Genetic Analysis of *Puccinia coronata* Corda f. sp. *avenae*

Resistance in Oat (*Avena sativa* L.)

A Thesis Submitted to the College of

Graduate Studies and Research

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

In the Department of Plant Sciences

University of Saskatchewan

Saskatoon

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ABSTRACT

In Western Canada, oat crown rust (caused by *Puccinia coronata* Corda f. sp. *avenae* Eriks) is considered the most economically important disease of cultivated oat (*Avena sativa* L.). Resistant oat varieties are an important control method for crown rust. *Avena sterilis* L. accessions have been the main source of crown rust resistance genes due to their reproductive compatibility with *A. sativa*. In order to discover new seedling resistance genes in *Avena sterilis* accessions, six genetic populations were generated by crossing AC Morgan (Reg. no.CV-369, PI 629113) with six *A. sterilis* accessions (PI311623-4, PI333561, PI334672-5, PI335562, PI375506 and PI375547). All F_2 populations and partial F_3 families were inoculated with isolate CR259 (race LQCB-91; virulent on *Pc35/38/39/40/55/59/60/61/63/91*) at the two leaf stage and rated 11 or 12 days post-inoculation. Resistance in PI334672-5 was the result of two resistance genes with one dominant resistance gene and one recessive resistance gene. Two dominant resistance genes were likely present in PI375547. Current F_2 tests revealed that both PI311623-4 and PI335562 contained two dominant genes, resulting in duplicate dominant epistasis. Based on current F_2 and F_3 data, resistance genes present in PI375506 are most likely one dominant resistance gene or one dominant resistance gene and one recessive resistance gene. In F_2 tests, PI333561 appeared to be heterogeneous. Assuming no heterogeneity, PI333561 would carry two resistance genes, one or both of which are incompletely dominant. PI333561 is the only accession resistant to all crown rust isolates contained in the Cereal and Flax Pathology Lab (University of Saskatchewan, Saskatoon, SK) and Cereal Research Centre (Agriculture and Agri-Food Canada, Winnipeg, MB) collections. As such, this accession likely carries valuable new seedling crown rust resistance genes.

Extensive use of seedling genes in breeding programs corresponds with high virulence frequency to those genes. Adult plant resistance (APR) is believed to be a durable rust management strategy. The crown rust resistance in the oat line MN841801 has been effective for more than 20 years. Research was conducted to detect APR quantitative trait loci (QTLs)

contributed by MN841801. A genetic map was generated in a population of 167 F_7-derived recombinant inbred lines (RILs) from a cross AC Assiniboia x MN841801 (AM). The map containing mostly Diversity Arrays Technology (DArT) markers consisted of 30 linkage groups spanning 955 cM. Two field environmental tests under a lattice design were conducted in Saskatoon, Saskatchewan. Only one QTL contributed by AC Assiniboia was detected on 2010 Saskatchewan field data. This detected QTL was the result of the seedling resistance gene *Pc68*, since it was mapped 4 cM away from the sequence characterized amplified region (SCAR) marker *Pc68-300*. No QTL contributed by MN841801 were detected in this study. Low oat genome coverage in the current genetic map of the AM population is the primary limitation to detecting APR QTLs contributed by MN841801. Single nucleotide polymorphism (SNP) markers from the first complete oat map will be important for improving the genetic map of this population and detection of APR QTL from MN841801. Additional field testing of the AM population with *P. coronata* isolate CR251 is recommended to improve the precision and accuracy of the phenotypic data.

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LIST OF ABBREVIATIONS

AAFC	Agriculture and Agri-Food Canada
ABC	ATP-binding cassette
AM	AC Assiniboia x MN841801
ANOVA	Analyses of variance
APR	Adult plant resistance
ATP	Adenosine-5'-triphosphate
Avr	Avirulence
Bgh	*Blumeria graminis* f.sp. *hordei*
CI	Coefficient
CIM MLE	Composite interval mapping based on maximum-likelihood estimates
CRC	Cereal Research Center
CTAB	Hexadecyltrimethylammonium bromide
CWA	Cell wall apposition
DArT	Diversity Arrays Technology
DS	Disease severity
EFR	EF-Tu receptor
EF-Tu	Elongation factor Tu
EST	Expressed sequence tag
ETI	Effector-triggered immunity
FDNA	Fungal DNA
flg	Flagellin
FLS2	Flagellin-sensing 2-like protein
GRIN	Germplasm Resources Information Network
HR	Homozygous resistant
HRM	High resolution melting
HS	Homozygous susceptible

IP	Infection peg
IT	Infection type
KO	Kanota x Ogle
LPS	Lipopolysaccharide
LRR	Leucine-rich repeat
LRR-RLK	Lucine-rich repeat-receptor-like kinase
MAMP	Microbe-associated molecular pattern
MB01	Manitoba in 2001
MB02	Manitoba in 2002
MN	MN841801-1 x Noble-2
NB	Nuclear binding domain
PAMP	Pathogen-associated molecular pattern
PCD	Programmed cell death
PCR	Polymerase chain reaction
PGN	Peptidoglycan
PR	Pathogenesis-related
PRR	Pattern-recognition receptor
PTI	PAMP-triggered immunity
q-PCR	Quantitative real time polymerase chain reaction
QTL	Quantitative trait locus
R	Resistance
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
ROS	Reactive oxygen species
SCAR	Sequence characterized amplified region
SEG	Segregating
SK10	Saskatoon, Saskatchewan in 2010
SK11	Saskatoon, Saskatchewan in 2011
SMA	Single marker analysis

SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism
SSR	Simple sequence repeat
USDA	United States Department of Agriculture
USDA-ARS	United States Department of Agriculture-Agricultural Research Service

1. Introduction

Oat (*Avena sativa* L.) is an important cereal crop in the Prairie Provinces of Canada (Statistics Canada, 2011). Approximately half of the world's oat exports are supplied by Canada (Agriculture and Agri-Food Canada, 2010) and demand for oat continues to increase because oat grains are recognized as very nutritious (Agriculture and Agri-Food Canada, 2010). In western Canada, oat crown rust (caused by *Puccinia coronata* Corda f. sp. *avenae* Eriks) is considered the most economically important disease in cultivated oats (McCallum *et al.*, 2007; Leonard, 2007; Chong *et al.*, 2008). The disease primarily causes severe yield and grain quality losses (Simons, 1970) while lodging and low cold resistance are additional negative effects caused by this disease.

Resistant oat varieties have been the main management method in North America, but fungicide application is becoming common (McCallum *et al.*, 2007). *Avena sterilis* L., which contains more than 45 effective resistance genes, has been a major source of crown rust resistance genes since the 1960s (McCallum *et al.*, 2007; Leonard, 2007). Through cross-pollination between *A. sativa* and *A. sterilis* resistance genes have been moved from wild oats to cultivated oats and identified by seedling tests (McCallum *et al.*, 2007).

The majority of known race-specific resistance genes used in oat breeding programs are involved in gene-for-gene resistance. This type of rust resistance is able to control *P. coronata* at the time of initial widespread deployment of the gene. However, in a relatively short period of time these genes are overcome by *P. coronata*. Other *Avena spp.* beyond *A. sterilis* have also been used to develop crown rust resistance (Rooney *et al.*, 1994; Aung *et al.*, 2010). However, Carson (2009a) noted that no evidence exists to demonstrate that seedling resistance genes from diploid or tetraploid *Avena* spp. are more durable than those resistance genes from *A. sterilis.* In the current study, six F_2 and partial $F_{2:3}$ populations developed from six *A. sterilis* accessions crossed with AC Morgan were evaluated in seedling tests to detect putative new resistance genes.

Partial resistance has been widely applied as a durable disease management strategy in other cereal crops (Dyck, 1987; Humphry *et al.,* 2006). The oat line, MN841801, has consistently demonstrated resistance to various *P. coronata* populations for more than 20 years (Chong, 2000; Leonard 2002). It is believed to potentially contain durable resistance genes. Chong (2000) concluded that MN841801 is carrying two adult plant resistance (APR) genes with additive effects. However, four major quantitative trait loci (QTLs) and three minor QTLs for APR contributed by MN841801-1 were detected in the cross MN841801-1 x Noble-2 (MN) (Portyanko *et al.*, 2005). Acevedo *et al.* (2010) validated these APR QTLs and discovered one new QTL from the same cross. A second project was conducted as part of this study to establish a recombinant inbred line (RIL) population from the cross AC Assiniboia x MN841801. Across two years of field tests using this RIL population, the presence of APR QTLs contributed by MN841801 were tested through QTL mapping.

This study consisted of two projects. In project one, the research hypothesis tested was that *Avena sterilis* accessions carry seedling crown rust resistance genes effective against isolate CR259 (LQCB-91) which is virulent against most commonly deployed crown rust resistance genes. The objectives to test this hypothesis were to evaluate the presence and genetic inheritance of resistance genes contained in six *Avena sterilis* accessions when inoculated with CR259. In project two, the research hypothesis was that oat line MN841801 contained APR loci that can be mapped. The objectives to test this hypothesis were to create a genetic linkage map in a recombinant inbred population derived from AC Assiniboia x MN841801 and identify QTLs controlling APR by measuring crown rust reaction in this population.

2. Literature Review

2.1 Oat

Cultivated oat (*Avena sativa* L.) belongs to the family *Poaceae*. Optimal growing conditions for oat are well drained soils, long warm days and adequate water supply. Suitable soil textures range from sandy loam to heavy clay. Oat has been marketed as one of the most important human grains. A dehulled oat kernel (groat) contains 12 - 20% protein and has 3 - 6% beta-glucan. Beta-glucan is a soluble fiber that has been shown in a number of studies to reduce the risk of coronary heart disease (Ludwig *et al.*, 1999; Maki *et al.*, 2007) and type II diabetes (Jenkins *et al.*, 2002). In 1997, the US Food and Drug Administration (FDA) approved the use of a health claim for oats stating that soluble oat fiber beta-glucan will lower the risk of coronary heart disease. Oat grains also contain a number of anti-oxidants, including avenanthramides which are unique to oat (Rines *et al.*, 2006). Studies have linked this compound with the prevention of atherosclerosis (Nie *et al.*, 2006).

Oat is an important crop in Canada, particularly in the Prairie Provinces. Approximately 1.8 million hectares of oat have been seeded each year in Canada over the past decade (Statistics Canada, 2011). In 2012, Canada produced 3.25 million tonnes of oat, making Canada the third largest oat producer in the world. In addition, Canada was the largest oat exporter. In 2012, 1.7 million tonnes were exported to the world cereal market (United States Department of Agriculture, 2012). The province of Saskatchewan produces approximately 1.4 million tonnes of oat on 600,000 hectares of land on an annual basis (Government of Saskatchewan, 2010).

There are three ploidy levels in *Avena spp.*, which are diploid, tetraploid and hexaploid. Wild oat (*A. sterilis* L.) and cultivated oat are hexaploids which carry the A, C and D genomes. Each genome contains seven base chromosomes. The ancestor of *A. sativa* is suspected to be *A. sterilis* (Loskutov and Rines, 2011). *Avena canariensis* is the diploid A-genome ancestor of the hexaploid *Avena* species whereas *A. ventricosa* is the diploid C-genome ancestor. Currently, the ancestral Avena diploid D-genome species is unknown

(Loskutov and Rines, 2011). *Avena strigosa* has been an important AA genome species which has contributed a large number of effective crown rust resistance genes (Rines *et al.*, 2006). Among tetraploid *Avena* species, the AABB, AACC and CCDD genomes are observed. *Avena murphyi* and *A. insularis* carry the AACC genome, *A. barbata* carries the AABB genome and *A. magna* carries the CCDD genome. *Avena magna* is also thought to be the tetraploid progenitor of the hexaploid genome (Loskutov and Rines, 2011). With respect to crown rust resistance, *A. barbata* has contributed effective crown rust resistance genes that have been transferred into cultivated oat (Carson, 2009a; Loskutov and Rines, 2011).

2.2 *Puccinia coronata*

2.2.1 Economic loss caused by *P. coronata*

Common diseases of oat include crown rust, stem rust, barley yellow dwarf virus, loose smuts, powdery mildew, Septoria leaf blight, Victoria blight, bacterial blights, soil-borne viruses and nematodes (Chong, 2003). In western Canada, oat crown rust (caused by *Puccinia coronata* Corda f. sp. *avenae* Eriks) is considered the most economically important disease in cultivated oats (McCallum *et al.*, 2007; Leonard, 2007; Chong *et al.*, 2008). The disease can cause severe yield and grain quality losses in North America, Europe, South America, the Middle East and Australia. Yield losses in North America can range from 10% to 40% (Simons, 1970). A severe rust epiphytotic in 1957 in Illinois resulted in 20% yield loss valued at $16.9 million (Endo and Boewe, 1958). Humphreys and Mather (1996) also noted that *P. coronata* infection is associated with poor grain filling. In addition to grain yield, seed weight and test weight were reported to be negatively correlated with crown rust severity (Holland and Munkvold, 2001; Long *et al.*, 2006).

Oat crown rust has additional negative effects on oat beyond yield and poor grain quality. Severe crown rust can result in weak straw and lodging, which makes the crop difficult to harvest (Endo and Boewe, 1958). Also, juvenile winter oats infected by *P. coronata* have reduced cold resistance (Murphy, 1939).

2.2.2 Life cycle and disease symptoms

There are two different types of rust pathogens: autoecious rusts and heteroecious rusts. *Puccinia coronata* belongs to the heteroecious group. Autoecious rust fungi are able to complete their life cycles on a single host species while two unrelated hosts are required to complete the life cycle of a heteroecious rust (Littlefield, 1981). The complete life cycle of *P. coronata* consists of five different spore stages: basidiospores, pycniospores, aeciospores, urediniospores and teliospores. The telial stage of this fungus was first discovered and named *P. coronata* in 1837. Later, a close relationship between *P. coronata* on *A. sativa* and on *Rhamnus cathartica* L. (common buckthorn) was observed. *Avena spp.* and *Rhamnus spp.* are the essential hosts for *P. coronata* which are required to complete its life cycle (Simons, 1970).

The life cycle of *P. coronata* starts from teliospores in spring. Positive (+) and negative (-) nuclei fuse together in the germinated teliospores to form a diploid stage. After nuclear fusion, meiosis occurs. Four haploid basidiospores, two of (+) mating type and two of (-) mating type, are produced. The newly produced basidiospores have genotypes different from that of the haploid nuclei in the parental teliospores as a result of random assortment of chromosomes and genetic recombination (Simons, 1970; Littlefield, 1981). Basidiospores are able to be transferred by wind a few hundred meters to infect the alternate *Rhamnus* host. When a basidiospore arrives at a *Rhamnus* leaf under suitable environmental conditions, the basidiospores germinate and form appressoria. A penetration peg is developed from the appressorium to penetrate the *Rhamnus* cuticle and epidermis using high turgor pressure (Simons, 1970; Agrios, 2004).

After one to two weeks, pycnia are formed from the haploid mycelium on the upper surface of the *Rhamnus* leaf. Pycnia have a mainly epiphyllous growth on leaves, petioles, young stems and floral structures. Pycniospores that are produced at the tips of sporophores within the pycnia are small, round, orange-yellow, and slightly raised from the leaf.

Flexuous hyphae among paraphyses are also initiated from pycnia. The pycniospores, which are considered as the male gametes, are capable of entering the flexuous hyphae functioning as female receptive structures. The pycniospores cannot mate with flexuous hyphae that originate from the same pycnia. Each pycnium is either (+) mating type or (-) mating type. In order to mate successfully, a pycniospore is transferred by insects to compatible flexuous hyphae of the opposite mating type (Littlefield, 1981).

Aecia develop beneath the pycnia and grow on the underside of the infected *Rhamnus* leaf. When the nucleus of the pycniospore migrates to the aecial primordium from the flexuous hyphae, dikaryotic sporogenous cells with two opposite mating type nuclei are developed and are named aeciospores. Because several different genotypes of pycniospores migrate to one aecial primordium, one aecium can contain a number of different genotypes of aeciospores. After receiving several different pycniospore nuclei, an aecium is formed on the underside of the infected leaf. The aecium looks round and has tightly packed clusters of small orange or yellow cups with long chains. Aeciospores are borne in chains and dispersed by wind (Littlefield, 1981).

Aeciospores are released from *Rhamnus* to nearby Avena plants. Appressoria produced from germinated aeciospores form sub-stomatal vesicles inside host plants after penetration through the stomata. A dikaryotic mycelium developed from a dikaryotic aeciospore grows into the host tissue (Littlefield, 1981). Later, uredinia develop and produce several generations of dikaryotic urediniospores. On the main host (*Avena spp.*), uredinia appear on leaf blades and occasionally on the sheaths and floral structures. Bright orange-yellow urediniospores are produced in round to oblong uredinia (pustules). Uredinia length ranges up to five millimeters. Symptoms on resistant varieties range from small, light-colored flecks to small to medium-sized pustules surrounded by chlorotic or necrotic areas (Simons, 1970). Urediniospores can be carried by wind long distances (up to 150 km) to infect other *Avena* plants. Major yield loss and poor grain quality are caused by urediniospore infection (Eversmeyer and Kramer, 2000).

Urediniospores are surrounded by self-germination inhibitors. The inhibitors can be digested by hydrolytic enzymes. Urediniospores germinate on an *Avena* leaf when moisture and temperature reach optimal levels. A germ tube is produced from a germinated urediniospore which grows and elongates away from light because of negative phototropism (Staples and Macko, 1984). Once the germ tube reaches the host stomata, it forms an appressorium. A penetration peg grows from the appressorium and hyphae elongate to produce haustorial mother cells after penetration into the host leaf. A haustorial apparatus is formed when the haustorial mother cell contacts the host mesophyll cells (Harder, 1984). Nutrients from host cells are taken up by the haustorium to produce a uredinium. Large urediniospores are produced from the uredinium, which can infect additional *Avena* plants. Later, dikaryotic telia are formed to produce teliospores. These teliospores, which are clavate-oblong with dark-brown cell walls, are able to tolerate cold and germinate the next spring (Simons, 1970; Littlefield, 1981; Agrios, 2004).

2.2.3 Pathogen host range

Avena spp. are the main hosts for *P. coronata* in the anamorphic or uredinial stage. This includes *A. sativa* and *A. sterilis* (McCallum *et al*., 2007), *A. fatua* L. and *A. barbata* (McCallum *et al*., 2007; Carson, 2009a), as well as, *A. strigosa* Schreb., *A. insularis* Ladiz. and *A. murphyi* Ladiz. (Rines *et al*., 2007). During the teleomorph stage of *P. coronata*, common alternate *Rhamnus spp.* hosts found in North America include *R. cathartica, R. frangula* L., and *R. alnifolia L'Hér* (Simons, 1970; Simons, 1985; Leonard, 2002). Most recently *R. lycioides* L. has been identified as being a host for *P. coronata* in Tunisia (Hemmami *et al*. 2006).

2.2.4 Epidemiology of *P. coronata*

Crown rust development is favored by warm (20-25˚C) and humid weather, which is typical weather in June and July in North America. Urediniospores are spread by wind from the United States to Western Canada (Carson, 2008). In North America, *P. coronata* has two

disease transmitting patterns (Simons, 1970). The first pattern does not require participation of the alternate *Rhamnus* host (Eversmeyer and Kramer, 2000). Winter in southern North America allows urediniospores to survive on winter oats. Those urediniospores from northern Mexico and southern Texas move northwardly across the Great Plains into the Canadian Prairies in the spring (Agrios, 2004). This migration route is called the *Puccinia* pathway. Urediniospores in southern North America lose their viability in hot summer temperatures, but urediniospores in the northern North America retain their viability because of the relatively cool summer temperatures (Simons, 1970). In the fall, urediniospores from northern North America are transmitted back to southern North America and cause disease infection on juvenile winter oats. The urediniospores on winter oats survive the warmer winters in southern North America (Eversmeyer and Kramer, 2000).

The alternate *Rhamnus* host is involved in the second transmitting pattern (Simons, 1970). Teliospores overwinter on infected oat residue. Germinated teliospores go through meiosis to produce basidiospores in spring (Simons, 1970). *Rhamnus* around fields is infected by basidiospores from infected oat field residue. Later, aeciospores from *Rhamnus* cause disease infection in oat fields. Aeciospores can be moved up to 2.5 km (Eversmeyer and Kramer, 2000). Infected *Avena spp.* then produce urediniospores, which cause further disease infection. Telia on infected oat residue that is left in fields allows the pathogen to overwinter (Simons, 1970).

In the first transmitting pattern, massive urediniospore numbers disperse through the *Puccinia* pathway and cause severe disease epidemics in susceptible oat fields (Eversmeyer and Kramer, 2000). The second transmitting pattern, which harbors aeciospores carrying new recombinant genotypes, has the potential to generate new *P. coronata* races that are virulent on resistant oat cultivars (Leonard, 2007; Eversmeyer and Kramer, 2000). Both patterns are important sources of primary inoculum (Eversmeyer and Kramer, 2000).

2.2.5 New pathogenic forms of *P. coronata*

According to Knott (1989), sexual recombination, random mutation and somatic hybridization are three sources of pathogenic variability in wheat rust diseases. *Puccinia coronata* has similar patterns of pathogenic variability. Murphy (1935) mentioned that *Rhamnus* is responsible for the development of new races of *P. coronata*. An aecium can produce aeciospores with a large diversity of genotypes because of the following three reasons. First, every year newly produced basidiospores have different genotypes than the haploid nuclei in the parental teliospores as a result of random assortment of chromosomes and genetic recombination. Second, pycniospores fuse with the flexuous hyphae of the opposite mating type. Third, several genotypically different nuclei of pycniospores are able to migrate to one aecial primordium. Thus, one aecium can contain a number of different aeciospores genotypes (Littlefield, 1981). These sexual processes result in greater race diversity and is evident when *P. coronata* is compared to *P. triticina* (Chong *et al.*, 2011; McCallum *et al.*, 2011). *Puccinia triticina* causes wheat leaf rust and lacks a sexual reproductive cycle in the *Puccinia* pathway of North America.

A new race of *P. coronata* could also be produced by heterokaryosis or random mutation. For example, *P. coronata* race 228 was initially avirulent on the oat variety 'Saia' and virulent on 'Ukraine' while race 393 had an opposite virulence pattern on these two oat varieties. When the two races were inoculated on the same plant, a new race (race 229) was found to be virulent on both Saia and Ukraine. Somatic hybridization through hyphal anastomosis created an opportunity to form a new virulent *P. coronata* race (Bartos *et al.*, 1969; Simons, 1970). Simons (1970) also noted that one mutation occurs naturally in every 2,200 *P. coronata* infections on resistant varieties. However, new virulent *P. coronata* races could be raised relative easily under the extremely high selection pressure created from long term use of prevailing commercial oat varieties that contain a few major seedling resistance genes.

2.2.6 Disease rating methods

Three different disease rating methods are widely used for evaluation of oat crown rust: visual rating, digital image rating, and fungal DNA (FDNA) detection. Visual assessment is the most common method and has many different rating scales depending on the crown rust research group. For estimating seedling resistance, the disease rating scale developed by Murphy (1935) is the most common one. For evaluation in fields, the modified Cobb scale and infection type (IT) is widely used (Peterson *et al.*, 1948; Chong *et al.*, 2011). Annual Canadian crown rust disease surveys are conducted using a 0-9 rating scale (Xue and Chen, 2011).

Disease severity (DS), infection type (IT) and coefficient of infection (CI) are three key parameters in visual disease rating. IT is commonly used when evaluating seedling resistance genes. DS and CI are commonly applied during field evaluations. DS represents the actual percentage of leaf area occupied by rust pustules using the modified Cobb scale. In the modified Cobb scale, 37% of the actual leaf area covered with rust pustules is considered 100% DS (Peterson *et al*., 1948). Coefficient (CI = DS x IT) is considered a better parameter to examine and represent actual rust damage in fields because CI has a higher correlation to yield and test weight loss (Murphy *et al.*, 1940; Table 2.1).

Table 2.1 Correlations between yield loss, test weight loss and three different crown rust estimation methods (from Murphy *et al*., 1940).

	Severity	Infection type	Coefficient
Yield loss	0.73	0.75	0.8
Test weight loss	0.68	0.64	0.74

Various research groups estimate disease severity from different parts of the infected host plant. According to Chisholm *et al.* (2006), flag leaf, flag minus one leaf and flag minus two leaves supply 43%, 23% and 7%, respectively, of the nutrients impacting grain yield. In field oat rust estimation, many researchers focus on the flag leaves since these leaves produce

the majority of the energy for seed production (Carson *et al.*, 2009a; Carson *et al.*, 2009b; Acevedo *et al.*, 2010; Cabral *et al.*, 2011). Other pathologists have also recorded symptoms on flag minus one leaf (Portyanko *et al.*, 2005; Aung *et al.*, 2010; Xue and Chen, 2011), as well as the three uppermost leaves on the oat plant (Leonard, 2002; Acevedo *et al.*, 2010).

Digital image analysis has been suggested as one method to overcome differences in rating results among individuals (Nutter *et al.*, 1993). Infected host leaves are collected and scanned which allows rust pustule coverage of infected leaves to be precisely measured using image analysis software (Diaz-Lago *et al.*, 2003; Jackson *et al.*, 2008). Another method suggested to improve the precision of rust rating is fungal DNA detection (Jackson *et al.*, 2006). DNA from rust infected leaves is extracted and FDNA is amplified by pathogen-specific primers and measured using quantitative real-time polymerase chain reaction (q-PCR). The FDNA method is claimed to provide greater mean difference between parents than visual measurements (Jackson *et al.*, 2006; Jackson *et al.*, 2007; Jackson *et al.*, 2008). Studies by Jackson *et al.* (2007; 2008) showed that quantitative real time polymerase chain reaction (q-PCR) of FDNA also increased accuracy and precision in quantitative trait locus (QTL) detection.

2.2.7 Disease control methods

There are four common ways to control oat crown rust: early seeding, *R. cathartica* removal, fungicide application and resistant cultivar use. Many uredinia overwinter on infected oats in the southern United States and Mexico. Through the *Puccinia* pathway, numerous urediniospores move from the United States to Canada in June. Rust diseases tend to cause more damage to crops when disease onset is earlier. Early seeding provides additional time for oat plant development before urediniospore arrival (McCallum *et al.*, 2007). *Rhamnus spp.* provide primary disease inoculum to oat fields earlier than urediniospore arrival from the southern United States and also have the potential to release new virulent races. Since *R. cathartica* commonly occurs in North America, removal of *R.*

cathartica close to oat fields is recommended, although it is relatively hard and laborious work (McCallum *et al.*, 2007).

According to the Government of Saskatchewan (2009), crown rust can be controlled by fungicides, such as Bumper 418EC (propiconazole), Tilt (propiconazole), Folicur (tebuconazole) and Headline EC (pyraclostrobin). Prices of these fungicides vary from $6.50/acre to $17.2/acre, based on 2008 data, which increases crop input costs. A concern with frequent fungicide application is the potential for *P. coronata* to develop resistance to these fungicides under high selection pressure (McCallum *et al.*, 2007). Resistant oat varieties have been the main management method in North America, but fungicide application is becoming common. Resistant varieties are estimated to have saved farmers $0.4 billion between 1995 and 2005 (McCallum *et al.*, 2007).

2.3 Host Resistance

2.3.1 Plant non-host defense

Plants are dependent on an intricate defense system to impede pathogen invasion (Chisholm *et al.*, 2006). Three general defense mechanisms are relied upon to prevent pathogen infection: preformed or passive defense, basal defense, and effector-triggered immunity (ETI). Preformed or passive defense and basal defense are classified as Type I non-host resistance. Type I resistance shows no visible symptoms, however several molecular changes such as pathogenesis-related (PR) gene expression still occur. Type I resistance can take the form of physical barriers (cell walls, cytoskeleton, actin microfilament etc.) and basal plant defenses that prevent further movement of the pathogen on the host. ETI is categorized as Type II non-host resistance. Type II resistance is the most common phenomenon of non-host resistance and is similar to the incompatible gene-for-gene interaction. A rapid hypersensitive response with cell death is a common aspect of Type II non-host resistance defense (Mysore and Ryu, 2004)

2.3.1.1 Preformed or passive defense

When favorable environmental conditions arise, pathogens start to infect plants. Preformed or passive defense is the first obstacle to prevent the pathogen from infecting host plants (Mysore and Ryu, 2004). Among pre-existing structural defenses, a thick cuticle can prevent direct penetration from some pathogens (Walters, 2011), as can the cell wall cytoskeleton (Mysore and Ryu, 2004). Stomatal closure can prevent bacteria from penetrating through stomata (Walters, 2011). Among pre-existing chemical defenses, many existing secondary metabolites act as antimicrobial compounds during infection. Phenolics, terpenoids, and nitrogen-containing organic compounds are three groups of secondary plant metabolites involved in pre-existing chemical defense (Walters, 2011).

2.3.1.2 Basal defense

When pathogens pass through preformed or passive defenses and continue to invade plants, pathogen-associated molecular patterns (PAMPs) from the pathogens are detected by pattern-recognition receptors (PRRs) on host cell surfaces and induce basal defense reactions (also called PAMP-triggered immunity (PTI) or innate immunity). PTI is able to repel a wide range of pathogens. PAMPs or microbe-associated molecular patterns (MAMPs) are highly conserved molecules released by pathogens that assist with infection of the host (Dodds and Rathjen, 2010). Disfunction of PAMP molecules results in the absence of pathogen recognition by the host and subsequent pathogen virulence (Naito *et al.*, 2008).

The most common MAMPs are flagellin (flg), elongation factor Tu (EF-Tu), lipopolysaccharide (LPS) and peptidoglycan (PGN) (Aslam *et al.*, 2009). Flagellin is the peptide component of the motility organ flagellum and is the best characterized PAMP from phytopathogenic bacteria (Aslam *et al.*, 2009). Flagellin in *Pseudomonas syringae* induces visible alkalization and elicits a type of necrotic or hypersensitive response in tomato plants (Felix *et al.*, 1999). Flg22 is a 22-amino-acid peptide, which is the most conserved part in the N-terminus of flagellin (Felix *et al.*, 1999) and is used to represent the presence of flagellin in many research experiments (Aslam *et al.*, 2009). Gomez-Gomez and Boller (2002)

discovered the leucine-rich repeat receptor-like kinase (LRR-RLK) flagellin-sensing 2-like protein (FLS2) gene. FLS2 is considered an important flg22 receptor in host plants and triggers defense genes PR1 and PDF1.2 (Chinchilla *et al.*, 2006; Aslam *et al.*, 2009). Felix *et al.* (1999) showed that the plant defense response can be quickly induced by FLS2 within a few minutes. Plants carrying FLS2 are resistant to *P. syringae* pv. *tomato* DC3000. The silencing of FLS2 results in susceptibility to *P. syringae* pv. *tomato* DC3000 (Zipfel *et al.*, 2004).

Elongation factor Tu (EF-Tu), which aggregates and forms on the bacterial cell surface, helps pathogens attach to host plants and is the most abundant bacterial protein (Kunze et al., 2004; Zipfel *et al.*, 2006; Zipfel, 2008; Aslam *et al.*, 2009). Elf18, a highly conserved N-acetylated 18 amino acid peptide, is used to represent EF-Tu in research (Kunze *et al.*, 2004). The LRR-RLK EF-Tu receptor (EFR) from host plants is able to recognize EF-Tu from pathogens in the host plasma membrane. Basal resistance is triggered and plant resistance genes such as PR1, PDF1.2 and PAL1 are activated (Zipfel *et al.*, 2006; Zipfel, 2008; Aslam *et al.*, 2009). Absence or silencing of EFR-1 causes failure of recognition of EF-Tu resulting in susceptible disease reactions (Zipfel *et al.*, 2006).

During PTI, certain plant structures are reinforced to stop the penetration of pathogens. Cell wall appositions (CWAs) are formed to reinforce plant cell walls and inhibit invading pathogens. Papillae, which are composed of phenolics, callose, peroxidases and cell wall material, are produced by plants beneath the site of the pathogen infection peg. Papillae also accumulate reactive oxygen species (ROS) to protect against pathogen entrance (Walters, 2011). Formation of tyloses helps block pathogens from spreading through xylem (Clerivet *et al.*, 2000).

PEN1, *PEN2* and *PEN 3* are three important genes that prevent pathogen penetration via PTI in *Arabidopsis*. Syntaxin, encoded by *PEN1*, is associated with papilla-related vesicles (Mysore and Ryu, 2004). In sites where pathogens attempt to penetrate, vesicles containing

syntaxin are docked at the target membrane and are associated with building papillae and an antifungal environment (Schweizer, 2007). *PEN2* is involved with cell walls (Mysore and Ryu, 2004) and encodes a peroxisome-associated glycosyl hydrolase that is associated with production of an antifungal compounds (Humphry *et al.,* 2006; Schweizer, 2007). *PEN3* encodes an adenosine-5'-triphosphate (ATP)-binding cassette multi-drug transporter (Humphry *et al.,* 2006) which is thought to be involved in transportation of the antifungal product of *PEN2* into the apoplast (Schweizer, 2007).

2.3.1.3 Effector-triggered immunity

During the long battle between plants and pathogens, pathogens have adapted by secreting a vast range of effectors into plant cells to modify host proteins (Greenshields and Jones, 2008). These effectors are able to detoxify host enzymes and overcome the toxic effect of preformed antimicrobial secondary metabolites and PTI (Walters, 2011). For example, oligosaccharides of chitin, which are produced through the action of plant chitinases, are an important PAMP. Increased levels of chitin oligosaccharide triggers medium alkalization and leads to PTI in host plants. *Cladosporium fulvum*, a fungal pathogen of tomoato causing tomato leaf mold, secrets an effector (Ecp6) to suppress activation of chitin PTI by specific binding to chitin oligosaccharides. Binding of chitin oligosaccharide by Ecp6 prevents medium alkalization and activation of PTI (Jonge *et al*., 2010).

For those pathogen effectors that successfully defeat PTI, plants have developed a second pathogen-sensing mechanism in the plant cytoplasm and cell membrane to recognize those effectors, trigger resistance (R) genes and constrain the invasion (Walters, 2011). In this second mechanism, a group of plant receptor proteins containing nuclear binding domains (NB) and leucine-rich repeats (LRR) are used to recognize the microbial effectors intracellularly and trigger downstream signaling pathways. This is called effector triggered immunity (ETI). In ETI, pathogen effectors change from virulence components to avirulence components. Thus, the genes producing these effectors are named avirulence (Avr) genes. For example, as a result of flagellin from *P. syringae* pv. *phaseolicola* being detected by

FLS2 in host PAMP-trigged resistance, the effector AvrPtoB from *P. syringae* pv. *phaseolicola* evolved to inhibit the basal defense triggered by flagellin (Torres *et al.*, 2006). In ETI, the Pto resistance protein in plants recognize AvrPtoB to subsequently trigger programmed cell death (PCD) (Abramovitch and Martin, 2005).

2.3.2 Gene-for-gene resistance

The majority of known race-specific resistance genes used in oat breeding programs are involved in gene-for-gene resistance. In the gene-for-gene system, an incompatible interaction results from a resistance gene in the host detecting an avirulence gene in the pathogen (Flor, 1956). Typically, resistance is dominant in the host and avirulence is dominant in the pathogen. Only a single incompatible interaction is needed for host resistance. Type II non-host resistance and race specific gene-for-gene resistance share some similarities. They both trigger hypersensitive reactions, active reactive oxygen species (ROS) and increase lignification in cell walls. Both gene-for-gene resistance and Type II non-host resistance rely on the recognition of avirulence genes or effectors to trigger defense mechanisms. Pathogens can avoid detection in gene-for-gene or Type II non-host by modifying effectors. Type II non-host resistance in the *Arabidopsis* pathogen *P. syringae* pv. *phaseolicola* is similar to gene-for-gene resistance mediated by RPS2. However, it cannot be concluded that Type II non-host resistance and gene-for-gene are the same. Type II non-host resistance governed by PEN1/ROR2 is different from a race-specific R gene. It is suspected that Type II non-host resistance and gene-for-gene resistance have different signal transduction pathways, but with significant amount of cross-talk or convergence between these two pathways (Mysore and Ryu, 2004)

2.4 Resistance Breeding

2.4.1 Breeding for crown rust resistance

Oat cultivars were susceptible to crown rust before the 1930s. The first resistant oat cultivar was released in 1935. From the mid-1940s to the mid-1950s, 'Victoria' and lines inheriting resistance from Victoria (*Pc2, Pc11* and other undefined resistance genes) were

widely deployed in North America. Eventually isolates of *P. coronata* overcame these resistant cultivars by the end of the 1950s. *Avena sterilis*, from which more than 45 effective resistance genes have been obtained, became a major source of crown rust resistance genes beginning in the 1960s (McCallum *et al.*, 2007; Leonard, 2007). Through cross-pollination of *A. sativa* and *A. sterilis*, resistance genes were moved from wild oats to cultivated oats and identified by seedling disease tests. Many seedling resistance genes to crown rust have been detected by this method (McCallum *et al.*, 2007). *Pc38* and *Pc39* were transferred from *A. sterilis* and released in the early 1980s. However, *P. coronata* defeated *Pc38* and *Pc39* in the late 1980s (Chong and Seaman, 1997; McCallum *et al.*, 2007). *Pc48* was widely used in the 1990s, but was overcome in 2001 (Chong and Zegeye, 2004). The cultivar 'AC Assiniboia' with the combination of *Pc38/39/68* was released in 1995. The first virulence on AC Assiniboia was detected in 1999 (McCallum *et al.*, 2007; Leonard, 2007). By 2005, virulence to *Pc68* was common in the Canadian *P. coronata* population (Chong *et al.*, 2008). Thus, a clear pattern of short lived resistance is evident in the oat - *P. coronata* pathosystem in Western Canada.

Other *Avena spp.* besides *A. sterilis* have been involved in developing crown rust resistance (Rooney *et al.*, 1994; Aung *et al.*, 2010). *Pc91* and *Pc94* are major crown rust resistance genes deployed in Canadian cultivars (Chong *et al.*, 2011). *Pc91* originated from *A. magna* (McMullen *et al.*, 2005) while *Pc94* was attained from *A. strigosa* (Fetch *et al.*, 2007). Carson (2009a) also suggested that *A. barbata* carries great potential for resistance genes. However, Carson (2009a) noted that there is no evidence to demonstrate that seedling resistance genes from diploid or tetraploid *Avena spp.* are more durable than those resistance genes from *A. sterilis.* Virulence to the *Pc91* has recently been reported (McCartney *et al.*, 2011) as have virulent isolates to *Pc94* (Chong *et al.*, 2011).

2.4.2 Limitations of seedling resistance genes

Seedling race-specific resistance genes are relatively easy to use in breeding programs (Carson, 2008; Graichen *et al.*, 2010). However, widespread commercial production of oat

varieties with seedling resistance genes leads to the development of virulence to those genes. Evidence has shown that widespread commercial production of resistant cultivars with few major resistances generates greater selection pressure for new virulent races (Chong *et al.*, 2011). In addition, some *P. coronata* races already exist with virulence to newly released resistance genes (Chong and Kolmer, 1993). At times those *P. coronata* races which develop virulence to a specific resistance gene also quickly become virulent to other resistance genes (Chong and Brown, 1996; Leonard, 2007). In these cases, *R. cathartica* is also thought to contribute to the breakdown of resistance gene pyramids. These limitations challenge oat breeders to consistently discover and deploy new seedling resistance genes into oat varieties to maintain crown rust resistance.

2.4.3 Breeding durable disease resistance

One classic durable gene-for-gene resistance gene is the *mlo* resistance in barley against powdery mildew. Powdery mildew of barley is caused by *Blumeria graminis* f.sp. *hordei* (Bgh). Presence of the MLO protein in host plants is essential and critical for powdery mildew colonization. Absence of this protein results in failure of the pathogen to enter epidermal host cells. The pathogen is stopped at the pre-invasive stage because host plants form callose-containing papillae in epidermal cells inhibiting infection and trigger cell death to terminate haustoria growth. Barley varieties without the MLO protein (*mlo* resistance) have consistently been resistant to Bgh for more than 25 years (Humphry *et al.,* 2006).

In *mlo*-mediated resistance research with *A. thaliana* it was shown that *mlo* resistance did not rely on salicylic acid, jasmonic acid, or ethylene-mediated signaling (Humphry *et al.,* 2006). Further, the genes *Ror1* and *Ror2* were required for full function of *mlo* resistance (Freialdenhoven *et al.*, 1996). Loss of non-host resistance genes *Ror1* or *Ror2* in barley inhibited *mlo* resistance. As noted previously, *PEN1*, *PEN2*, and *PEN3* are three essential genes required to prevent penetration by many different pathogen in PTI (Mysore and Ryu, 2004). *Ror2* in barley is an orthologue of the Arabidopsis PEN1 syntaxin, but the identity of *Ror1* still remains unknown (Humphry *et al.,* 2006). Additionally, Humphry *et al.* (2006)

listed several similarities between *mlo* resistance and non-host resistance and concluded that the *mlo* resistance is a type of non-host resistance or innate immunity (Humphry *et al.,* 2006), and not typical of ETI-based resistance genes.

The adult plant resistance gene *Lr34* is a durable leaf rust resistance gene in wheat (Dyck, 1987). This gene is present in many Canadian wheat cultivars and has not been overcome by a virulent leaf rust race despite years of widespread deployment (Kolmer *et al*., 2007). Krattinger *et al*. (2009) cloned *Lr34* and discovered that it produced an ATP-binding cassette (ABC) transporter and, therefore, was not related to other ETI-based resistance genes.   Cloning of the *Lr34* gene revealed that the resistant *Lr34* allele contains a three base pair deletion not present in the susceptible *Lr34* allele (Krattinger *et al*., 2009).   In wheat leaf rust resistance breeding, *Lr34* has been successfully pyramided with other leaf rust resistance genes and other disease resistance genes. Orthologous *Lr34* genes are currently screened for in *Oryza sativa* and *Sorghum bicolor* (Krattinger *et al*., 2010). Oat researchers hope to discover durable resistance genes like *Lr34* or *mlo* for the control of crown rust, and to combine these genes with seedling resistance genes to control *P. coronata* over the long term (McCallum *et al.*, 2007).

3. Genetic Analysis of Crown Rust Resistance in *Avena sterilis* Accessions

3.1 Introduction

Oat crown rust (caused by *Puccinia coronata* Corda f. sp. *avenae* Eriks) is considered the most economically important disease in cultivated oat (*Avena sativa* L.) in Western Canada (McCallum *et al.*, 2007; Leonard, 2007; Chong *et al.*, 2008). Moderate to severe yield losses are caused by this disease. Since ideal weather conditions for oat growth also favor crown rust development, theoretically fields with high yield potential can suffer significant yield losses. Pathogen growth is maximal during humid days in June and July when temperatures reach 20-25˚C.

In North America, disease resistance breeding is used to manage oat crown rust. *Avena sterilis* L. accessions have been the main source of crown rust resistance genes due to its reproductive compatibility with *A. sativa*. Through cross-pollination and seedling tests, resistance genes have been transferred from *A. sterilis* into cultivated oats and have been evaluated (McCallum *et al.*, 2007). The resistance transferred from *A. sterilis* is typically expressed in the early seedling stage based upon a gene-for-gene interaction. Consequently, the rust resistance genes from *A. sterilis* have been effective for only a few years before being overcome by new *P. coronata* isolates (McCallum *et al.*, 2007; Chong *et al.*, 2008). Recently, there have been studies on the identification and transmission of novel resistance genes from other *Avena spp*. One current major effective crown rust seedling gene, *Pc91*, was originally transferred from tetraploid *A. magna* Murphy et. Terrel. (McMullen *et al.*, 2005). A second important gene, *Pc94*, was obtained from diploid *A. strigosa* Schreb. (Fetch *et al.*, 2007). Carson (2009a) also suggested that tetraploid *A. barbata* Pott ex Link holds great potential as a source of resistance genes. In this study, six *A. sterilis* accessions were chosen for study and discovery of putatively new seedling resistance genes.

3.2 Materials and Methods

3.2.1 Population development

Six *A. sterilis* accessions (PI311623-4, PI333561, PI334672-5, PI335562, PI375506 and PI375547) were obtained from the Germplasm Resources Information Network (GRIN), National Genetic Resource Program, Agricultural Research Service, United States Department of Agriculture (USDA) (Table 3.1). These accessions demonstrated good resistance to crown rust isolates CR13, CR185, CR223, CR241, CR249, CR254, CR257, CR258 and CR259 (Dr. Curt McCartney, unpublished data; see Appendix A for race nomenclature of these isolates). These nine isolates together are virulent to twenty-nine crown rust seedling resistance genes (*Pc14/35/38/39/40/45/46/47/48/52/54/55/56/58/9/60/61/63/67/68/70/71/91/94/96/101/102/103/104*), some of which are widely used in North American oat cultivars (Dr. James Chong, unpublished data). Since these six *A. sterilis* accessions were resistant to these isolates, they had potential value for discovering new major resistance genes. In the winter of 2008, the six *A. sterilis* accessions were used as male parents in crosses with the cultivar AC Morgan (Kibite and Menzies, 2001), which was susceptible to all known Canadian isolates of *P. coronata*.

Table 3.1 Original collection information for six *Avena sterilis* accessions from Germplasm Resources Information Network, National Genetic Resource Program, Agricultural Research Service, United States Department of Agriculture.

Line	Collection Location	Collecting Institution	Received Date
PI311623-4	Northern, Israel	Hebrew University	Feb. 8, 1966
PI333561	Haifa, Israel	Hebrew University	Aug. 1, 1968
PI334672-5	Northern, Israel	Hebrew University	Aug. 1, 1968
PI335562	Haifa, Israel	Hebrew University	Aug. 1, 1968
PI375506	Tel Aviv, Israel	Tel Aviv University	Feb. 23, 1972
PI375547	Northern, Israel	Tel Aviv University	Feb. 23, 1972

F$_1$ hybrids, F$_2$ populations and F$_{2:3}$ families from these six crosses were developed. AC Morgan panicles were emasculated just as they emerged from the flag leaf. Stigmas were pollinated 3 to 5 days after emasculation. The F$_2$ and subsequence F$_{2:3}$ families were developed from as few F$_1$ plants as possible. A single F$_1$ plant developed the segregating populations for the crosses AC Morgan x PI335562, AC Morgan x PI375506 and AC Morgan x PI375547. More than one F$_1$ plant developed the segregating populations for the crosses AC Morgan x PI311623-4, AC Morgan x PI333561 and AC Morgan x PI334672-5. The population development of AC Morgan x PI333561 is described as an example of *A. sterilis* accession population development in this study. Twelve PI333561 plants and three AC Morgan plants were planted. One panicle was emasculated on each AC Morgan plant for crossing. Those panicles were named X pan-1, X pan-2 and X pan-3. Pollen from several different PI333561 plants was used at each time of pollination (Table 3.2).

Table 3.2 Detailed information for crosses made between three AC Morgan female parents and several PI333561 male parents at the University of Saskatchewan in 2008.

Panicle name	Male plants	Pollination dates	Seed set
AC Morgan X pan-1	PI333561	Apr 30th , May 1st and May 2nd	2
AC Morgan X pan-2	PI333561	May 3rd	1
AC Morgan X pan-3	PI333561	May 13th and May 14th	6

3.2.2 Disease evaluation

The *P. coronata* isolate used in this study was CR259 (race LQCB-91), supplied by Dr. James Chong (Cereal Research Center, Agriculture and Agri-Food Canada, Winnipeg, Manitoba, Canada). This isolate was derived from a single pustule and is virulent on seedling resistance genes *Pc35/38/39/40/55/59/60/61/63/91*. The urediniospores of isolate CR259 were increased by inoculating the urediniospores on the susceptible cultivar AC Morgan. Urediniospores collected from infected leaves were dried in a desiccator in a fridge and then stored in a -80 °C freezer.

Plants were grown in chambers with a 16 hour photoperiod. Day temperature was 21°C with 85% humidity while night temperature was 19°C with 85% humidity. Seedling oat plants were inoculated at the complete two leaf stage with urediniospores. Urediniospores were heat-shocked in a 40°C water bath for five minutes after removing from a -80 °C freezer. Four root trainers of plants (32 plants per trainer) were sprayed with a fine suspension of 0.01g urediniospores in 900 mL Bayol35 oil, a light mineral oil (Esso Canada, Toronto, ON). According to Simons (1970), high moisture and dark, along with temperatures between 18 to 22°C, are critical for urediospore germination. Thus, the sprayed plants in this study were incubated in a 100% humidity chamber for at least 17 hours in the dark (18°C). After inoculation, plants were moved back to growth chambers.

Infection types (ITs) were scored using a 0-4 rating scale at 11 or 12 days post-inoculation (Murphy, 1935). ITs of "0", ";", "1", and "2" were considered resistant and ITs of "3" and "4" were susceptible. Pustule sizes and leaf color around pustules were two key parameters in this scale. Necrosis and chlorosis of host tissues around pustules, caused by a hypersensitive reaction or plant resistance genes, indicated a strong resistant defense reaction in hosts. Light green leaf color around pustules indicated that haustoria successfully established in plant tissues and absorbed nutrients from host cells. Those urediniospores surrounded by light green leaf color reflected a susceptible reaction in hosts.

3.2.3 Statistical analysis

In the F_2 generation, two hundred and twenty four plants were evaluated in each cross. Individuals in each cross were categorized into either the resistant group or susceptible group. The Chi-square test with the Yates correction term was used to determine the goodness-of-fit to expected segregation ratios. When testing populations with only two classes (i.e. the resistant group and the susceptible group), the Chi-square test with the Yates correction term is considered more accurate than the general Chi-square analysis (Strickberger, 1985). Since the purpose of this study was to identify new putative broadly effective resistance genes, the populations that appeared to carry single genes in the F_2 generation were further evaluated in

$F_{2:3}$ family tests. Twenty-four plants were tested in each F_3 family and at least one hundred and twenty families were evaluated in each cross. According to the number of resistant and susceptible plants in each family, each family was classified as homozygous resistant (HR), segregating (SEG) or homozygous susceptible (HS). Segregation ratios (HR: SEG: HS) in $F_{2:3}$ family tests were evaluated against expected ratios using the general Chi-square test. The 2 x 2 contingency test was conducted to determine the homogeneity of F_2 or F_3 results derived from different F_1 plants of the same cross. If F_2 or $F_{2:3}$ data obtained from different F_1 hybrids of the same cross were determined to be homogeneous, all F_2 or F_3 data from the same cross was pooled together.

3.3 Results

3.3.1 Disease rating for parental lines and F_1 hybrids

Resistant parental lines, the susceptible parental line (AC Morgan) and one standard crown rust gene differential (*Pc91*) were used as checks in the F_2 generation and F_3 family tests. As shown in Table 3.3, it was confirmed that all resistant parental lines were highly resistant (IT) to crown rust isolate CR259, while AC Morgan and the differential *Pc91* were highly susceptible to the CR259 isolate.

3.3.2 Chi-square analysis of disease reaction in six different crosses

3.3.2.1 Chi-square analysis of AC Morgan x PI311623-4

In the cross AC Morgan x PI311623-4, the F_1 hybrids were resistant to the crown rust isolate CR259 indicating resistance was inherited in a dominant manner (Table 3.3). F_2 plants from the first F_1 plant segregated to 96:10 (resistant:susceptible), which fit the 15:1 ratio (X^2 = 1.33, P = 0.25) (Table 3.4). F_2 plants from the second F_1 plant segregated to 102:10 (resistant:susceptible), which also fit a 15:1 ratio (X^2 = 0.95, P = 0.33) (Table 3.4). In the 2 x 2 contingency test, F_2 results from the first and second F_1 plants were pooled together (X^2 = 0.02, P = 0.9). The overall F_2 population consisted of 198 resistant plants and 20 susceptible plants, which fit a 15:1 ratio (X^2 = 2.7, P = 0.1). The data is consistent with PI311623-4 carrying two dominant genes controlling resistance, resulting in duplicate dominant epistasis.

Table 3.3 Crown rust disease ratings for six *Avena sterilis* accessions, oat cultivar AC Morgan, F$_1$ hybrids from the six crosses and the *Pc91* crown rust resistance gene differential.

Line	Disease Rating					
	0	;	1	2	3	4
PI311623-4 (Inbred)	12					
AC Morgan x PI311623-4 F$_1$ hybrids	4					
PI333561 (Inbred)	7	25				
AC Morgan x PI333561 F$_1$ hybrids		1	3			
PI334672-5 (Inbred)	88	42	2			
AC Morgan x PI334672-5 F$_1$ hybrids	1	3				
PI335562 (Inbred)	3	5	1			
AC Morgan x PI335562 F$_1$ hybrids	1	1	1			
PI375506 (Inbred)	25	29	21	3		
AC Morgan x PI375506 F$_1$ hybrids	2	1	1			
PI375547 (Inbred)	1	40	40	22		
AC Morgan x PI375547 F$_1$ hybrids		2	2			
AC Morgan (Inbred)					87	384
Pc91 differential (Inbred)					54	400

Table 3.4 Segregation ratio and chi-square analysis of crown rust reaction in the oat cross AC Morgan x PI311623-4.

Generation	Total	Resistant	Susceptible	Expected Ratio	X^2	P
F$_1$	4	4	0			
F$_2$ (F$_{1-1}$)	106	96	10	15:1	1.33	0.25
F$_2$ (F$_{1-2}$)	112	102	10	15:1	0.95	0.33
F$_2$ (pooled)	218	198	20	15:1	2.7	0.1

3.3.2.2 Chi-square analysis of AC Morgan x PI333561

In the cross AC Morgan x PI333561, all F_1 hybrids were resistant, indicating resistance to CR259 was inherited in a dominant manner (Table 3.3). In the F_2 generation test, 224 seeds derived from three F_1 plants were evaluated. F_2 plants from the first F_1 plant (F_1 Plant1 from AC Morgan x PI333561 X-pan3) segregated 76:5 (resistant:susceptible), which closely fitted a 15:1 ratio ($X^2 = 0.04$, $P = 0.84$), and F_2 plants derived from the second F_1 plant (F_1 Plant2 from AC Morgan x PI333561 X-pan3) segregated 66:6 (resistant:susceptible), which also fitted with the 15:1 ratio ($X^2 = 0.24$, $P = 0.63$) (Table 3.5). However, F_2 progeny from the third F_1 plant (F_1 Plant3 from AC Morgan x PI333561 X-pan3) segregated 47:10 (resistant:susceptible), which fit both a 3:1 ratio ($X^2 = 1.32$, $P = 0.25$) and a 13:3 ratio ($X^2 = 0.004$, $P = 0.95$) (Table 3.5).

Table 3.5 Segregation ratio and chi-square analysis of crown rust reaction in the oat cross AC Morgan x PI333561.

Generation	Total	Resistant	Susceptible	Expected Ratio	X^2	P
F_1	4	4	0			
F_2 (F_{1-1})	81	76	5	15:1	0.04	0.84
F_2 (F_{1-2})	72	66	6	15:1	0.24	0.63
F_2 (F_{1-3})	57	47	10	3:1	1.32	0.25
				13:3	0.004	0.95
F_2 (F_{1-7})	204	190	14	15:1	0.047	0.83
F_2 (F_{1-8})	208	171	37	3:1	5.39	0.02
				13:3	0.07	0.79
F_2 (F_{1-9})	208	149	39	3:1	1.6	0.21
				13:3	0.369	0.54
F_2 ($F_{1-3\&9 \ pooled}$)	245	196	49	3:1	3.01	0.08
				13:3	0.18	0.67

Based on the 2 x 2 contingency tests, F_2 results from the first F_1 plant could not be pooled with the F_2 data from the third F_1 plant (Table 3.6). There are two interpretations of this data. First, PI333561 could be a heterogeneous mixture with lines variable for the number of genes and/or the gene action controlling resistance to CR259. Second, the pollen used to create the third F_1 plant was derived from a heterozygous plant. Because seed from the original male parents was not separately harvested, it was not possible to evaluate selfed progeny seeds from the third parent and determine the existence of heterozygosity. Because of the fairly small F_2 populations used and to help resolve the two possible ratios identified in the F_2 population derived from the third parent, larger F_2 populations were developed from three additional F_1 plants (F_{1-7}, F_{1-8} and F_{1-9}).

Table 3.6 *P*-values of the 2 x 2 chi-square contingency tests for crown rust reaction among all F_2 generation tests in the oat cross AC Morgan x PI333561.

P -value	F_2 (F_{1-1})	F_2 (F_{1-2})	F_2 (F_{1-3})	F_2 (F_{1-7})	F_2 (F_{1-8})	F_2 (F_{1-9})
F_2 (F_{1-1})	1	0.61	0.03	0.83	0.01	0.003
F_2 (F_{1-2})		1	0.12	0.68	0.06	0.02
F_2 (F_{1-3})			1	0.01	0.97	0.60
F_2 (F_{1-7})				1	0.001	<0.001
F_2 (F_{1-8})					1	0.46
F_2 (F_{1-9})						1

Results from the retest confirmed the same segregation ratio of 3:1 or 13:3 for the F_2 population derived from the ninth F_1 parent (F_1 Plant7 from AC Morgan x PI333561 X-pan1) (Table 3.5). This data could also be pooled with the original test (Table 3.6) and together a 3:1 or 13:3 ratio was observed (Table 3.5). Moreover, F_2 plants from the seventh F_1 plant (F_1 Plant1 from AC Morgan x PI333561 X-pan1) segregated 190:14 (resistant:susceptible),

which closely fit a 15:1 ratio ($X^2 = 0.047$, $P = 0.83$) (Table 3.5). F_2 plants from the eighth F_1 plant (F_1 Plant1 from AC Morgan x PI333561 X-pan2) segregated 171:37 (resistant:susceptible), which closely fit a 13:3 ratio ($X^2 = 0.07$, $P = 0.79$) (Table 3.5). According to Table 3.2 and Table 3.5, different panicles and even different F_1 seeds from the same panicles gave rise to F_2 populations with different segregation ratios. Heterogeneity may exist within the PI333561 parental lines. This is hard to explain given the consistent results observed with the original isolate screening on the line (Table 3.7). The most likely conclusion is that two genes exist within the line, one gene is dominant and the other gene is incompletely dominant. The expression of the incompletely dominant gene is likely variable and could be influenced by environmental conditions.

Table 3.7 Crown rust ratings for individual PI333561 plants against a range of oat crown rust isolates.

Isolate	Time	No. Plants	Disease Rating
CR192	Fall 08	3	0 0 ;
CR13	Summer 09	3	2 2 2
CR185	Summer 09	3	; 0 0
CR223	Summer 09	3	2 1^+ 2
CR241	Summer 09	4	1 1 ; 0
CR254	Summer 09	4	1 1 1 1
CR257	Summer 09	4	; ; ; 0
CR258	Summer 09	4	0 0 0 0
CR259	Summer 09	4	; ; ; ;
CR185	Fall 09	2	0 ;
CR258	Fall 09	3	1 ; ;
CR259	Fall 09	3	1 1 ;

3.3.2.3 Chi-square analysis of AC Morgan x PI334672-5

In the cross of AC Morgan x PI334672-5, the F_1 hybrids were resistant to the crown rust isolate CR259 indicating resistance was also inherited in a dominant manner (Table 3.3). F_2 plants from the first F_1 plant segregated 105:26 (resistant:susceptible), which either fit a 3:1 ratio ($X^2 =$ 1.59, $P = 0.21$) or the 13:3 ratio ($X^2 = 0.044$, $P = 0.83$) (Table 3.8). F_2 plants from the second F_1 plant segregated 58:22 (resistant:susceptible), which also either fit the 3:1 ratio ($X^2 = 0.15$, $P = 0.7$) or the 13:3 ratio ($X^2 = 3.47$, $P = 0.06$) (Table 3.8). Based on the 2 x 2 contingency test, F_2 results from the first and second F_1 plants could be combined ($X^2 =$ 1.66, $P = 0.2$). The overall F_2 population consisted of 163 resistant plants and 48 susceptible plants, consistent with either a 3:1 ratio ($X^2 = 0.46$, $P = 0.5$) or a 13:3 ratio ($X^2 = 1.96$, $P = 0.16$) (Table 3.8). The F_2 data suggested there was 1 dominant resistance gene or 2 resistance genes with dominant and recessive epistasis to CR259 in this cross.

F_3 families derived from the first F_1 plant segregated 26:32:5 (resistant:segregating:susceptible), which is fit a 7:8:1 ratio ($X^2 = 0.38$, $P = 0.83$) (Table 3.8). F_3 families derived from the second F_1 plant segregated 17:30:3 (resistant:segregating:susceptible) which also fit a 7:8:1 ratio ($X^2 = 2.09$, $P = 0.35$) (Table 3.8). After combining both F_3 families, a ratio of 43:62:8 was observed which still matched the 7:8:1 ratio ($X^2 = 1.5$, $P = 0.47$) (Table 3.8). From the F_2 population and F_3 family tests, it appears that PI334672-5 contains one dominant resistance gene and one recessive resistance gene effective against the isolate CR259.

3.3.2.4 Chi-square analysis of AC Morgan x PI335562

In the F_1 hybrid test of the cross AC Morgan x PI335562, all plants were resistant indicating CR259 resistance was inherited as a dominant trait (Table 3.3). F_2 plants from the F_1 plant segregated 195:15 (resistant:susceptible), which fit a 15:1 ratio ($X^2 = 0.15$, $P = 0.7$) (Table 3.9). The F_2 data for PI335562 suggests it carries 2 duplicate dominant epistatic genes which control resistance to CR259.

Table 3.8 Segregation ratio and chi-square analysis of crown rust reaction in the oat cross AC Morgan x PI334672-5.

Generation	Total	Resistant	SEG	Susceptible	Expected Ratio	X^2	P
F_1	4	4					
F_2 (F_{1-1})	131	105		26	3:1	1.59	0.21
					13:3	0.044	0.83
F_2 (F_{1-2})	80	58		22	3:1	0.15	0.7
					13:3	3.47	0.06
F_2 (Pooled)	211	163		48	3:1	0.46	0.5
					13:3	1.96	0.16
F_3 (F_{1-1})	63	26	32	5	1:2:1	14.02	<0.01
					7:8:1	0.38	0.83
F_3 (F_{1-2})	50	17	30	3	1:2:1	9.84	<0.01
					7:8:1	2.09	0.35
F_3 (Pooled)	113	43	62	8	1:2:1	22.75	<0.01
					7:8:1	1.5	0.47

Table 3.9 Segregation ratio and chi-square analysis of crown rust reaction in the oat cross AC Morgan x PI335562.

Generation	Total	Resistant	Susceptible	Expected Ratio	X^2	P
F_1	3	3	0			
F_2	210	195	15	15:1	0.15	0.7

3.3.2.5 Chi-square analysis of AC Morgan x PI375506

In the cross of AC Morgan x PI375506, all F_1 hybrids from this cross were resistant and indicated that resistance to CR259 was inherited as a dominant trait (Table 3.3). F_2 plants

from F_1 plants segregated 160:42 (resistant:susceptible), which fit either a 3:1 ratio ($X^2 = 1.69$, $P = 0.19$) or a 13:3 ratio ($X^2 = 0.43$, $P = 0.51$) (Table 3.10). The data suggested PI375506 carried one dominant resistance gene or two resistance genes with dominant and recessive epistasis. F_3 families segregated 43:56:17 (resistant:segregating:susceptible), which neither fit a 1:2:1 ratio ($X^2 = 11.79$, $P = 0.003$) nor a 7:8:1 ratio ($X^2 = 14.36$, $P = 0.001$) (Table 3.10). Thus, the number of genes involved in PI375506 resistance is likely to be one dominant resistance gene or one dominant resistance gene and one recessive gene. Further testing with a larger sample size may resolve this.

Table 3.10 Segregation ratio and chi-square analysis of crown rust reaction in the oat cross AC Morgan x PI375506.

Generation	Total	Resistant	SEG	Susceptible	Expected Ratio	X^2	P
F_1	4	4		0			
F_2	202	160		42	3:1	1.69	0.19
					13:3	0.43	0.51
F_3	116	43	56	17	1:2:1	11.79	0.003
					7:8:1	14.36	0.001

3.3.2.6 Chi-square analysis of AC Morgan x PI375547

In the cross of AC Morgan x PI375547, the F_1 hybrids from this cross were resistant to crown rust isolate CR259 indicating resistance was inherited as a dominant trait (Table 3.3). F_2 plants segregated 125:58 (resistant:susceptible) which was slightly significant when tested against a 3:1 ratio ($X^2 = 4.02$, $P = 0.045$) (Table 3.11). Although the F_2 data did not support that 1 gene controlled resistance to CR259 in this cross, segregation distortion could account for the observed F_2 ratio. In the F_3 family test, a segregation ratio of 25:55:10 (resistant:segregating:susceptible) was observed which did not fit a 1:2:1 ratio ($X^2 = 9.44$, $P = 0.009$) nor a 7:8:1 ratio ($X^2 = 10.87$, $P = 0.004$). When homozygous resistant and segregating groups were pooled, F_3 families segregated 80:10 (resistant:susceptible) which fit a 15:1 ratio

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($X^2 = 2.85$, $P = 0.09$), suggesting that two resistance genes were segregating with segregation distortion towards susceptibility in both the F_2 and F_3 populations (Table 3.11).

Table 3.11 Segregation ratio and chi-square analysis of crown rust reaction in the oat cross AC Morgan x PI375547.

Generation	Total	Resistant	SEG	Susceptible	Expected Ratio	X^2	P
F_1	4	4		0			
F_2	183	125		58	3:1	4.02	0.045
					13:3	19.29	<0.01
F_3	90	25	55	10	1:2:1	9.44	0.009
					7:8:1	10.9	0.004
		80		10	3:1	8.53	0.003
					15:1	2.85	0.09

3.4 Discussion

As shown in previous research, rust resistance genes from *A. sterilis* have been a rich source of resistance, but these genes have been effective for only a few years before being overcome by new *P. coronata* isolates (McCallum *et al.*, 2007; Chong *et al.*, 2008). Other *Avena spp.*, such as *A. magna* and *A. strigosa*, have also been mined for major crown rust resistance genes, with *Pc91* and *Pc94* genes being examples of these efforts (McMullen *et al.*, 2005; Fetch *et al.*, 2007; Carson, 2009a). However, widespread use of interspecific transfer of rust resistance genes between species of different ploidy is limited due to the inherent difficulties associated with this approach. Embryo rescue, the ability to form synthetic hexaploids between diploid and tetraploid lines, and silencing of resistance genes once transferred into hexaploid oat are significant barriers encountered during interspecific transfer of resistance (Rines *et al.*, 2007). Further, Carson (2009a) indicated there is no evidence demonstrating that seedling resistance genes from diploid or tetraploid *Avena spp.* are more durable than resistance genes from *A. sterilis.*

Due to the relatively easier task of crossing *A. sterilis* to cultivated oat, evaluation of crown rust resistance to isolate CR259 in F_2 populations and F_3 families from crosses between AC Morgan and six different *A. sterilis* accessions was undertaken to identify novel crown rust resistance. Several different gene action modes controlling resistance were revealed through this process. Resistance in PI334672-5 was clearly demonstrated to be the result of two resistance genes with one dominant resistance gene and one recessive resistance gene. There was also a strong likelihood that two resistance genes were present in PI375547. F_2 tests revealed that both PI311623-4 and PI335562 contain two dominant genes, resulting in duplicate dominant epistasis. Based on current F_2 and F_3 data, resistance genes involved in PI375506 are most likely due to one dominant resistance gene, or one dominant resistance gene and one recessive resistance gene. In F_2 tests, PI333561 appeared to be heterogeneous. Therefore, the crosses of AC Morgan x PI333561 derived from different F_1 plants were considered as independent crosses. The PI333561 male parent used to create the first F_1 plant was resistant to ten crown rust isolates representing considerable virulence variability (Dr. Curt McCartney, unpublished data). Assuming no heterogeneity, PI333561 would carry two resistance genes, one or both of which are incompletely dominant. Therefore, PI333561 likely contains new seedling resistance genes for crown rust. Additional research is recommended on PI333561-derived resistance.

While these crosses may contain valuable new resistance genes to crown rust, these genes will need to be assessed for their interaction with currently available crown rust genes being used in cultivated oat. Gene silencing has been noted on two occasions with crown rust resistance derived from *A. sterilis*. Wilson and McMullen (1997) reported that the *Pc62* gene was silenced by *Pc38* (or a gene linked to *Pc38*) that was introduced from *A. sterilis*. The silencing effect associated with *Pc38* was also noted when it was combined with *Pc94* (Chong and Aung, 1996). Rines *et al.* (2007) noted a similar effect when working with *A. strigosa* crown rust resistance, but in this case the suppressor affected the crown rust resistance gene being transferred into cultivated oat (and not one already present in the cultivated oat line). No explanation could be found for why the suppressor did not express

itself in the originating *A. sterilis* accession. However, via repeated backcrossing they were able to alleviate this effect which indicated that a gene (suppressor) linked to the crown rust gene, and not the resistance gene itself was responsible for the suppressive effect. Silencing of introduced resistance genes may be more common when using *A. sterilis* as opposed to other *Avena spp*. Initial crosses between various *A. barbata* resistance lines and oat varieties 'Otana' and 'Ogle' have shown no such silencing issues (Carson 2009a).

Considering the extensive effort and difficulty of identifying and transferring crown rust resistance into cultivated oat it is essential to consider the best deployment strategy to maximize their longevity. The use of multilines has been proposed in the past to provide more durable resistance (Mundt, 2002). Multilines are able to reduce the initial inoculum levels and inhibit the spread of disease within the field as incompatible (resistant) host plants are encountered by the different virulences within the pathogen population (Garrett and Mundt, 1999). Carson (2009b) examined the crown rust load on a multiline containing 10 susceptible component lines versus the individual lines. Despite the susceptibility in the component lines, the multiline consistently showed lower levels (approximately 30%) of crown rust infection in comparison to the component lines. However, an undesired effect of the multiline was that it selected for new virulence combinations in the pathogen population effective against a greater range of resistance (up to all 10 component lines). Browning and Frey (1981) reported a similar finding in oat crown rust. This is certainly not a desired outcome and would suggest that mulitlines may not be a good strategy to maintain durable resistance. However, the use of several new resistance genes in the multiline (as opposed to defeated genes) may inhibit the development of multiple virulences to a greater degree since races would not already be present that have virulence against the new resistance genes. Release of pure-line varieties containing different resistance genes may also reduce the speed at which multiple virulence develops (as the proximity of different virulent races is much larger), the ultimate result would likely be the same.

A better source of durable resistance is non-race specific (also called APR or partial resistance) resistance which has proven to be durable in barley (*mlo* resistance; Humphry *et*

al., 2006) and wheat (*Lr34* resistance; Kolmer *et al*., 2007).   This is the subject of the next section.

4. Linkage and QTL Mapping of Crown Rust Resistance in the Cross AC Assiniboia / MN841801

4.1 Introduction

Approximately 1.8 million hectares of oat (*Avena sativa* L.) have been seeded annually in Canada over the past decade (Statistics Canada, 2011). The province of Saskatchewan has produced approximately 1.4 million tonnes of oats on 600,000 hectares of land annually over this time period (Government of Saskatchewan, 2010). Oat crown rust (caused by *Puccinia coronata* Corda f. sp. *avenae* Eriks) is considered the most economically important disease (McCallum *et al.*, 2007; Leonard, 2007; Chong *et al.*, 2008). Resistant oat varieties are an important control strategy for crown rust. An estimated $400 million in lost production was prevented from 1995 to 2005 because of breeding of resistant oat cultivars (McCallum *et al.*, 2007).

Although yield loss from crown rust has been reduced, breeding for resistance to this disease is challenging. Major single gene seedling resistance is widely and frequently used in North American rust resistant oat varieties (McCallum *et al*., 2007). Such race-specific resistance genes are relatively easy to utilize in breeding lines when compared to adult plant resistance (APR) (Carson, 2008; Graichen *et al*., 2010). Unfortunately, extensive use of seedling resistance genes in breeding programs results in the eventual selection of *P. coronata* races with virulence to those genes. For instance, *P. coronata* races in Western Canada defeated previously effective seedling resistance genes such as *Pc38* and *Pc39* in the late 1980s, *Pc48* in 2001 and *Pc68* in 2005 (McCallum *et al*., 2007). Virulence to the currently effective *Pc91* seedling resistance gene has been reported (McCartney *et al*., 2011; Table 4.1). Virulent isolates to another major seedling resistance gene, *Pc94*, were also recently identified (Chong *et al*., 2011; Table 4.1). Continued widespread use of cultivars carrying single race-specific seedling genes will likely continue this trend.

Table 4.1 Frequency of virulence on six oat crown rust differentials and two putative new resistance genes by crown rust isolates collected from wild oat (*Avena fatua* L.) and cultivated oat (*A. sativa*) in commercial fields in Manitoba and eastern Saskatchewan between 2007 and 2009 (Chong *et al*., 2011).

Pc gene	Virulence Frequency (%)					
	Wild oat			Cultivated oat		
	2007	2008	2009	2007	2008	2009
Pc38	94.7	92.9	93.2	97.7	98	100
Pc39	94.1	94.2	88.7	96.9	95.9	100
Pc68	45.9	42.3	43.9	70.8	81.6	81.8
Pc91	0	0	0.5	0	0	0
Pc94	1.8	0	0.5	0.8	0	0
Pc96	17.6	5.8	2.7	7.7	0	0
Temp_pc97	1.8	0	1.8	3.1	0	0
Temp_pc98	1.2	0	1.4	0	2	4.5

Research on APR to wheat leaf rust resulted in the identification of the *Lr34* resistance gene from the Brazilian cultivar Frontana (Dyck *et al*., 1966). This gene has been widely incorporated into many wheat cultivars around the world and since 1966 has not yet been overcome by a virulent leaf rust race (Kolmer *et al.*, 2007). It is possible that durable resistance genes similar to *Lr34* may be present in the oat genepool. The oat line, MN841801, has consistently demonstrated resistance to various *P. coronata* populations for more than 20 years (Chong, 2000; Leonard 2002). Chong (2000) concluded that MN841801 is carrying two APR genes with additive effects. However, QTL mapping described four major QTLs and three minor QTLs for APR contributed by MN841801-1 in the cross MN841801-1 x Noble-2 (MN) (Portyanko *et al.*, 2005). Acevedo *et al.* (2010) validated these APR QTLs and discovered one new QTL from the same cross.

The present study was conducted to: (1) create a genetic linkage map in a population created from the cross AC Assiniboia x MN841801 (AM), and (2) detect and characterize QTLs for APR to crown rust.

4.2 Materials and Methods

4.2.1 Plant materials

A population of one hundred sixty-seven F_7-derived recombinant inbred lines (RILs) was developed from the AM cross made at the Cereal Research Center (CRC), Agriculture and Agri-Food Canada (AAFC) (Winnipeg, Manitoba). AC Assiniboia was bred at the CRC and registered in 1996. This variety is susceptible to current *P. coronata* populations, even though it contains three crown rust seedling resistance genes (*Pc38/39/68*) (Brown *et al*., 2001). MN841801 is a breeding line with adult plant resistance developed at the University of Minnesota in the early 1970s (Leonard, 2002).

4.2.2 Disease inoculation

4.2.2.1 Fungal isolates and inoculum production

The *P. coronata* isolate CR251 (race BRCB) is virulent to all seedling resistance genes in both parents, but avirulent to adult plant resistance in MN841801 (Chong, 2000). The isolate was obtained from Dr. Chong at the CRC. Urediniospores of isolate CR251 were increased by inoculating the *P. coronata* susceptible cultivar AC Morgan. AC Morgan was released by the Lacombe Research Centre, AAFC in 1999 and is susceptible to most races of crown rust (Kibite and Menzies, 2001). Urediniospores collected from infected leaves were dried in a desiccator kept at 4°C and then stored in a -80 °C freezer.

4.2.2.2 Naturally (multi isolate / race) infected buckthorn nursery

The AM population was sown at the University of Saskatchewan Horticulture Crown Rust Nursery in Saskatoon, SK in 2010 (SK10). Three rows of buckthorn (*Rhamnus cathartica* L.) are present along two edges of the field with one row down the middle of the field (Figure 4.1). In early spring, previously harvested infected oat straw bearing telia were

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placed under the buckthorn to initiate aecial infection. Aeciospores from the buckthorn subsequently infected the nearby spreader rows. Urediniospores were then produced in spreader rows and initiated infection of the entire oat nursery.

In SK10, three replicates were planted in an incomplete block design (lattice design). Thirty seeds per line were sown in a single hill. Hills were planted 0.3 m from each other. In addition, AC Assiniboia, AC Morgan, AC Ronald, CDC Boyer, CDC Dancer, CDC Orrin, HiFi, Leggett, MN841801 and four crown rust gene differentials (*Pc91*, *Pc94*, *Pc96* and *Pc97*) were randomly planted in each replicate as checks for monitoring disease development and determining optimal disease rating date. In order to provide even disease inoculum and create the environmental conditions necessary for disease development, the blocks were surrounded by spreader rows. AC Morgan was planted as the spreader.
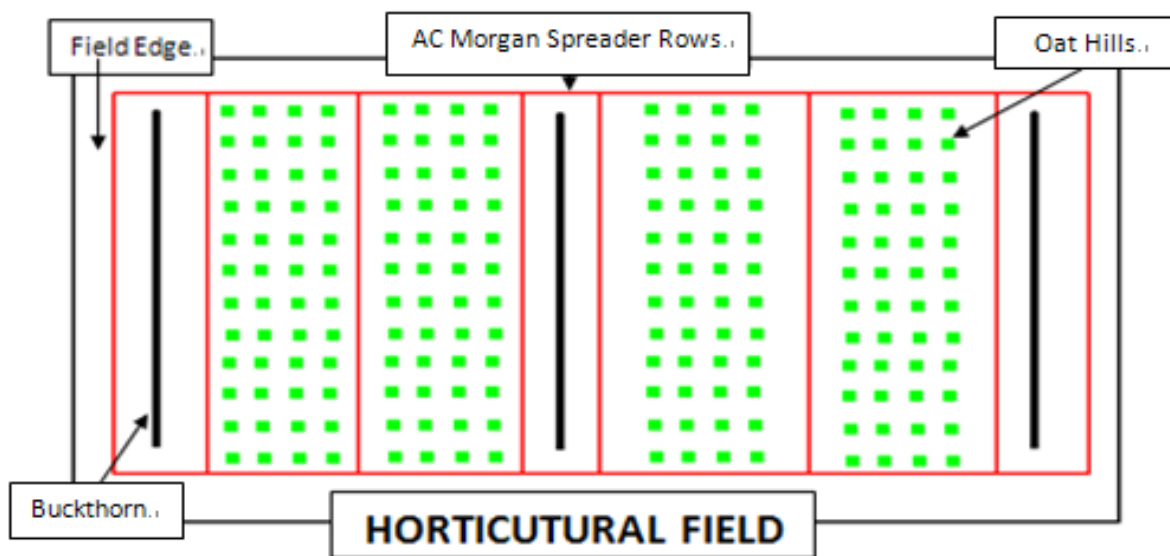
Figure 4.1 Design of the Horticulture Crown Rust Nursery used for the SK10 experiment at the University of Saskatchewan.

4.2.2.3 Single isolate inoculated disease nurseries

According to Jackson *et al.* (2008), a single race field inoculation can reduce the confounding effects of multiple race field inoculations. Thus, disease resistant QTLs are more easily discovered. The AM population was sown in the Preston Field Nursery at the

University of Saskatchewan in Saskatoon, Saskatchewan in 2011 (SK11). The test was planted as three replicates using the same design as the SK10 nursery. The same checks and spreader rows as the SK10 test were planted in SK11.

In SK11, when plants in the spreader rows reached the fourth or fifth leaf stage, three inoculations were done within 10 days. At each inoculation, 0.3 g of crown rust urediniospores from isolate CR251 were mixed with 300 ml Bayol35 oil, a light mineral oil (Esso Canada, Toronto, ON) and sprayed onto spreader rows with a Herbiflex hand-held sprayer (Micron Sprayers Ltd., Bromyard, UK). Water was immediately sprayed onto the spreader rows after inoculation to provide enough moisture for urediniospore germination. Because urediniospores only germinate in darkness, each spreader row was covered by a 0.61 m wide and 45.72 m long dark plastic tarp for at least 13 hours. Orange pustules on some spreader rows were observed after the third inoculation.

4.2.3 Disease evaluation

In this study, disease estimation focused on the flag leaves. During the summer when urediniospore development reaches maximum levels, crown rust disease severity (DS) and infection type (IT) were estimated by visually scoring at least five flag leaves in each hill. The modified Cobb scale was used in adult plant disease rating (Peterson *et al.*, 1948). All ITs were subsequently converted into a numerical value: R=0.2, MR=0.4, MRMS=0.6, MS=0.8, S=1. Coefficient (CI) of each hill was calculated with the formula: CI = DS x IT.

4.2.4 DNA extraction, marker development and analysis

One hundred sixty-seven $F_{7:10}$ RILs from the AM cross were selected for DNA extraction. DNA extraction was performed by using a modified CTAB extraction protocol (Murray and Thompson, 1980). For each line, DNA was extracted from five to six coleoptiles. DNA samples from the first ninety lines were selected for Diversity Arrays Technology (DArT) marker analysis (Diversity Arrays Technology Pty. Ltd, Yarralumla, Australia).

In an attempt to improve the genomic coverage of the linkage map, specifically in regions previously identified with APR QTL, a number of alternative marker sources were used. Fourteen additional single nucleotide polymorphism (SNP) markers, distributed randomly across the oat genome, were screened on all one hundred and sixty-seven RILs by Dr. Jackson at the Small Grains and Potato Germplasm Research Unit, United States Department of Agriculture-Agricultural Research Service (USDA-ARS) (Aberdeen, ID).

Eight additional Sequences Characterized Amplified Region (SCAR) markers were developed from expressed sequence tags (EST) of restriction fragment length polymorphism (RFLP) markers on linkage groups 3 and 26 in the MN841801-1 x Noble-2 map (MN map; Portyanko *et al*., 2005; Figure 4.2). Linkage group 14 in the new Kanota x Ogle DArT linkage map (KO map) (Tinker *et al*., 2009) was suspected to represent the same region of the oat genome as linkage group 13 in the MN map. Thirty-four SCAR markers were generated based on DArTs and RFLPs from the linkage group 14 of the KO map (Figure 4.3). PCR primers for each SCAR were designed using Primer3 v.0.4.0 software for standard PCR reactions and listed in the Appendix B (Rozen and Skaletsky, 2000). Amplified product sizes ranged from 150bp to 250bp. Primers were designed to be 18bp to 27bp in length with an annealing temperature between 55ºC to 65 ºC and a GC content from 40% to 60%.

High Resolution Melting (HRM) analysis of eleven markers designed based on three ESTs of RFLP markers in the MN map and three ESTs of RFLP markers from the KO map were generated. PCR primers for each HRMs listed in the Appendix B were designed using Primer3 v.0.4.0 software (Rozen and Skaletsky, 2000). Amplified product sizes ranged from 150bp to 200bp. Primers were designed to be 18bp to 21bp in length. An annealing temperature arranged from 57ºC to 62ºC. Percentage of GC in primers is from 40% to 60%.
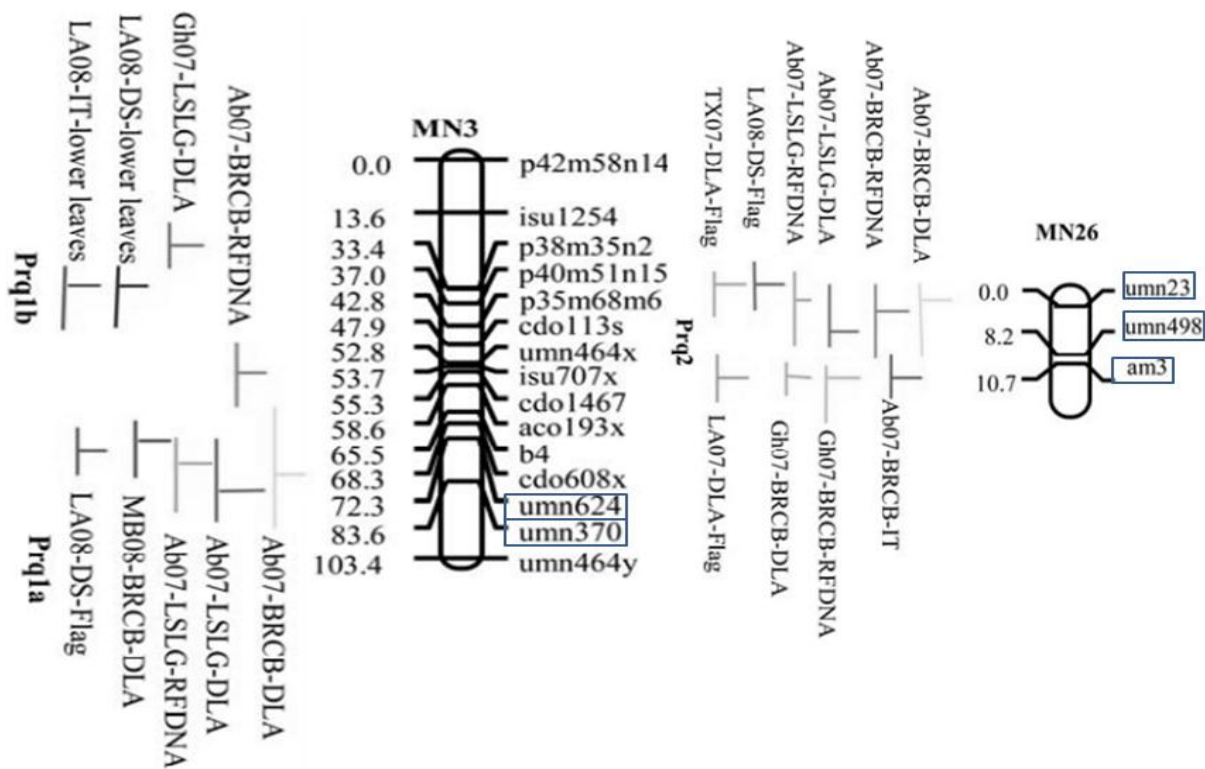
Figure 4.2 Linkage groups 3 and 26 in the oat MN map containing previously identified APR QTLs (modified from Acevedo *et al*., 2010). Markers in blue color boxes were converted into SCAR markers or HRM markers.
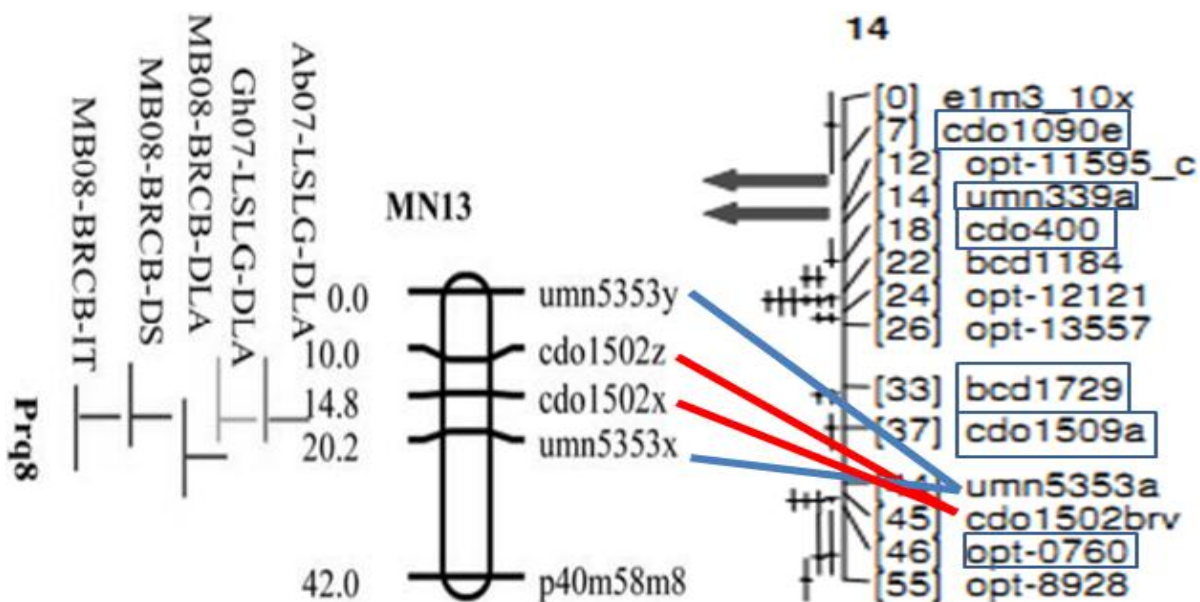
Figure 4.3 Comparison of linkage group 13 in the oat MN map and linkage group 14 in the new oat KO map (modified from Tinker *et al*., 2009 and Acevedo *et al*., 2010). Markers in blue color boxes were converted into SCAR markers or HRM markers.

Finally, one Single-Strand Conformation Polymorphism (SSCP) marker based on marker UMN498W, located on linkage group 13 in the MN map, was tested (Appendix B). One Simple Sequence Repeat (SSR) marker, AM3, provided by Dr. Scoles (University of Saskatchewan, Saskatoon, SK) was also evaluated. The APR wheat leaf rust resistance gene *Lr34* confers a partial resistance phenotype at the adult plant stage. The SSR marker swm10 is closely linked to *Lr34*, where the indel SNP marker caIND11 is located in the gene sequence of *Lr34* (Bossolini *et al.*, 2006; Dakouri *et al.*, 2010). An SSR marker (swm10) and one indel SNP marker (caIND11) were tested for detection of a homologous *Lr34* gene in *Avena* (Bossolini *et al.*, 2006; Dakouri *et al.*, 2010). Finally, one SCAR marker, *Pc68-300*, tightly linked with the *Pc68* oat crown rust resistance gene was evaluated on the population to determine the nature of a putative APR QTL detected in this study (Scoles and Eckstein, 2004).

4.2.5 Phenotypic data analysis

The inoculation methods and *P. coronata* populations in SK10 and SK11 were different, so the two field tests in this study were considered as independent experiments. Statistical analysis of disease reaction data was carried out using the SAS software package, v. 9.2 (SAS Institute Inc., 2008) and R software v. 2.12.1. The two measures of crown rust reaction were crown rust disease severity (DS) and coefficient (CI). The Shapiro-Wilk test was applied to estimate the normality of the trait distributions (Shapiro and Wilk, 1965). RILs, replication and block effect were considered as random effects in the statistical model for the normality test. Square root (sqrt (trait+0.5)) (sqrt), log (log10 (trait+2)) (log) and arcsine square root (arsine (sqrt (trait/100)) (asin) data transformation methods were used to normalize DS and CI data when frequency curves were not in the normal distribution.

Analyses of variance (ANOVA) were performed for DS and CI for each field test. Genetic variance (σ^2_G) for DS and CI was calculated using the formula:

$$\sigma^2_{G} = (MS_G - r*\sigma^2_{GE} - \sigma^2_e)/r*e,$$

where MS_G was mean square of genotype, σ^2_{GE} was the variance of interaction of genotype and environments, σ^2_e was the error residual of the model used, r was the number of replicates in each field test and e was the number of total environmental tests. The model for ANOVA and genetic variance considered genotype as a fixed effect, and replication and block as random effects.

4.2.6 Linkage map construction and detection of APR QTLs

DNA marker data was used to generate a linkage map using CarthaGene v. 1.0 R (de Givry *et al*., 2005). Linkage groups were determined using a minimum LOD score of 3.0 and a maximum distance between markers of 30 cM was used to generate initial linkage groups. The Kosambi mapping function was used to estimate distances. A combination of the commands "build", "greedy", "flips", "polish" and "generate" were used to determine and generate an optimal marker order within linkage groups.

QTL mapping of APR was done on transformed data of DS and CI from each field disease nursery experiment. The single-trait composite interval mapping based on maximum-likelihood (CIM MLE) was conducted using QGene v. 4.0 (Joehanes and Nelson, 2008). A stepwise cofactor selection was used, with a maximum number of 5 cofactors, the F to add = 0.05 and the F to drop = 0.05. A permutation test with 1000 iterations was conducted to determine a significance threshold for each trait. Single marker analysis (SMA), which is a simple t-test between alternate alleles for each unlinked marker, was used to determine association ($P < 0.05$) between unlinked markers and traits.

4.3 Results

4.3.1 Phenotypic distribution

DS and CI on AC Assiniboia were significantly higher than on MN841801 (Table 4.2). Distribution curves of the 167 RILs for DS and CI for SK10 and SK11 were not normally distributed (Table 4.2) and skewed toward the resistant parent (Figure 4.4 and Figure 4.5).

The distribution curves indicate that DS and CI were quantitatively inherited traits. From the ANOVA test, genotypes were a significant source of variability for DS and CI.

Table 4.2 Means and variance components of oat parents and recombinant inbred lines for DS, IT and CI at the SK10 and SK11 oat crown rust field nursery experiments.

Environment	Trait[1]	σ^2_G	Parents		RILs		
			AC Assiniboia	MN841801	Means	STD	Range
SK10	DS	402.7	47.8	11	29.6	23.4	2-99
	IT[2]		0.9	0.6	0.73	0.19	0.3-1
	CI	275.9	41.9	7.2	22.3	20.3	1.5-99
SK11	DS	340	55.6	10.6	34.7	24.6	1-99
	IT		1	0.4	0.74	0.28	0.2-1
	CI	431.7	55.6	3.9	30	26.3	0.2-99

[1]DS: disease severity, IT: infection type, CI: coefficient

[2]Infection type based on leaf color surrounding pustules at flag leaves in the Peterson *et al*., 1948 diseases rating scale.

A.

SK10 DS

B.

SK11 DS
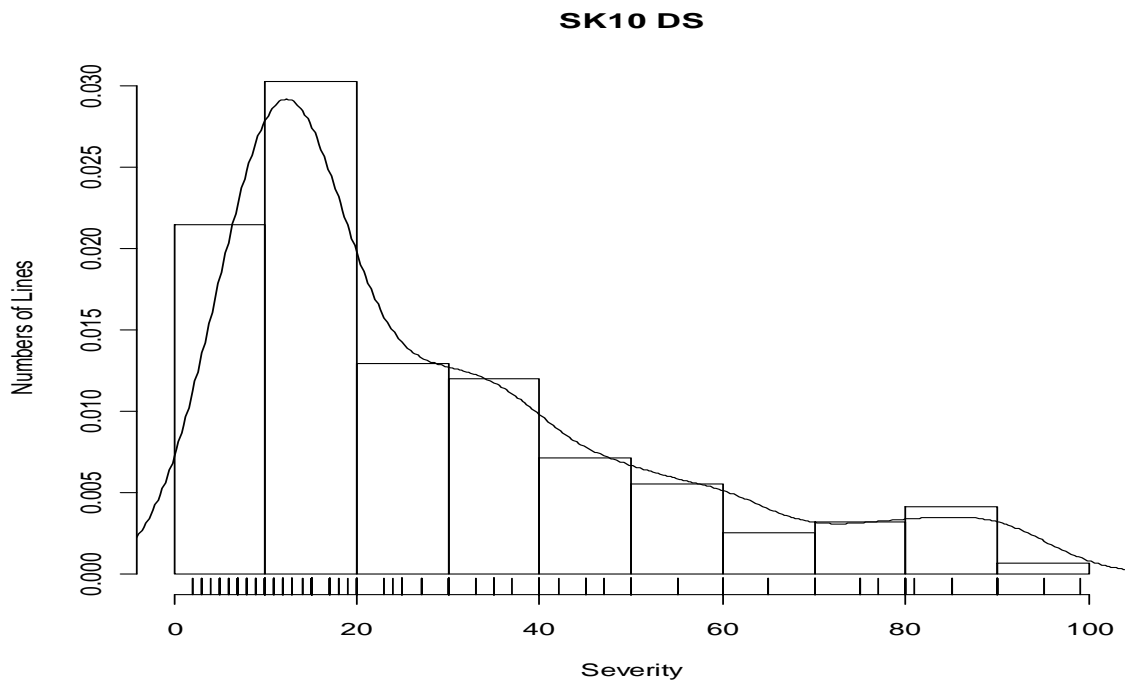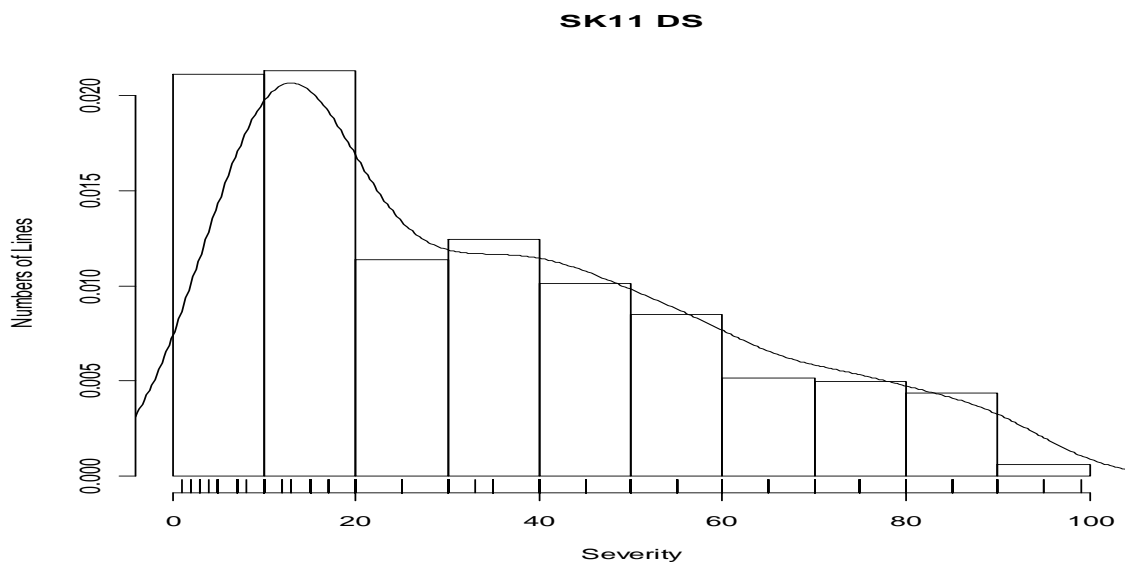
Figure 4.4 Frequency distributions for DS for 167 F_7-derived RILs of the oat AM cross in SK10 (A) and SK11 (B) crown rust field nursery experiments. The line represents the negative binomial distribution and the bars represent the number of plants in DS categories spanning ten units.

A.

SK10 CI

B.

SK11 CI
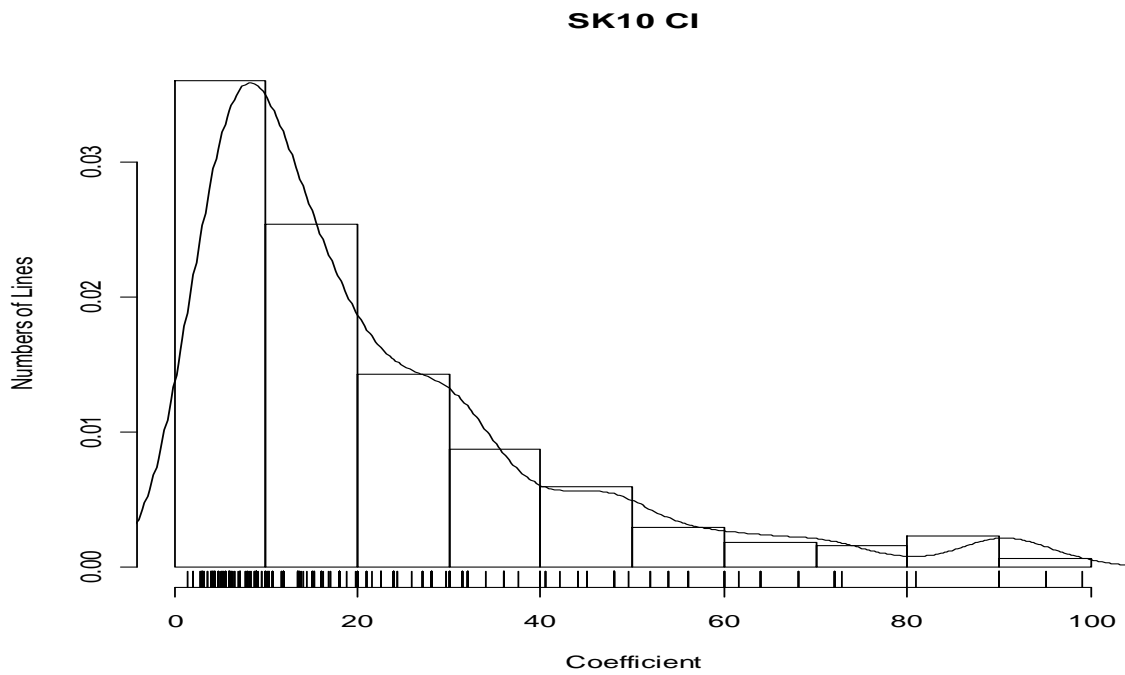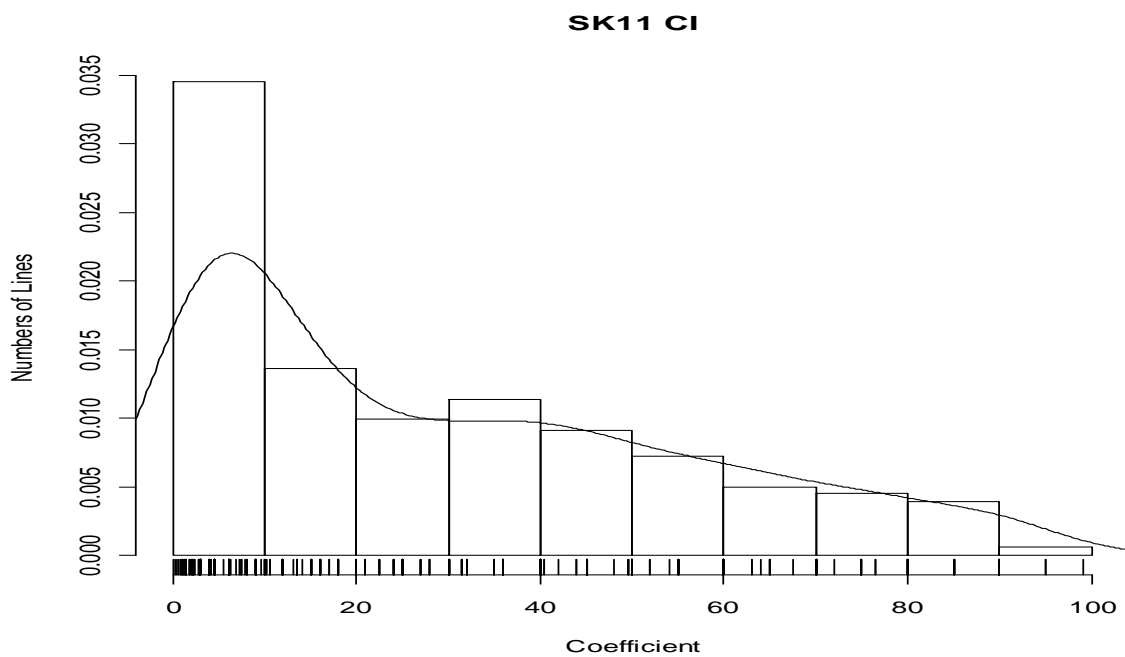
Figure 4.5 Frequency distributions for CI for 167 F_7-derived RILs of the oat AM cross in SK10 (A) and SK11 (B) crown rust field nursery experiments. The line represents the negative binomial distribution and the bars represent the number of plants in DS categories spanning ten units.

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A generalized linear model was created by treating RILs as a fixed effect and block as a random effect. Square root (sqrt (trait+0.5)), log (log10 (trait+2)) and arcsine square root (arcsine (sqrt (trait/100)) transformations of the data were investigated to normalize DS and CI data. In Table 4.3, raw data and transformed data for each disease nursery field environment are listed. Both the square root and log transformation methods normalized the SK10 DS and SK11 DS data (Figure 4.6). Both methods were able to improve the SK10 CI and SK11 CI data, but neither could normalize the data (Figure 4.6). However, since the transformed CI data was closer to a normal distribution than the raw data it was used for QTL analysis. Thus, both the square root transformed data and log transformed data for both DS and CI data at SK10 and SK11 were used in QTL mapping.

Table 4.3 *P*-value for normality tests on the raw and transformed data for DS and CI at each oat crown rust nursery field experiment using the Shapiro-Wilk method.

Environment	DS				CI			
	Raw data	sqrt	asin	log	Raw data	sqrt	asin	log
SK10	<.001	0.1	<.001	0.35	<.001	0.044	<.001	0.02
SK11	<.001	0.32	0.02	0.08	<.001	<.001	<.001	<0.01
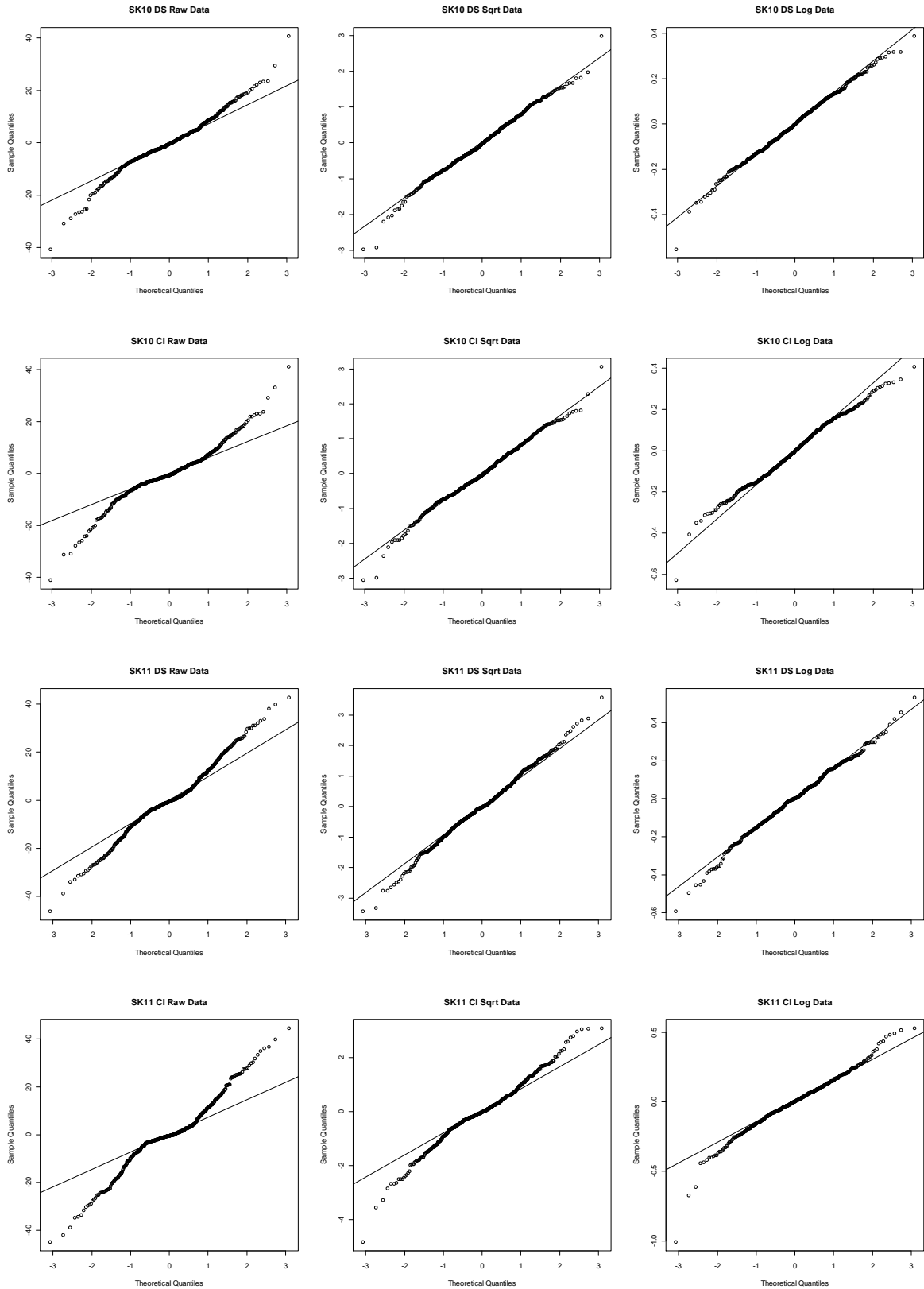
Figure 4.6 QQ line and QQ norm on raw data, sqrt transformed data and log transformed data for DS and CI in SK10 and SK11 oat crown rust field nursery experiments.

4.3.2 Linkage map

A total of 553 loci, including 493 DArT, 41 SCAR, 16 SNP, 1 SSCP and 2 SSR markers, were screened across the AC Assiniboia x MN851801 population. After removing poor quality markers, 30 linkage groups spanning 955 cM of the oat genome were generated using 240 markers (43.48%) (Appendix C). Thirteen markers were unlinked. Because of short map length and limited SNPs, all DArTs with Q>77 and the following SNPs were retained to increase genome coverage. Two DArTs (oPt-2660 and oPt-11217) and 1 SNP (7964) showed an excess of the MN841801 genotype and 1 SNP (5435) showed an excess of the AC Assiniboia genotype. Two SNPs (c12516_2 and c841_2) with 12% and 14% missing data were also assigned to this map.

The SCAR marker *Pc68-300*, linked to the oat *Pc68* seedling crown rust resistance gene, was successfully added into the AM map. The *caIND11* and *swm10* markers linked to the *Lr34* APR crown rust resistance gene were not polymorphic on the AM population despite testing by agarose gel electrophoresis, capillary electrophoresis and polyacrylamide gel electrophoresis. Seven HRMs markers were polymorphic between AC Assiniboia and MN841801. However, these HRM markers were difficult to evaluate on the RILs. None of HRM markers were included in the map.

Eight SCAR markers were developed from sequenced RFLP markers in linkage group 3 and 13 in the MN map. None of the SCAR markers showed polymorphisms between AC Assiniboia and MN841801 using agarose gel electrophoresis. One SSCP marker designed from marker UMN498W located on linkage group 13 in the MN map was polymorphic on the AM population. Thirty-four SCAR markers were generated from the new KO DArT linkage map. Only one SCAR marker (oPt0760W) was polymorphic between the parents when tested by agarose gel electrophoresis.

4.3.3 QTLs for crown rust resistance

Raw data and transformed data of DS and CI in four different field disease nursery experiments were used in QTL mapping. Only one QTL, contributed by AC Assiniboia, was detected in the SK10 experiment (Figure 4.7). The permutation test indicated a threshold significance level of 3.06 for the trait. The QTL detected demonstrated a LOD score in excess of 8. This QTL was 4 cM away from the marker Pc68-300 (Figure 4.7; Table 4.4), making it likely that this QTL was the result of the *Pc68* seedling resistance gene carried by AC Assiniboia.
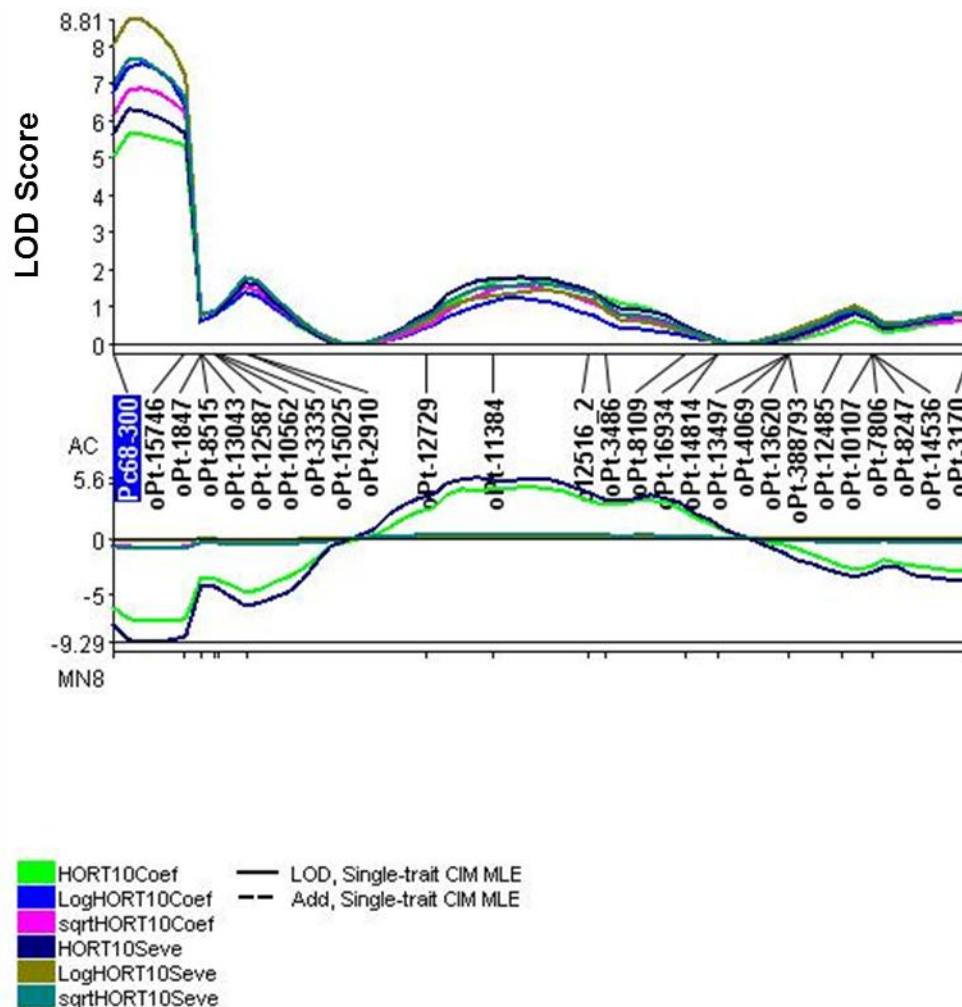
Figure 4.7 Overlapping QTLs for DS and CI detected in the SK10 oat crown rust field nursery experiment using both raw data and transformed data of DS and CI.

Table 4.4 Summary of crown rust resistance QTL identified based on DS and CI measured on 167 RILs from the oat AM cross in the SK10 oat crown rust field nursery experiment.

Trait Data	QTL marker	Peak/interval[1]	Linkage group	QTL name	LOD[2]	R^2x100[3]	Additive[4]
CI	*Pc68-300*	4 / 0 - 10	30	*Pc68*	5.7	0.15	-7.6
CI (log)	*Pc68-300*	4 / 0 - 10	30	*Pc68*	7.6	0.19	-0.2_1
CI (sqrt)	*Pc68-300*	4 / 0 - 10	30	*Pc68*	6.9	0.18	-0.8_1
DS	*Pc68-300*	4 / 0 - 10	30	*Pc68*	6.4	0.17	-9.6
DS (log)	*Pc68-300*	4 / 0 - 10	30	*Pc68*	8.7	0.22	-0.15_1
DS (sqrt)	*Pc68-300*	4 / 0 - 10	30	*Pc68*	7.7	0.2	-0.9_1

[1]Peak of QTL (in cM) and interval spanned by the QTL (in cM).

[2]LOD: log of the odds

[3]phenotypic variance of oat crown rust data explained by the QTL

[4]additive effects from transformed datasets were back-transformation in order to interpret with original scale.

Removal of the *Pc68* effect was accomplished in 2011 by inoculation of the nursery with the CR251 isolate. No QTLs contributed by MN841801 were detected based on SK11 data. The same AM population was also tested with the CR251 isolate at the AAFC Nolette Field Station in Winnipeg, Manitoba in 2001 and 2002 (MB01 and MB02) (Dr. James Chong, unpublished data) and no QTLs were detected using this data. Because five QTLs (*Prq1a*, *Prq2*, *Prq3*, *Prq7* and *Prq8*) contributed by MN841801-1 were detected using the MN map and inoculation with the CR251 isolate in field studies at Aberdeen, ID and Glenlea, MB (Acevedo *et al.*, 2010) (Table 4.5), markers closely linked to the most consistent of these loci (*Prq1a*, *Prq1b*, *Prq2* and *Prq8*) in the MN map were tested in the AM population. Many of the markers in the MN map were RFLPs and so were converted to SCARs and evaluated in the AM population. However, no significant QTL were detected in the AM population.

On linkage group 3 of the MN map, *Prq1a* (near markers cdo608x, b4, isu707x and cdo1467) was detected in seven of fourteen tests and *Prq1b* (close to markers p35m68m6 and p38m35n2) was found in four of fourteen tests (Portyanko *et al.*, 2005; Acevedo *et al.*, 2010; Figure 4.8; Appendix D). Linkage groups 17 and 25 in the AM map have three common SNPs (c1361_1, c841_2 and lrc16503_1) with linkage group 3 in the MN map, where *Prq1a* and *Prq1b* were mapped (Figure 4.9). However, no QTLs were detected in this part of the oat genome in this study.
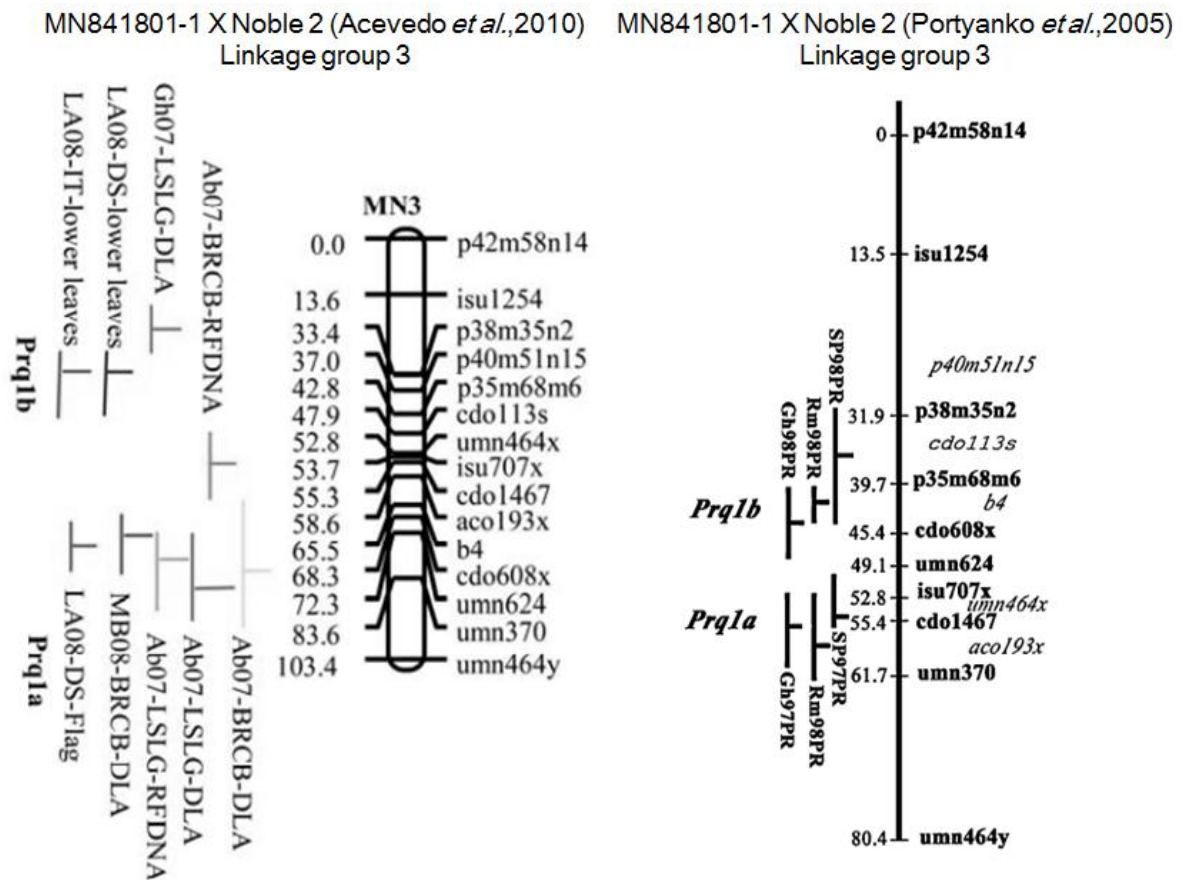
Figure 4.8 Linkage group 3 from the oat MN cross showing two oat crown rust APR QTLs (*Prq1a* and *Prq1b*) associated with crown rust resistance detected in ten field tests and four greenhouse tests.
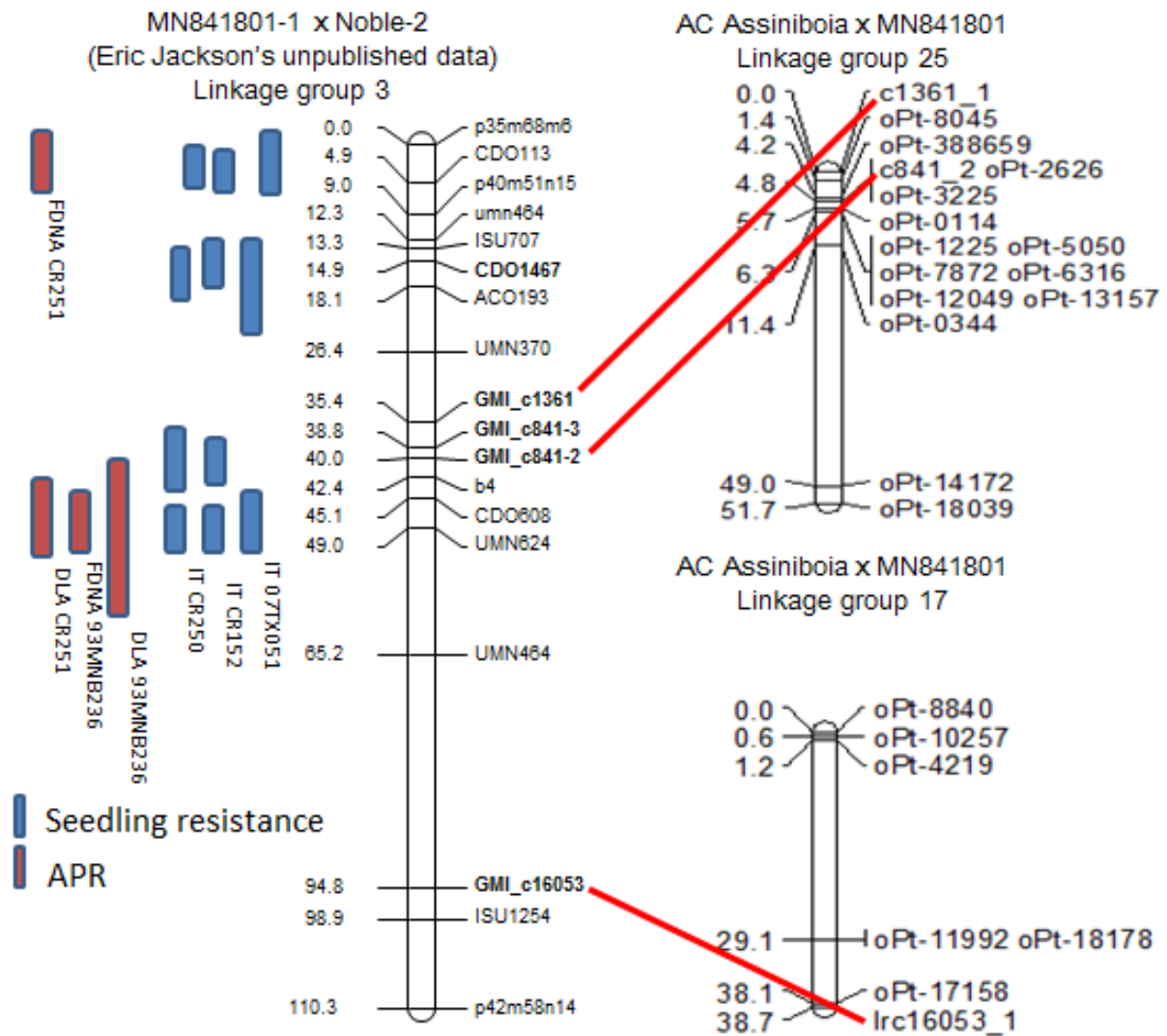
Figure 4.9 Comparison of linkage group 3 from the oat MN cross and linkage groups 17 and 25 from the oat AM cross. Seedling oat crown rust resistance QTLs in linkage group 3 of the MN map courtesy of Dr. Acevedo (unpublished data).

On linkage group 26 of the MN map, *Prq2* (close to markers umn498, umn23 and AM3) was detected in ten of fourteen tests (Portyanko *et al.*, 2005; Acevedo *et al.*, 2010; Appendix D). The AM3 SSR marker was not polymorphic between AC Assiniboia and MN841801. Three SCARs based on EST sequences from umn23 and umn498 were also not polymorphic. One SSCP marker (UMN498W) could be mapped (Figure 4.10). The SSCP marker UMN498W in this study and the RFLP marker umn498 in the MN841801-1 x Noble-2 map may not be interrogating the same genomic region in the oat genome.
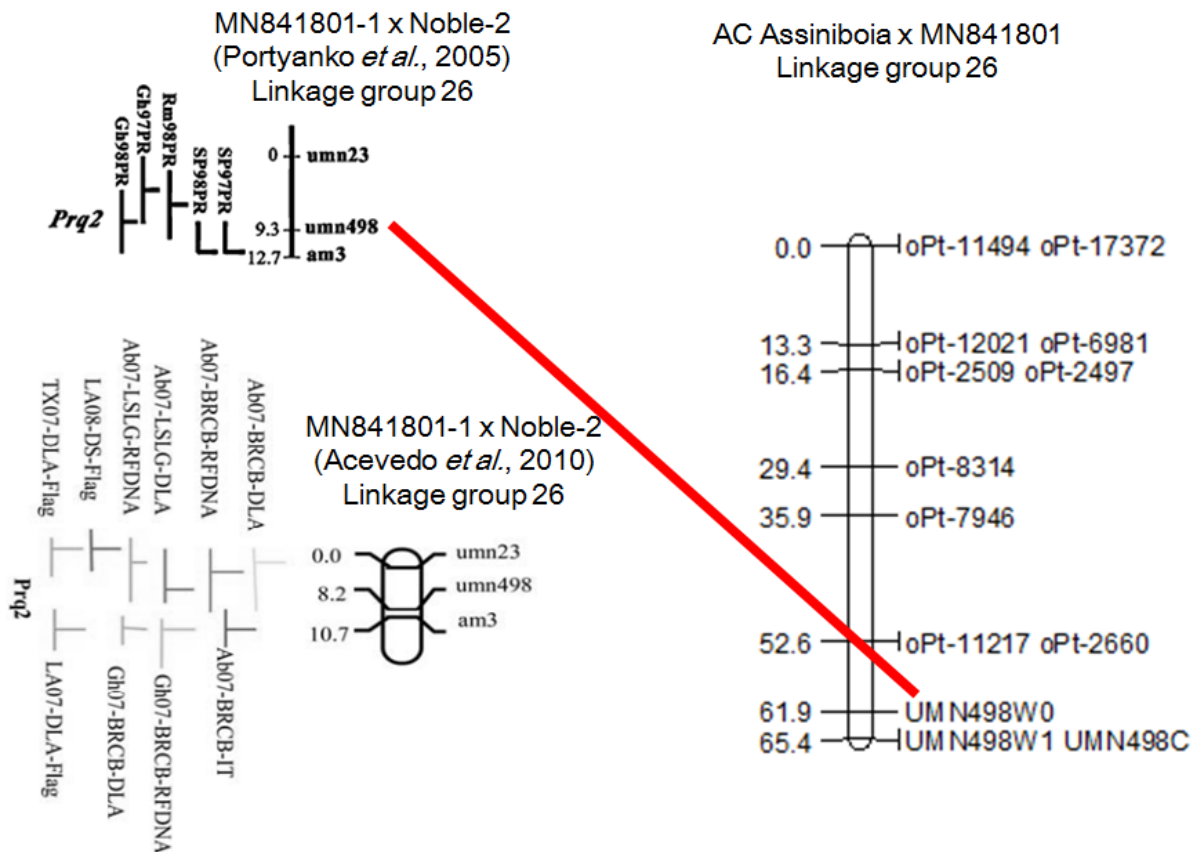
Figure 4.10 Comparison of linkage group 26 from the oat MN map, containing one APR QTL (*Prq2*) associated with crown rust resistance, and linkage group 26 from the oat AM cross.

On linkage group 13 of the MN map, *Prq8* (close to markers cdo1502x and umn5353x) was identified in three of fourteen tests (Portyanko *et al*., 2005; Acevedo *et al*., 2010; Appendix D). Since there were no available EST sequences for umn5353x, umn5353y, cdo1502x and cdo1502y, it was impossible to develop SCARs for comparison to the AM map. Linkage group 14 in the new KO map contains two RFLP markers, umn5353a and cdo1502brv, suspected to share homology with linkage group 13 in the MN map. With the assistance of two SCARs developed from DArT markers present on linkage group 14 in the new KO map, linkage groups 18 and 21 were identified in the AM (Figure 4.11). However, *Prq8* was not detected in this study. It is possible that linkage group 13 in the MN map might not be homeologous with linkage group 14 in the new KO map.
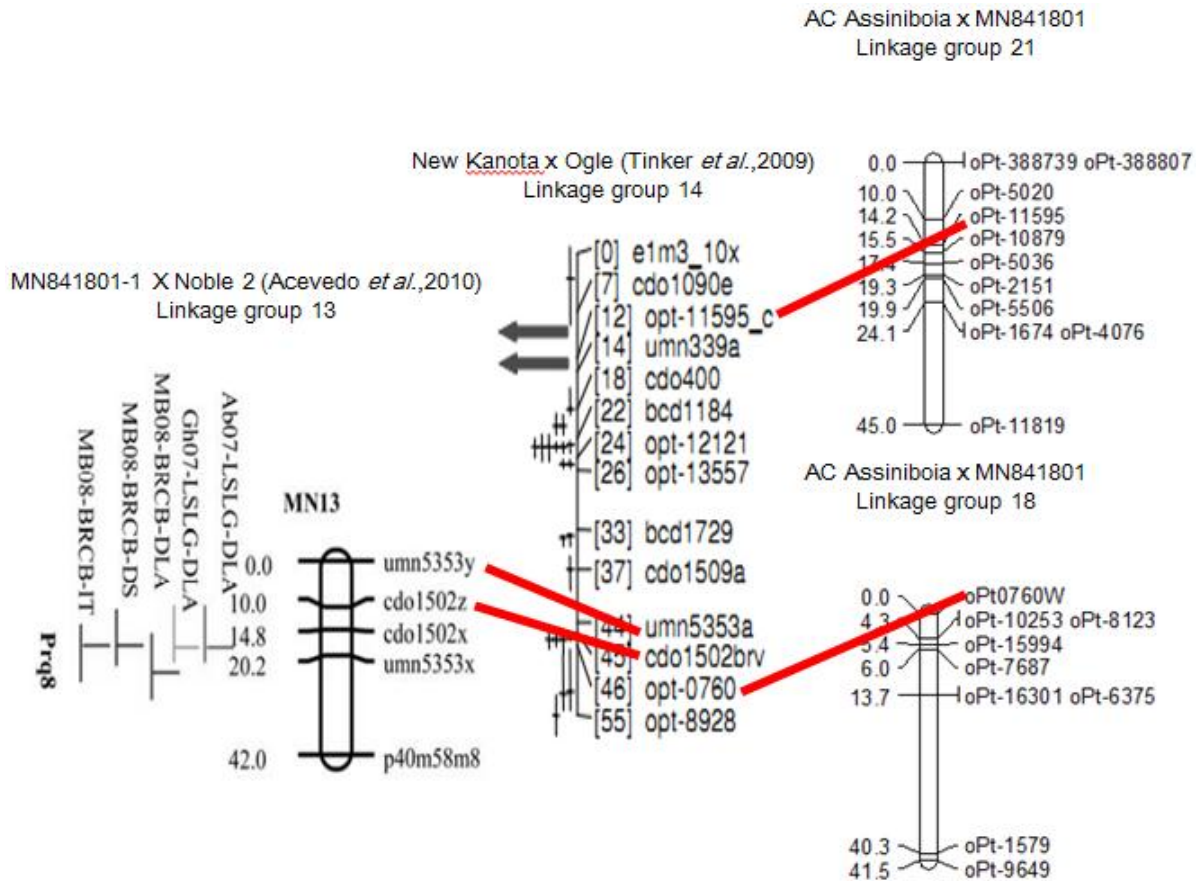
Figure 4.11 Comparison of linkage group 13 from the oat MN map, containing one APR QTL (*Prq8*) associated with crown rust resistance, with linkage group 14 from the new KO map and linkage groups 18 and 21 from the oat AM map.

Table 4.5 Crown rust severity of oat cultivars AC Assiniboia, AC Morgan, Noble-2 and Portage, and oat lines MN841801 and MN841801-1 in different crown rust experiments.

| Oat Line	Rust isolate	Rated leaves	Average Severity	References
AC Assiniboia	Buckthorn	Flag leaf	47.8	SK10
	CR251	Flag leaf	55.6	SK11
			29.2	unpublished data[4]
AC Morgan	CR251	Flag leaf	74.1	SK11
MN841801	Buckthorn	Upper three leaves	10.3	Leonard, 2002
		Flag leaf	11	SK10
	CR251	Flag leaf	10.6	SK11
			3.95	unpublished data[4]
MN841801-1	CR251	Flag leave	1.5[2]	Acevedo *et al*., 2010
		Upper three leaves	0.2[3]	Acevedo *et al*., 2010
	Buckthorn	Flag leaf minus one	9.9[1]	Portyanko *et al*., 2005
Noble-2	CR251	Flag leave	18.8[2]	Acevedo *et al*., 2010
		Upper three leaves	36.8[3]	Acevedo *et al*., 2010
	Buckthorn	Flag leaf minus one	40[1]	Portyanko *et al*., 2005
Portage	Buckthorn	Upper three leaves	55.7	Leonard, 2002

[1] Average of rust severity in SP97PR and SP98PR.

[2] DLA severity in the field test at Aberdeen, ID in 2007.

[3] DS in the field test at Manitoba in 2008.

[4] Unplished data collected from Dr. J. Chong at AAFC-Winnipeg in 2001 and 2002.

4.4 Discussion

The success of identifying genetic loci associated with crown rust resistance in oat depends on both the nature of the resistance and, to a less defined degree, the marker density and coverage of the oat genome. In genetic resistance governed by one or a few genes there has been greater success in identifying loci linked to resistance. Hoffman *et al.* (2006) were

able to identify the *Pc58* seedling resistance complex (a cluster of 3 genes) using a 441 marker linkage map created from the 'Ogle' x TAM O-301 population.   Due to the dominant nature of this resistance, phenotyping was easily and clearly scored and identification of race specific interactions with the *Pc58* region was possible.   As with any mapping study, good phenotypic information was essential to successful mapping.

When resistance is more quantitative in nature due to a greater number of genetic loci involved in the trait, and each loci becomes more environmentally influenced, the ability to identify all relevant loci becomes more difficult. For example, Zhu and Kaeppler (2003) used a 272 marker linkage map derived from a 'Ogle' x MAM17-5 population to identify QTL linked to MAM17-5 resistance (which was more quantitative than *Pc58* resistance). They identified two QTLs consistently over a two year period that explained 48-70% and 9-14% of the resistance, respectively. Crown rust evaluation was done in the field on adult plants which made the phenotypic data more variable than, for example, seedling tests conducted in the greenhouse in populations where resistance is governed by a single major gene.   Despite additional variability in the phenotypic data, the relatively simple inheritance of the MAM17-5 resistance (one major gene and a minor gene) allowed for detection of the resistance loci.

In contrast to these studies, the APR carried by MN841801 is clearly quantitative in nature. Portyanko *et al.* (2005) identified four major and three minor QTL using a 230 marker linkage map created in a population derived from MN841801-1 x Noble-2. A total of three field environments (using a mixture of isolates) and two greenhouse tests (using a single crown rust isolate) were used in this study.   The quantitative nature of this resistance was additionally indicated by the low heritability estimates obtained from both the field (0.30) and greenhouse data (0.44).   Individual QTL detected from field data explained only 2.9-16.8% of the variation with only one QTL detected across all three field sites.   The inconsistent detection of *Prq1a, Prq1b, Prq2* and *Prq7* led the authors to suggest that larger population size and additional field experiments were needed to confirm the detected QTLs

(Portyanko *et al.*, 2005). Using an additional seven field and two greenhouse tests, the same population was evaluated and the same seven QTL were detected along with one additional QTL identified (Acevedo *et al.*, 2010). Heritability estimates based on disease leaf area measurements from field trials ranged from 0.14-0.38 and again QTL were not consistently detected across environments with explained variance ranging from 4.7-36.1% for individual QTL.

The large influence of environment on MN841801 resistance is one possible explanation why no QTL were detected in this study. That is, different environmental conditions between the AM and MN experiments may not have allowed detection of QTL. Thus, future field experiments that tested both the MN and AM populations concurrently would be helpful in eliminating environmental effects on QTL detection, evaluating the QTLs and understanding resistance effects contributed by those QTLs. In addition, different crown rust visual rating methods were used between the two studies. The pathology team of Dr. Chong in Manitoba and the current Saskatoon research study evaluated only the flag leaves. Acevedo *et al.* (2010) visually assessed the three uppermost leaves and digitally scanned the flag leaves. However, the different estimation methods should not create drastically different estimates of disease severity and subsequent lack of detection of five QTLs. The Acevedo *et al*. (2010) study also used the CR251 isolate in greenhouse tests to minimize phenotypic variability, so greenhouse testing with this isolate could also be used to evaluate the AM population. This would help to eliminate environmental effects interfering with APR QTL detection and clearly demonstrated whether failure to detect these QTL is caused by environmental effects or poor oat genome coverage. Use of this isolate was very helpful at removing the confounding effect of the *Pc68* gene in the SK10 disease nursery field experiment (i.e. the only QTL detected in the current study).

Low oat genome coverage in the current map created with the AM population is strongly believed to be the major limitation to detecting APR QTLs contributed by MN841801. According to Oliver *et al.* (unpublished data), the first complete consensus oat map consists

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of 21 linkage groups with a total map length 1,839 cM. O'Donoughue *et al.* (1995) predicted the complete oat map should be approximately 2,932 cM. In comparison, the AM map was much shorter. It contains 30 linkage groups spanning only 955 cM, which is about 32.6% coverage of the estimated oat genome. APR QTLs are likely located in the unmapped portion of the oat genome in the AM population and it is expected that increasing the current map length by adding additional markers would help detect APR QTLs in future research. Anchored markers from the complete oat map will help to quickly improve the genome coverage of the AM map.

While low oat genome coverage is a logical reason for the lack of QTL detection, it is harder to understand why they were detected in the MN population, which could also be argued to suffer from low genome coverage. Although the total marker number used to create the maps in the AM and MN populations is fairly similar (230 versus 240, respectively), the AM map is only 63% the size of the MN map (955 cM versus 1,509 cM, respectively) (Portyanko *et al.*, 2005). In addition to this size disadvantage, the two maps may also be covering different areas of the oat genome. The MN map is composed primarily of RFLP markers (Portyanko *et al.*, 2001) whereas the current map is based on DArT markers. Creation of maps in *Triticum monococcum* using DArTs and SSR indicated that the two marker types tended to produce independent clusters along the linkage groups (Jing *et al.*, 2009). A similar pattern may occur within oat between RFLPs and DArTs. As noted by Tinker *et al.* (2009), DArT markers are located across the genome, but are not uniformly distributed and some oat regions may not contain any DArT markers.

Comparison of linkage maps between different oat mapping populations has been limited by the lack of common markers between populations (Rines *et al.*, 2006). As a result, current studies face great challenges in evaluating the same QTLs in different populations, even if they share the same QTL donor parent. Difficulty in comparing the AM map and the MN map is not rare. This issue is due in part to the markers used to create the maps. For example, the 'Ogle' x TAM O-301 (OT) map, which is based predominantly on RFLP

markers, contains numerous markers that cross hybridize to multiple loci and makes marker placement difficult (Portyanko *et al.*, 2001). The same problem arises when comparing markers across maps.   When the OT map was compared to the 'Kanota' x 'Ogle' (KO) map many of the markers gave equivocal KO linkage group assignments (Portyanko *et al.*, 2001). Rines *et al.* (2006) also pointed out the difficulty in correctly assigning map positions to markers that may be distributed across homeologous loci in the hexaploid genome, making map comparison complicated. In the current study, without a large number of markers in common between the AM map and the MN map, it is impossible to make the assumption that linkage groups carrying the converted SCAR markers in the AM map are the same linkage groups carrying the APR QTLs in the MN map. Accurate comparisons of the crown rust resistance in the AM population with the MN population will require an improved AM linkage that spans the entire genome and common genetic markers that unequivocally mark the same positions in the MN maps, or a reference oat consensus map.

The short linkage map with low oat genome coverage is a reasonable explanation for the failure to detect APR QTLs in this study. The AM genetic map needs to be expanded to cover the complete oat genome in order to detect APR QTL from MN841801. SNPs from the first complete oat map will be important in accomplishing this goal. Additional field or greenhouse testing of the AM population with *P. coronata* isolate CR251 is also recommended (perhaps in conjunction with the MN population) to improve the precision and accuracy of the phenotypic data.

5. General Discussion

Oat is an important cereal crop that is well adapted to the cooler northern climate of the Prairie Provinces in Western Canada. As an important exporter of oat to the world market, diseases such as crown rust impact the ability to supply quality oat are of concern. Crown rust is the most important disease of oat in Western Canada and as a result, two different genetic studies related to crown rust resistance were investigated in this thesis. The first investigated the genetic inheritance of potentially novel seedling resistance genes introduced from *A. sterilis* into cultivated oat. The second study investigated the genetic control of APR present in MN841801 which has shown durable crown rust resistance for several decades.

As the alternate host of *P. coronata* is present in North America, sexual recombination is a common occurrence in the pathogen population which produces new virulence combinations and results in a limited period of effectiveness for oat crown rust seedling resistance genes, typically around seven years (Chong *et al.*, 2011; McCallum *et al.*, 2007; McCallum *et al.*, 2011). In order to develop resistance against new virulent races, crown rust resistance from *Avena spp.* such as *A. strigosa, A. magna* and *A. barbata* have been used (Rines *et al.*, 2007; Carson, 2009a). Genetic inheritance of novel seedling resistance transferred from six different *A. sterilis* accessions was investigated in this thesis using a crown rust isolate (CR259) that is virulent against almost all currently used crown rust genes (except *Pc94*). Simple genetic inheritance was observed in all cases (2 or fewer genes), although the epistatic interaction between the pairs of resistance genes in some populations requires clarification.

Due to the effectiveness of these resistance genes against CR259, they represent a valuable set of tools to deal with crown rust. The resistance genes present in lines such as PI333561 should initially be the focus of further investigations as it is expected that either gene from PI333561 would provide effective resistance. It would also be valuable to determine if these, and the other resistance genes, differ in the crown rust race spectrum

against which they are effective. One would expect that there would be different resistance spectrums present in the six *A. sterilis* accessions that were used in the study. This will require efforts to continue building the crown rust differential set of oat and the nomenclature system initiated by Fleischmann and Baker (1971) and most recently advanced by Chong *et al.* (2000).

Avena sterilis continues to be a rich source of crown rust resistance genes. To date, over 40 different resistance genes have been identified from *A. sterilis*, with the most recently named resistance gene additions being *Pc97* and *Pc98* (Chong *et al.*, 2008). The relative ease of using this gene pool, in comparison to diploid and tetraploid species, and the lack of evidence that durability of *A. sterilis* resistance genes is different from other species (Carson, 2009a) will ensure its continued use as a major crown rust resistance gene pool.

When using *A. sterilis* as a source of crown rust resistance genes there have been several reports that the introgressed regions from *A. sterilis* have a silencing effect on crown rust genes already present in the cultivated oat parent. This effect was noted by Wilson and McMullen (1997) when *Pc38*, derived from *A. sterilis*, was introduced into a cultivated oat line already carrying *Pc62*.   Chong and Aung (1996) made a similar observation with *Pc38* when it was combined with *Pc94* resistance.   While no explanation has been provided for these observations, some have suggested that the different resistance genes may be competing for the same signal transduction pathways that are part of the defense reaction following pathogen recognition (Rines *et al.*, 2007). Potential suppression by the resistance genes identified in this study will need to be determined and if noted, a backcrossing scheme may be required to remove this activity.   This approach was successfully used to remove suppressors linked to resistance genes being introduced into cultivated oat from *A. strigosa* (Rines *et al.*, 2007).

APR offers a proven method of controlling oat crown rust over extended periods of time not seen with race-specific resistance.   Varieties such as 'CDC Dancer' and 'CDC Boyer'

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consistently show moderate levels of crown rust infection under strong disease pressure which provides good yield potential under these situations. Similar to these varieties, MN841801 has provided good APR since the 1970s (Chong, 2000; Leonard 2002) and has been the focus of several QTL mapping studies (Portyanko *et al.*, 2005; Acevedo *et al.*, 2010).

The second study of this thesis also undertook to identify APR QTLs in a population derived from MN841801. Over two years of study no APR QTLs were detected in the population. Several factors are thought to be responsible for this. Firstly, the low heritability of this trait, as observed from data in the Portyanko *et al.* (2005) and Acevedo *et al.*, (2010) studies, make QTL detection difficult. Secondly, poor genome coverage obtained with DArT markers in this study means that APR QTLs lying in the unmapped regions will go undetected. This is an issue that has previously been noted for DArT markers used with oat (Tinker *et al.*, 2009) To deal with this issue, an attempt to validate those QTLs previously detected for APR in MN841801 was made by screening QTL-linked markers from the MN population on the AM population used in this study. This also proved unsuccessful and highlights one of the difficulties in working with oat. Lacking a common marker system makes it difficult to compare and cross reference different maps. Difficulties with assignment of markers to specific loci on a given map, due to hybridization of markers to multiple locations within the hexaploid oat genome, increases the probability that different regions of the oat genome are being compared across maps (Rines *et al.*, 2006). It is therefore possible that the converted markers from the MN map that were used in this study interrogated different areas of the oat genome.

This limitation in oat genetic mapping is being addressed through the Collaborative Oat Research Enterprise (CORE) working group. The goal of this North American initiative is to create a set of genomic tool, including a comprehensive oat consensus map, which will assist the oat breeding and research community. Initial work focused on extensive EST sequencing and SNP identification for incorporation onto a genotyping platform (Oliver *et al.*, 2011). Currently a physically anchored 21 linkage group consensus map with

unambiguous marker positions is being generated, along with a 6,000 SNP Illumina genotyping assay, which will help avoid ambiguity when comparing QTLs and map positions across different populations. Additionally, use of doubled haploids in oat and creation of maps from such population (Tanhuanpaa *et al*., 2008 and 2012) will improve mapping via the creation of fixed genomes.

Continuing to explore different durable resistance strategies will be necessary for effective control of oat crown rust. Searching for, or perhaps creating via mutations, non-race specific resistance such as *mlo* resistance in barley (Humphry *et al.,* 2006) is one option. Other durable resistance genes such as *Rpg1* in barley which have been effective for over 60 years (Brueggeman *et al.*, 2002) need to be better understood so similar modes of action of resistance genes can be identified in other crop species. Similarly, identifying an APR gene such as *Lr34* would be another desirable way to improve resistance to crown rust (Kolmer *et al.*, 2007). Although studies with other pathogens have shown the effectiveness of gene pyramids over one or two years (Singh *et al.*, 2001; Castro *et al.*, 2003), the effectiveness of these pyramids over longer periods of time remain to be seen. A related strategy involving the use of multilines containing different resistance genes indicates that such gene deployment strategies does not hinder the development of newly virulent races effective against a wide range of resistance genes (Carson 2009b)

In the short term, continuing to exploit *A. sterilis* as a source of major resistance genes and attempting to find markers for these and APR from lines like MN841801 will be valuable tools in the management of oat crown rust.

6.0 References

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APPENDIX A

Nine Oat Crown Rust Isolates / Races

Table A 1. Detailed information for nine oat crown rust isolates / races used in this study.

Isolate	Race	Virulence to *Pc* Genes
CR13	SJQL-96	*Pc14/39/40/45/46/47/48/52/54/55/60/71/96/101/102/103/104*
CR185	NBFB	*Pc35/40/46/58/59/103*
CR223	NGCB-94	*Pc39/40/46/55/94/*
CR241	DSGB	*Pc38/39/46/47/48/52/55/63/67/70/71*
CR249	DQBG-94	*Pc38/39/46/55/56/63/94/104*
CR254	LRBG	*Pc38/39/55/56/60/61/63/67/68/70/71/104*
CR257	BRBG-94	*Pc38/39/55/56/60/63/67/68/70/71/94*
CR258	NTGG	*Pc38/39/40/46/48/52/55/56/63/67/68/71*
CR259	LQCB-91	*Pc35/38/39/40/55/59/60/61/63/91*

Adult Plant Resistance QTL Markers

Table B 1. Detailed information for markers linked to adult plant resistance QTL and tested in this study.

Primer pair	Marker Type	Tested Loci	Linkage	Original Marker Type	Reference	Electrophoresis	Forward Primer (5' - 3')	Reverse Primer (5' - 3')
AM3	SSR	am3	MN26	SSR	Li *et al.*, 2000	Agarose, Capillary, PAGE	CTGGTCATCC TCGCCGTTCA	CATTTAGC CAGGTTGC CAGGTC
BCD1729_262W	SCAR	bcd1729	KO14, MN12	RFLP	Tinker *et al.*, 2009; Portyanko *et al.*, 2005	Agarose	AGATACTGG CCAAGTTGCT A	CCATCAAT CTTTCAGA TCGT
BCD1729_262_1	SCAR	bcd1729	KO14, MN12	RFLP	Tinker *et al.*, 2009; Portyanko *et al.*, 2005	Agarose	AGATACTGG CCAAGTTGCT A	CCCTTATG GTATGGCT ACG
BCD1729_262_2	SCAR	bcd1729	KO14, MN12	RFLP	Tinker *et al.*, 2009; Portyanko *et al.*, 2005	Agarose	TGATGTAAG CGAATCTCTC C	CCATCAAT CTTTCAGA TCGT

BCD1729_375W	SCAR	bcd1729	KO14, MN12	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	CTCAGTCCTC CACTCCAGA A	GTGGGTAA ATGGTGGT GTTG
BCD1729_375_1	SCAR	bcd1729	KO14, MN12	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	CTCAGTCCTC CACTCCAGA A	AGGCATGC AATAGACT GACC
BCD1729_375_2	SCAR	bcd1729	KO14, MN12	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	CAGCTTTCAA GGGATCTGC	GTGGGTAA ATGGTGGT GTTG
caIND11	indel SNP	*Lr34*		indel SNP	Dakouri *et al*., 2010	Agarose, Capillary	GTCTCCCAAT CTGCATGCTC	TACCTCCC AAAAGCCA GTTG
CDO400_437W	SCAR	cdo400	KO14	RFLP	Tinker *et al*., 2009	Agarose	AACGAGACA CGCGGATTTA	CCATCCAG AAGCAGAT AGCC
CDO400_437_1	SCAR	cdo400	KO14	RFLP	Tinker *et al*., 2009	Agarose, HRM	AACGAGACA CGCGGATTTA	GCGTGTTA AAAACGAC GAGA

CDO400_437_2	SCAR	cdo400	KO14	RFLP	Tinker *et al*., 2009	Agarose	GGGCCACTTC TCTGCTTTAA T	CCATCCAG AAGCAGAT AGCC
CDO1090_394W	SCAR	cdo1090	KO14, MN10	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	CGAACTGTTC CACAAAGCA C	GGAGGACC CCAACATC TTC
CDO1090_394_1	SCAR	cdo1090	KO14, MN10	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose, HRM	CGAACTGTTC CACAAAGCA C	GGCAGTCT TCGTATGC ACCT
CDO1090_394_2	SCAR	cdo1090	KO14, MN10	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	GCTTAGTCGA CCTCCTCCAT C	GGAGGACC CCAACATC TTC
CDO1090_678W	SCAR	cdo1090	KO14, MN10	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	AGGTGCTGG GTGACAAGG T	CAACGAAC TGTTCCAC AAAGC
CDO1090_678_1	SCAR	cdo1090	KO14, MN10	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	TGGGTGACA AGGTTGAAA AA	CATGGTCT TCTTGCTG GACA

							Forward Primer	Reverse Primer
CDO1090_678_2	SCAR	cdo1090	KO14, MN10	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose, HRM	ACCCTGAGA ACGCCATCAT	CTTGAGCA TGCGGTGG AT
CDO1090_678_3	SCAR	cdo1090	KO14, MN10	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose, HRM	GAGCATTGA TGAGGATGA GGA	CCAACAGC AGTCAGAA TGACA
CDO1090_678_4	SCAR	cdo1090	KO14, MN10	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose, HRM	GATGGAGGA GGTCGACTA AGC	CGAACTGT TCCACAAA GCAC
CDO1509_365W	SCAR	cdo1509	KO14, MN12	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	TGACATCAG CAGTTTCAAG G	CTGATCAG TTTGAGGC CAAG
CDO1509_365_1	SCAR	cdo1509	KO14, MN12	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	TGACATCAG CAGTTTCAAG G	GGTTAGCC TAATTTGG AGCA
CDO1509_365_2	SCAR	cdo1509	KO14, MN12	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	TTCACATCGA ACCTTTCACT	CTGATCAG TTTGAGGC CAAG

CDO1509_443W	SCAR	cdo1509	KO14, MN12	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	TCCTCAAGTG CGGCAAG	TTGCCTTTT CAGCCTTG G
CDO1509_443_1	SCAR	cdo1509	KO14, MN12	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	TCGAGCGTCC TCAAGTG	TTGCACGA GACCTGGA G
CDO1509_443_2	SCAR	cdo1509	KO14, MN12	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	CATGAGGCC AAGCAGAAA G	CGTGGTAC ATGTGCTT GTCA
CDO1509_443_3	SCAR	cdo1509	KO14, MN12	RFLP	Tinker *et al*., 2009; Portyanko *et al*., 2005	Agarose	TAGGCTCCCC ACCAAGATT	TCTTGCCTT TTCAGCCTT G
oPt0760W	SCAR	oPt0760	KO14	DArT	Tinker *et al*., 2009	Agarose	AGGCAAGCA GGTAAAGAA GT	TCTTAGTTG TCCTCGTG CAT
oPt0760_1	SCAR	oPt0760	KO14	DArT	Tinker *et al*., 2009	Agarose	GCAAGCAGG TAAAGAAGT GC	CGGTCAGC ACTGGACA ATAG

oPt0760_2	SCAR	oPt0760	KO14	DArT	Tinker *et al*., 2009	Agarose	TTGAGGAGT ACGTCGCTAT G	TCGGACTG CACTGCTA TTTT
oPt0760_3	SCAR	oPt0760	KO14	DArT	Tinker *et al*., 2009	Agarose	AGTGCAGTG CGAGCAAGT AT	TAATGCAT CACCGGCT GTAG
oPt0760_4	SCAR	oPt0760	KO14	DArT	Tinker *et al*., 2009	Agarose	CCAAGAAGA AAGGGAGCT T	TCTTAGTTG TCCTCGTG CAT
Pc68-300	SCAR	*Pc68*		SCAR	Scoles and Eckstein, 2004	Agarose		
swm10	SSR	*Lr34*		SSR	Bossolini *et al.*, 2006	Agarose, Capillary	GCCTACTTTG ACGGCATAT GG	CCATCTTG ACATACTT TGGCCTTC C
UMN23W	SCAR	umn23	MN26	RFLP	Portyanko *et al*., 2005	Agarose	TGGACCAGG AGAGCTCTG AA	GGAACGAA CTCTTCAG CTTC

UMN23_1	SCAR	umn23	MN26	RFLP	Portyanko *et al.*, 2005	Agarose, HRM	TGGACCAGGAGAGCTCTGAA	GGTTTTCAGGCCAGGACAAT
UMN23_2	SCAR	umn23	MN26	RFLP	Portyanko *et al.*, 2005	Agarose,HRM	CTGGCCTGAAAACCAAACAT	GGAACGAACTCTTCAGCTTCT
UMN339W	SCAR	umn339	KO14	RFLP	Tinker *et al.*, 2009	Agarose	GCAGCTTCTGGAATTTTGATG	GATCATGGTTGGCCTTTCAA
UMN339_1	SCAR	umn339	KO14	RFLP	Tinker *et al.*, 2009	Agarose,HRM	GCAGCTTCTGGAATTTTGATG	TCCTTTGGAGCCATAACCAC
UMN339_2	SCAR	umn339	KO14	RFLP	Tinker *et al.*, 2009	Agarose	GATTGGAAGCGAGATGAAGC	CTGCAAGATGAGTTCGATCC
UMN339_3	SCAR	umn339	KO14	RFLP	Tinker *et al.*, 2009	Agarose	TTATGGTTTGACGCTTGGTG	CAATGATTCATGGGATCACG

UMN339_4	SCAR	umn339	KO14	RFLP	Tinker *et al*., 2009	Agarose	CGTGATCCCA TGAATCATTG TT	GATCATGG TTGGCCTTT CAA
UMN370	SCAR	umn370	MN3	RFLP	Portyanko *et al*., 2005	Agarose	TGTGGTCTAC TTGCCGCTTA	CTGCTGCA AACGAAAA GACA
UMN498W	SCAR	umn498	MN26	RFLP	Portyanko *et al*., 2005	Agarose, SSCP	GGAGTACTA CGGCGGTGA GA	ATCGTCAT TTGCGCAT GATT
UMN498_1	SCAR	umn498	MN26	RFLP	Portyanko *et al*., 2005	Agarose, HRM	CCGCAGGGA GGAATCTACT A	CTACAATC CATGGGCT CGAT
UMN498_2	SCAR	umn498	MN26	RFLP	Portyanko *et al*., 2005	Agarose, HRM	ACGTGAGGC TGATGCAGG T	CGAACATG ATCACACG CATA
UMN624	SCAR	umn624	MN3	RFLP	Portyanko *et al*., 2005	Agarose, HRM	AATGCATGT GTGAAGCAA GC	CAGGAATC AAACCCCA AATG

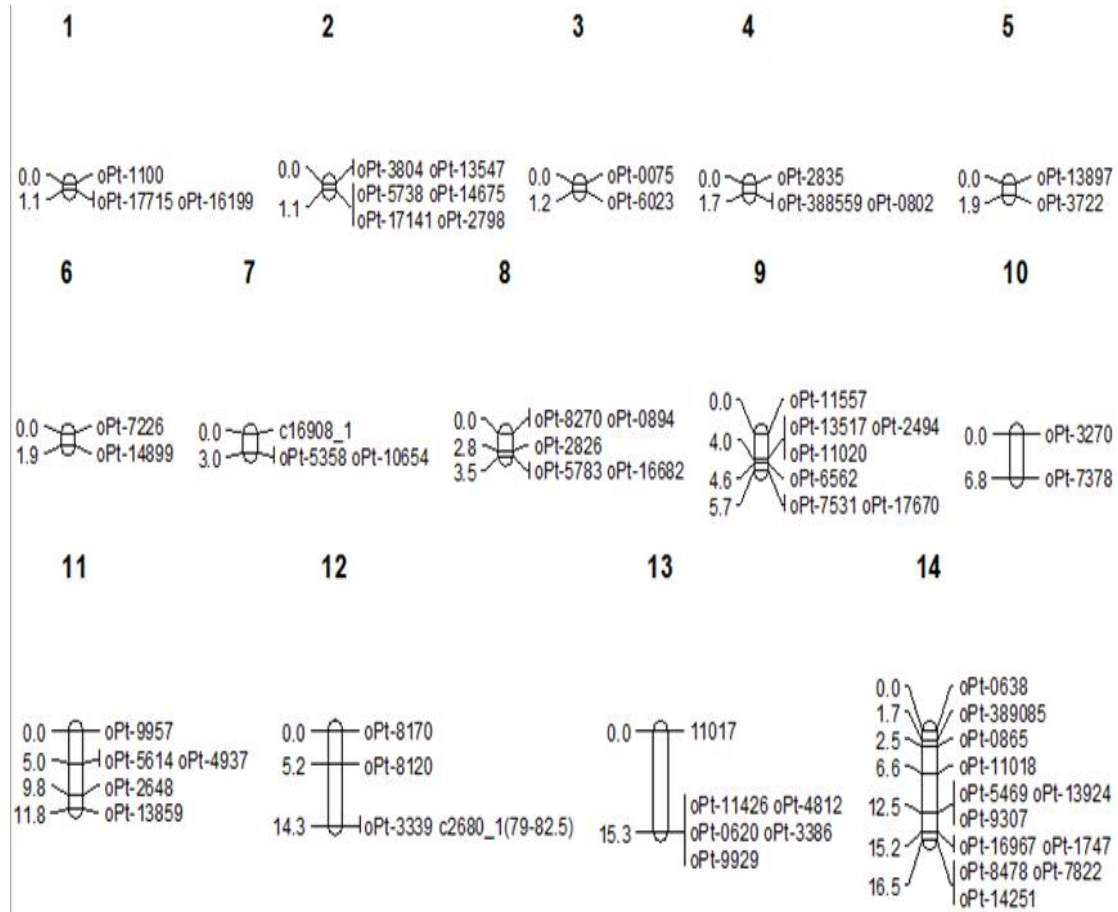**Linkage Map for the AC Assiniboia x MN841801 Population**

Figure C1. A 30 linkage group genetic map created using the AC Assiniboia x MN848101 population spanning 955 cM with 240 markers. Genetic distance is indicated in centiMorgans to the left of each linkage group and marker designations are indicated to the right of each linkage group. Linkage group designatuions are indicated as sequencial numbers above each group.
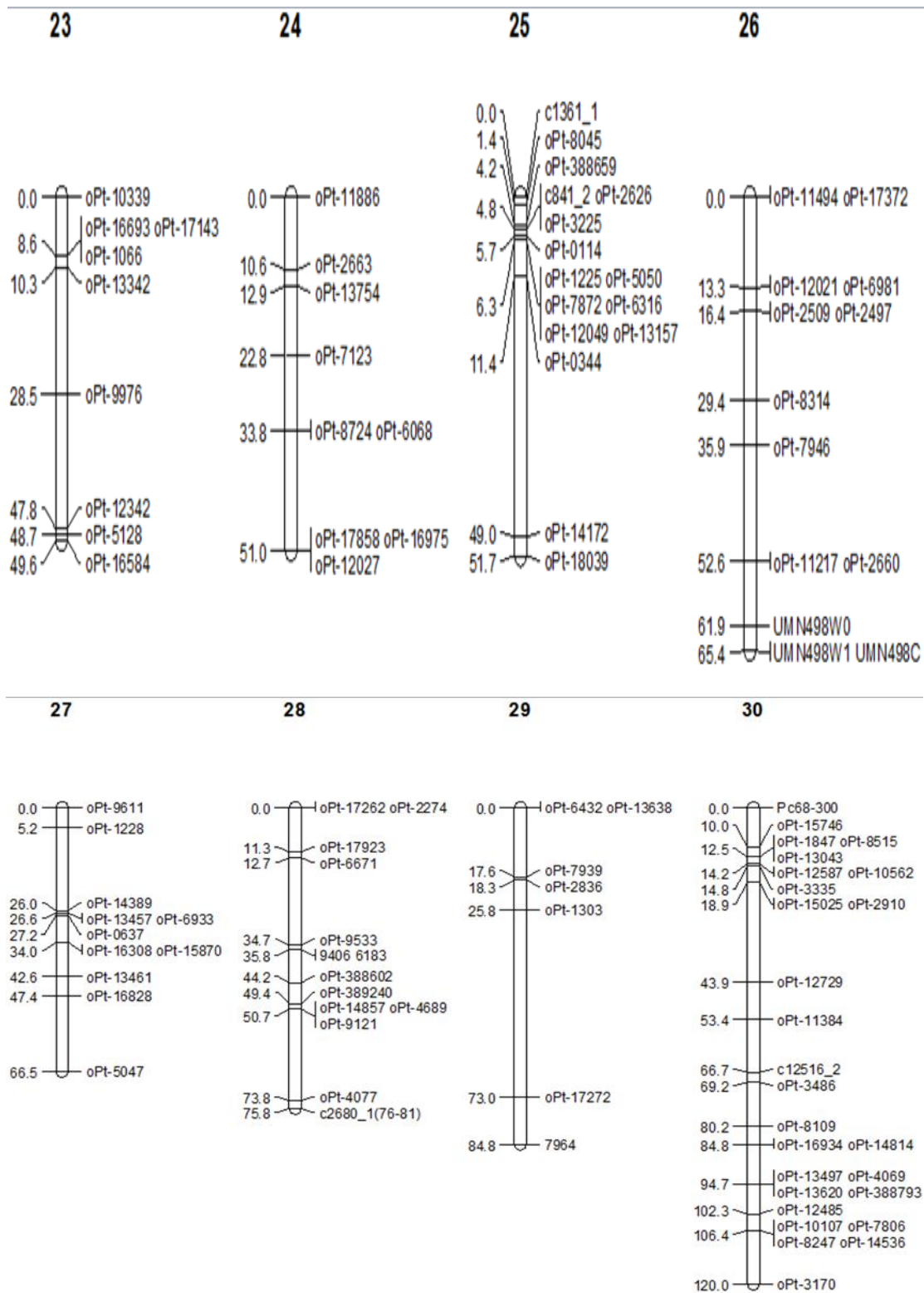
Figure C1. (cont.)

Adult Plant Resistance QTL Identified in Prior Studies

Table D1. Test identifier, environment, LOD score, R^2 values, disease rating methods and linkage groups associated with QTLs located on the MN map (Portyanko *et al.*, 2005; Acevedo *et al.*, 2010).   DS: Disease severity, DLA: Diseased leaf area, F: Flag leaf, F-1: Flag leaf minus 1.

Test Identifier	Environment	LOD	R^2 x 100	Rating method	QTL	Linkage Group	Reference
SP97PR	Field	4	7	DS-F-1	*Prq1*a	MN3	Portyanko *et al.*, 2005
Rm98PR	Field	3.4	6.2	DS-F-1	*Prq1*a	MN3	Portyanko *et al.*, 2005
Ab07-BRCB	Field	3.4	7.1	DLA	*Prq1*a	MN3	Acevedo *et al.*, 2010
Ab07-LSLG	Field	4.2	11.7	DLA	*Prq1*a	MN3	Acevedo *et al.*, 2010
LA08	Field	3.9	9.3	DS-F	*Prq1*a	MN3	Acevedo *et al.*, 2010
MB08-BRCB	Field	8.9	20	DLA	*Prq1*a	MN3	Acevedo *et al.*, 2010
Gh97PR	Greenhouse	11	26.8	DS-F	*Prq1*a	MN3	Portyanko *et al.*, 2005

Table D1. (cont.)

Test Identifier	Environment	LOD	R^2 x 100	Rating method	QTL	Linkage Group	Reference
SP98PR	Field	5.9	12.1	DS-F-1	*Prq1b*	MN3	Portyanko *et al.*, 2005
Rm98PR	Field	18.5	5.2	DS-F-1	*Prq1b*	MN3	Portyanko *et al.*, 2005
Gh98PR	Greenhouse	10	23.7	DS-F	*Prq1b*	MN3	Portyanko *et al.*, 2005
Gh07-LSLG	Greenhouse	2.7	7.1	DLA	*Prq1b*	MN3	Acevedo *et al.*, 2010
SP97PR	Field	4.5	9.6	DS-F-1	*Prq2*	MN26	Portyanko *et al.*, 2005
Rm98PR	Field	11.9	16.8	DS-F-1	*Prq2*	MN26	Portyanko *et al.*, 2005
SP98PR	Field	9.2	14.8	DS-F-1	*Prq2*	MN26	Portyanko *et al.*, 2005
Ab07-BRCB	Field	13.4	36	DLA	*Prq2*	MN26	Acevedo *et al.*, 2010
Ab07-LSLG	Field	2.6	7.1	DLA	*Prq2*	MN26	Acevedo *et al.*, 2010
LA07	Field	3.8	9.2	DLA-F	*Prq2*	MN26	Acevedo *et al.*, 2010
TX07	Field	3.5	8.8	DLA-F	*Prq2*	MN26	Acevedo *et al.*, 2010

Table D1. (cont.)

Test Identifier	Environment	LOD	R^2 x 100	Rating method	QTL	Linkage Group	Reference
Gh97PR	Greenhouse	9	17.8	DS-F	*Prq2*	MN26	Portyanko *et al.*, 2005
Gh98PR	Greenhouse	9	17.8	DS-F	*Prq2*	MN26	Portyanko *et al.*, 2005
Gh07-BRCB	Greenhouse	10.4	31.7	DLA	*Prq2*	MN26	Acevedo *et al.*, 2010
Ab07-LSLG	Field	3.5	9.4	DLA	*Prq8*	MN13	Acevedo *et al.*, 2010
MB08-BRCB	Field	4.4	11.5	DLA	*Prq8*	MN13	Acevedo *et al.*, 2010
MB08-BRCB	Field	12.9	26	DS	*Prq8*	MN13	Acevedo *et al.*, 2010