* in cultivated oat (*Avena sativa* L.). Mol. Breed. 14: 349-361.

Wight, C.P., Penner, G.A., O'Donoughue, L.S., Burrows, V.D., Molnar, S.J., and Fedak, G. 1994. The identification of random amplified polymorphic DNA (RAPD) markers for day length insensitivity in oat. Genome 37: 910-914.

Wilson, W.A., and McMullen, M.S. 1997. Dosage Dependent Genetic Suppression of oat crown rust resistance gene *Pc*-62. Crop Sci. 37: 1699-1705.

Winfield, M.O., Arnold, G.M., Cooper, F., Le Ray, M., White, J., Karp, A., and Edwards, K.J. 1998. A study of genetic diversity in *Populus nigra* subsp. *betulifolia* in the Upper Severn area of the UK using AFLP markers. Molecular Ecology 7: 3-10.

Wise, R.P., and Ellingboe, A.H. 1985. Fine structure and instability of the *Ml-a* locus in barley. Genetics 111: 113-130.

Xu, M.L., Melchinger, A.E., Xia, X.C., and Lübberstedt, T. 1999. High-resolution mapping of loci conferring resistance to sugarcane mosaic virus in maize using RFLP, SSR and AFLP markers. Mol. Gen. Genet. 261: 574-581.

Yu, G.X., and Wise, P. 2000. An anchored AFLP-and retrotransposon-based map of diploid *Avena.* Genome 43: 736-749.

Zadoks, J.C., Change, T.T., and Konzak, C.F. 1974. A decimal code for the growth stages of cereals. Weed Res. 14: 415-421.

Zamani, I., Kovacs, G., Gouli-Vavdinoudi, E., Roupakias, D.G., and Barnabas, B. 2000. Regeneration of fertile doubled haploid plants from colchicine-supplemented media in wheat anther culture. Plant Breeding 119: 461-465.

Zhu, J., Gale, M.D., Quarrie, S., Jackson, M.T., and Bryan, G.J. 1998. AFLP markers for the study of rice biodiversity. Theor. Appl. Genet. 96: 602-611.

Zhu, S., and Kaeppler, H.F. 2003. Identification of quantitative trait loci for resistance to crown rust in oat line MAM17-5. Crop Sci. 43: 358-366.

Zhu, S., Kolb, F.L., and Kaeppler, H.F. 2003. Molecular Mapping of genomic regions underlying barley yellow dwarf tolerance in cultivated oat (*Avena sativa* L.). Theor Appl Genet. 106: 1300-1306.

Zillinsky, F.J., and Derick, R.A. 1960. Crown rust resistant derivatives from crosses between autotetraploid *Avena strigosa* and *A. sativa.* Can. J. Plant Sci. 40: 366-370.

APPENDICES

Appendix 1. Nutrient media preparation

1. Weigh 25g of sucrose and 8g of agar and add the two into 1 liter of distilled water.
2. Heat the mixture to 85˚C by stirring until completely dissolved.
3. Reduce the temperature to 75˚C and add 3g of Gamborg*s B-5 basal medium (Manufacture: SIGMA G-5893) by stirring until completely dissolved.
4. Adjust the pH to 6.0 by adding 1N Sodium hydroxide (NaOH).
5. Dispense the medium into glass vials (8ml/35 ml vial).
6. Sterilize the medium in an autoclave at 15psi and 121˚C for 20 min.

Appendix 2. Protocol for chromosome counting

1. Collect young root tips of about one centimetre length and put them in cold distilled water in labelled vials.

2. Put the vials in ice and keep the samples in a refrigerator (~4°C) for 24 hours.

3. Fix the samples by transferring them into labelled vials with 3:1, 95% ethanol to glacial acetic acid.

4. The samples could be examined after five days but they could also be stored for a long time at this stage.

5. After the 5^{th} day, hydrolize the samples for seven minutes in 1N HCl in a water bath heated to 60°C.

6. Transfer each samples into Fuelgan stain and let them sit in there for about 20 minutes until the root cap turns purple.

7. The specimens will be ready for inspection under a microscope.