Agriculture Development Fund (ADF)
Project Final Report

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Project Title:	Development of markers linked to oat crown rust resistance to help breed improved varieties for Saskatchewan producers	
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Abstract *(maximum 500 words)*

Detail an outline on overall project objectives, methods, key findings and conclusions for use in publications and in the ministry's database. The abstract should address the following (usually 1–2 sentences per topic):

- Key aspects of the literature review
- Problem under investigation or research question(s)
- Clearly stated hypothesis or hypotheses
- Methods used (including brief descriptions of the study design, sample, and sample size)
- Study results
- Conclusions

Oat is considered a healthy cereal due to nutritional compounds found within the grain, including beta-glucan (which has been shown to reduce the risk of heart disease), antioxidant avenanthramides (that may protect against coronary heart disease) and up to 20% protein which provides a better balance of essential amino acids compared to other cereals and is considered to be gluten-free. As a result of these desirable attributes, oat remains a significant western Canadian crop that has been seeded on an average of 3.6 million acres since 2018. This has translated to the oat industry having a direct impact on the Canadian economy of $1.4 billion and 7,000 jobs annually since 2018, with an indirect impact of $4.2 billion.

One of the largest threats to oat production in Canada is the fungal pathogen *Puccinia coronata* which causes crown rust. Yield losses in Canada have been reported at 5.1-11.2%, with losses up to 50% reported in the U.S. While agronomic practices, such as crop rotation, early seeding and fungicide application can reduce crown rust severity, incorporating genetic resistance is an economically advantageous strategy for conventional producers and is critical for organic producers.

Sustainable Canadian Agricultural Partnership

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To help breeders develop oat varieties with improved resistance to crown rust, this project aimed to develop molecular markers linked to seeding and adult resistance genes which would allow for easier selection and pyramiding of resistance. Bi-parental oat populations segregating for seven different Pc seedling resistance genes (*Pc40*, *Pc46*, *Pc62*, *Pc63*, *Pc67*, *Pc98*, *Pc101*) and adult plant resistance (APR) derived from 'CDC Dancer' were developed and evaluated for crown rust in growth chambers or field nurseries to understand the inheritance of resistance. Populations were subsequently genotyped using the Illumina Infinium SNP Assays and QTL mapped to identify the specific locations within the oat genome which were responsible for resistance. Each seedling resistance gene was mapped to a unique region of the oat genome while eight QTL associated with APR were across multiple years and crown rust nurseries, suggesting that these QTL are stable and thus good targets for selection. Finally, KASP assays were developed for four of the resistance genes which will allow for high throughput evaluation for the presence of these resistance genes in oat breeding programs. This project has provided important knowledge regarding the location of crown rust resistance genes within the oat genome, critical information for breeders wishing to pyramid several genes to provide more effective resistance, and provided tools (in the form of KASP assays) to allow efficient breeding for crown rust resistance. Ultimately, varieties with improved crown rust resistance will improve grower returns, via greater yield per acre and lower cost of production, and increase returns to oat millers, via the maintenance of grain plumpness and test weight.

Extension Messages *(3 to 5 bullet point in plain language)*

Provide key outcomes and their importance for producers/processors and the relevant industry sector.

- the genomic locations of six Pc seedling resistance genes (*Pc40*, *Pc46*, *Pc62*, *Pc63*, Pc98, *Pc101*) were identified,
- none of the resistance genes reside at a common region within the oat genome, allowing them to be incorporated in different combinations in future oat varieties and thus potentially providing better crown rust resistance,
- molecular-marker assays for four of these genes are available which will help oat breeders to incorporate these genes into future oat varieties,
- crown rust resistance present in 'CDC Dancer' was governed by multiple genes, each with less influence on crown rust resistance than any of the individual seedling crown rust genes. The resistance in CDC Dancer, although providing less resistance than the Pc genes, is more durable.

Introduction *(maximum 1,500 words)*

Provide a brief project background and rationale.

Oat is considered a healthy cereal due to nutritional compounds found within the grain, including beta-glucan. Beta-glucan is a soluble fiber that has been shown to lower plasma cholesterol and reduce the risk of heart disease (Queenan et al., 2007; Liatisa et al., 2009). This has resulted in health claims being established in Canada (Health Canada, 2010), the European Union (EFSA, 2010) and the United States (U.S. FDA, 1997). Oat grain also contains several antioxidant compounds, including the polyphenolic avenanthramides, which have anti-inflammatory effects that may protect against coronary heart disease (Meydani, 2009). Oat contains 12-20% protein which is rich in globulins and contains more lysine and threonine than other cereals, providing a better balance of essential amino acids (Klose and Arendt 2012). Although soy, pea and wheat protein are commonly used for plant-based protein products, oat is increasingly considered a viable protein alternative (Mel and Malalgoda, 2021). This is not only due to the nutritional benefits of oat, but also because it is able to be consumed by most people suffering from celiac disease and is thus considered to be gluten-free (Peraaho et al., 2004). This has supported the growth of gluten-free products developed from companies such as MGM Seeds (Saskatoon) and Avena Foods (Regina).

As a result of these desirable attributes, oat remains a significant western Canadian crop that has been seeded on an average of 3.6 million acres since 2018 (Statistics Canada, 2023). Due to the low input costs and competitive nature of oat it has also become the second most widely grown organic crop in Canada at 280,000 acres, only behind wheat at 360,000 acres (Canadian Organic Trade Association, 2020). Over the past five years, 50% of the 3.9 million metric tonnes (MT) of oat produced annually in Canada has been exported to the U.S., destined for the food market. In addition to the export of raw oat, oat is critical to the domestic milling industry. Total annual domestic milling in Canada is close to 1,225,000 MT, with western Canada being home to Richardson Milling, Grain Millers, Patterson Global Foods, Ardent Mills, Canadian Oats Milling, Emerson Milling, MGM Seeds and Avena Foods, or about 90% of milling production (Strychar, 2021). The direct impact of the oat industry on the Canadian economy amounted to $1.4 billion and 7,000 jobs annually since 2018, with an indirect impact of $4.2 billion (LMC International, 2023).

The largest on-going threat to oat production in Canada (and worldwide) is the fungal pathogen *Puccinia coronata* Corda f. sp. *avenae* Eriks which causes oat crown rust. In Canada, yield losses averaged 5.1% from 2001 to 2005 (Chong et al. 2008) with the highest loss reported at 11.2% (McCallum et al. 2007). Similarly, yield losses of 50% and 35% were reported in Minnesota and South Dakota in 2014 (USDA 2014). In addition, crown rust can weaken straw causing plants to lodge (Endo and Boewe 1958). While cultural control methods, such as crop rotation and early seeding, and fungicide application can reduce crown rust severity, incorporating genetic resistance is an important component to an integrated management strategy. Finally, the significant acreage devoted to organic oat production relies heavily on genetic resistance.

Nearly 100 crown rust resistance genes, the vast majority being seedling resistance genes, have been reported in oat (Gnanesh et al. 2014). Despite the effectiveness of these genes to combat crown rust, there has been a history of these genes being defeated with 10 years of widespread deployment in cultivars due to the development of new virulent races of the pathogen (McCallum et al. 2007, Nazareno et al. 2017). As such, gene pyramiding and use of adult plant resistance (APR) are considered viable methods to deal with this issue. For example, the APR present in the Minnesota line MN841801 has been effective since the 1970s (Leonard 2002).

In order to effectively and efficiently implement these approaches, it is necessary to genetically map the location of both seedling and APR genes within the oat genome and develop markers to these genes. Crown rust resistance associated with seedling genes, such as *Pc39* (Zhao et al. 2020), *Pc45* (Gnanesh et al. 2015), *Pc54* (Admassu-Yimer et al. 2022), *Pc91* (McCartney et al. 2011) and the unnamed gene derived from *A. strigosa* (Rines et al. 2018), as well as, APR genes from MN841801 (Lin et al. 2014), are examples of genes with mapped locations within the oat genome. The CDC oat breeding program is currently using markers linked to these genes in order to incorporate and select for crown rust resistance. This is not only cost and time efficient, but it also allows identification of individual genes within an oat line containing several resistance genes which normally would not be possible using crown rust nurseries (as the line would simply appear resistant) or because crown rust isolates with the appropriate virulence combinations may not exist to differentiate individual genes.

With the decreasing cost associated with genotype-by-sequencing (GBS) markers, the availability of the 3K and 6K Infinium SNP genotyping platform, a consensus oat map integrating both marker types (Bekele et al. 2018) and a sequenced oat genome (Kamal et al. 2022), it is now possible to not only develop markers linked to oat crown rust genes, but also understand their chromosome locations and more importantly, the allelic relationships among the vast number of reported genes. Such information will allow oat breeders to understand which combinations of resistance genes can be pyramided together and test the effectiveness of such pyramids. Although the presence of some genes has been known for decades, their current distribution within oat germplasm and effectiveness is unclear. For example, although the *Pc45* gene was reported in 1971 (Fleischmann et al. 1971) it remains a useful gene within regions of western Canada and Ontario (J. Menzies, personal comm.). It is likely that other such useful genes exist within the current pool of reported genes.

To build on Saskatchewan's (and Canada's) position as a supplier of premium quality oat to current U.S. markets (and developing markets in Mexico and China) requires developing varieties with improved crown rust resistance. This will provide value to growers, through improved yield and reduced input costs (i.e. reduced fungicide use) which will help oat remain a viable crop within a grower's rotation, and to millers/food processors, through higher select ability (i.e. good plumpness and test weight).

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Objectives and Progress *(add additional lines as needed)*

Please list the original objectives and/or revised objectives if ministry-approved revisions have been made to original objectives. A justification is needed for any deviation from original objectives.

Objective	**Progress** *(i.e., completed/in progress)*
1. Evaluate crown rust reaction in bi-parental oat populations	A total of 16 bi-parental populations and a diversity panel population, all segregating for crown rust resistance, were used to assess the genetic control of resistance governed by seven Pc seedling resistance genes (*Pc40*, *Pc46*, *Pc62*, *Pc63*, *Pc67*, *Pc98*, *Pc101*) and an adult plant resistance (APR) derived from 'CDC Dancer'. One or two genes were indicated to control resistance associated with the seedling Pc resistance genes, while polygenic control of resistance was indicated for the APR. All Pc genes were inherited in a dominant manner, except for *Pc67* which was recessively inherited.
2. QTL mapping of crown rust resistance	Genotyping of seven bi-parental populations, each segregating for a different Pc seedling gene, with the Oat 6K or USDA SoyWheOatBar 3K Infinium SNP Assay, and subsequent QTL mapping demonstrated a single genomic location was associated with resistance governed by *Pc40*, *Pc46*, *Pc62*, *Pc63* and *Pc98*, while two loci were linked to resistance governed by *Pc101*. A definitive genomic location for *Pc67* was not determined. No two Pc genes were identified at the same genomic location, indicating that pyramiding of the genes was possible. QTL mapping of the APR present in 'CDC Dancer' identified 12 QTL across the USask and AAFC-Morden nurseries, with eight QTL in common. None of the QTL appeared to be associated with seeding Pc genes.
3. Development of high-throughput marker assays	Four KASP assays have been developed for *Pc40*, *Pc46*, *Pc62* and *Pc98* which will permit efficient incorporation of these genes within breeding programs. By assessing the KASP assays for *Pc46* and *Pc62* on a diverse collection of oat lines (via the CORE panel) there is good evidence that the assays are quite specific to their related Pc genes. KASP assays will also be available within the next year for *Pc63* and *Pc101*.

Methodology *(maximum of five pages)*

Specify project activities undertaken during this reporting period. Include approaches, experimental design, tests, materials, sites, etc. Please note that any significant changes from the original work plan will require written approval from the ministry.

Genetic Mapping Populations

Bi-parental populations segregating for seedling (Pc genes) and adult plant resistance (APR) to crown rust were created for this project. The genes represent a mixture of five older genes (*Pc40*, *Pc46*, *Pc62*, *Pc63*, *Pc67*), two newer seedling genes (*Pc98*, *Pc101*), and one source of APR (from 'CDC Dancer'). Details on the populations used are outlined in Table 1. Some populations were used for QTL mapping, while other populations were used to confirm the inheritance of resistance and in some cases that the markers identified in the mapping population accurately select for resistance. The CORE Diversity Panel was a set of diverse oat germplasm that was included to determine how accurately the KASP markers linked to specific Pc genes could detect the presence of those genes.

Table 1. Details on the oat genetic populations to be used for mapping of crown rust resistance.

Population (R x S)	Generation	Pop. Size	Pc Gene	Function
Pc40 x 'AC Morgan'	F5:6	210	*Pc40*	Mapping
Pc40 x 'Kasztan'	F2 & F2:3	156	*Pc40*	Confirmation
Pc40 x 'Bingo'	F2 & F2:3	155	*Pc40*	Confirmation
Pc46 x 'AC Morgan'	F5:6	202	*Pc46*	Mapping
Pc46 x 'Kasztan'	F2 & F2:3	199	*Pc46*	Confirmation
Pc46 x 'Ajay'	F2:3	200	*Pc46*	Confirmation
Pc62 x 'AC Morgan'	F5:6	198	*Pc62*	Mapping
Pc62 x 'Kasztan'	F2 & F2:3	143	*Pc62*	Confirmation
Pc63 x 'Bingo'	F2 & F2:3	155	*Pc63*	Mapping
Pc67 x 'AC Morgan'	F5:6	220	*Pc67*	Mapping
Pc67 x 'Bingo'	F2 & F2:3	156	*Pc67*	Confirmation
Pc98 x 'Bingo'	F2 & F2:3	168	*Pc98*	Mapping
Pc98 x 'Kasztan'	F2 & F2:3	154	*Pc98*	Mapping
Pc101 x 'AC Morgan'	F2 & F2:3	148	*Pc101*	Mapping
Pc101 x 'Kasztan'	F2 & F2:3	156	*Pc101*	Confirmation
'CDC Dancer' x 'AC Morgan'	F4:7	163	APR	Mapping
CORE Diversity Panel	RILs	114	*Pc46* & *Pc62*	Marker Accuracy Testing

Greenhouse Phenotyping of Seedling Resistance

Seedlings from each segregating population were grown in controlled environment chambers in root trainers potted with Sunshine Mix#3, comprised of 70-80% Canadian Spaghum peat moss, vermiculite and dolomite limestone (Sun Gro Horticulture Canada Ltd., Seba Beach, AB). Seedlings were watered as needed and grown at a 22-23°C daytime temperature, 18-20°C night temperature and an 18-hour day length. Seedlings at the one leaf stage were inoculated by spraying urediniospores (4 mg/450 µL) suspended in Bayol® on the leaves. Approximately 10 seedlings from each RIL in the F5:6 RIL populations, and 20 F2:3 families from each of F2 plants in each F2:3 population were inoculated. The inoculated plants were incubated in a humidity chamber (maintained at 100% relative humidity) overnight at 15-17°C and then returned to the previous growing conditions. Crown rust infection types (ITs) were scored 12 days after inoculation on a 0 to 4 scale (Murphy 1935): 0 (immune), ; (fleck), 1 and 2, resistant; 3 and 4, susceptible. After rust scoring, infected leaves from F2 and F2:3 plants were trimmed and the plants were maintained with leaves being collected at the fourth

leaf-stage for DNA collection. Plants were eventually grown to maturity at which point seed was collected. Chi-square analyses were performed for each population to determine the goodness-of-fit of the observed crown rust reactions against the expected segregation ratio for a single gene. The F5:6 populations were tested against a 1 resistant: 1 susceptible ratio, the F2 populations were tested against a 3 resistant: 1 susceptible ratio, and the F2:3 families were tested against a 1 homozygous resistant: 2 segregating: 1 homozygous susceptible ratio.

Field Phenotyping of Adult Plant Resistance

Lines segregating for APR were grown in a three-replicate lattice design. Lines were tested in 2018, 2019, 2020 and 2022 at AAFC-Morden Research and Development Centre (AAFC-MRDC, Morden MB) and sown as 1 m rows with a row spacing of 0.34 m. Lines were tested in 2018, 2020 and 2022 at the University of Saskatchewan (Saskatoon, SK) and sown as hills with a row spacing of 0.3 m and spaced 0.6 m apart along the seed row. A spreader row of 'AC Morgan' (U of S) or 'Makuru' (AAFC-MRDC) was sown to increase inoculum in the nursery.

Field nurseries were inoculated with a mixture of field isolates collected from across eastern Saskatchewan and Manitoba the prior year. This mixture contained more than 100 races and was tested to ensure it is virulent on the seedlings of the parents. Two to three inoculations were done within 10 days when plants in the spreader rows reached the four-leaf stage. At each inoculation, 0.3 g of crown rust urediniospores were mixed with 300 ml light mineral oil (Bayol®, Esso Canada, Toronto, ON.) and sprayed onto spreader rows with a Herbiflex hand-held sprayer (Micron Sprayers Ltd., Bromyard, UK). At the U of S nursery water was sprayed onto the spreader rows after the Bayol had evaporated and covered with white plastic sheets to simulate dew formation and ensure germination of the urediniospores and infection of the spreader rows. The Morden field nursery was irrigated using overhead irrigation once a week, starting just before inoculation until the plants are assessed for crown rust reactions, to ensure dew formation leading to crown rust infection and spread. In Saskatoon, irrigation was applied once a week throughout the season and then 3 times per week in the week preceding rating.

Crown rust was rated on flag leaves. Disease severity (DS) was assessed using the modified Cobb scale (Peterson et al. 1948). Pustules were defined by infection type (IT): resistant (R), moderately resistant (MR), moderately susceptible, and susceptible (S) (Stubbs et al. 1986). All ITs were converted to a numerical value as follows: R = 0, RMR = 0.1667, MR = 0.3333, MRMS = 0.5, MS = 0.6667, MSS = 0.8333, and S = 1, such that a coefficient of infection (CI) can be calculated according to the formula: CI = (DS × IT) / 100.

DNA Extraction, Genotyping, QTL Mapping

DNA was extracted from seedlings (coleoptile from RILs or 4 leaf stage leaves from F2:3 lines) using a CTAB extraction method (Procunier et al. 1991; Anderson et al. 1992) or from freeze-dried leaf tissue using the DNeasy Plant DNA extraction kit (Qiagen, Toronto, Canada). Mapping populations were genotyped with the Oat 6K Infinium SNP Assay or the USDA SoyWheOatBar 3K Infinium SNP Assay (due to the Oat 6K Assay being discontinued) at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND) on the iSELECT Genotyping BeadChip (Illumina, Inc., San Diego, CA).

Marker phase was determined following the method of Tinker et al. (2014). Markers significantly deviating from the expected 1:1 segregation ratio (P value < 1e-4) were removed (Gardner et al., 2016; Zuo et al., 2019). The remaining set of markers were filtered to retain those that met the criteria of <20% heterozygosity and <20% missing data. Additionally, lines with more than 30% missing data were removed from the data set. JoinMap v.4 software (Van Ooijen, 2006) was used to group and calculate the order and position of markers within each linkage group (LG) through the Maximum Likelihood algorithm. LGs were assigned to consensus map merge groups described in Chaffin et al. (2016).

QTLs were mapped using MapQTL 5 (Van Ooijen, 2004). Interval mapping (IM) was performed using an initial mapping step size of 1 cM. The initial LOD threshold of different LGs were estimated with permutation tests using 10,000

permutations. Potential QTL peak markers identified were then chosen as cofactors to perform the multiple-QTL mapping (MQM) to identify further QTLs (Romero et al., 2018; He et al., 2016).

KASP Assays

Ten SNP markers were selected for KASP marker design based on their co-location to markers underlying QTL associated with Pc genes. Co-location of such markers were identified with the help of the oat consensus map (Bekele et al., 2018). Sequence data for the markers was obtained from the T3/Oat website (Blake et al., 2016) and submitted to the commercial LGC KASP-by-Design service (LGC, Biosearch Technologies) for design of the KASP assay.

KASP assays were used to genotyping the parents and the progeny of the mapping population and the validation populations. The KASP genotyping reactions consisted of 5ul genomic DNA (10ng/ul), 5ul 2X Master mix, and 0.14ul Primer mix in a total volume of 10ul. PCR reactions were performed on ABI StepOnePlus instrument using the 96-well PCR plate. The thermal cycling conditions of the assay were adjusted according to the GC% content of the assay following the KASP thermal cycling conditions protocols (https://biosearch-cdn.azureedge.net/assetsv6/KASP-thermal-cycling-conditions-all-protocols.pdf).

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Results and Discussions *(maximum of 30 pages (not including figures or tables))*

Describe research accomplishments during the reporting period under relevant objectives listed under "Objectives and Progress" section. Please accompany a written description of results with tables, graphs and/or other illustrations. Provide discussion necessary to the full understanding of the results. Where applicable, results should be discussed in the context of existing knowledge and relevant literature. Detail any major concerns or project setbacks.

CORE Diversity Panel

The 114 oat genotypes comprising the CORE diversity panel were evaluated for crown rust reaction against the four crown rust isolates used in the project to assess the presence of Pc genes, that is, CR223, CR249 (at both low and high temperatures), CR254 and CR257 (Supplemental Table 1 in Appendix). This data was used to determine how accurately the KASP assays linked to *Pc46* and *Pc62* were able to identify the presence of these genes. Because resistance to a given Pca isolate could be governed by crown rust resistance genes other than the ones being studied in this project (and the Pc gene complement in the CORE panel is undefined), marker accuracy assessment was determined using only the susceptible genotypes (i.e. genotypes which would have no confounding resistance genes) within the CORE panel. This work was not part of the original proposal, but it was deemed to have value and was included. Due to the considerable time required to complete this assessment for each gene, only *Pc46* and *Pc62* were completed. Similar assessments will continue after the project is complete for the other Pc genes studied.

Pc40

The *Pc40* differential line (Pendek*2/CAV 4997) (Chong et al., 2000) was crossed with three crown rust susceptible cultivars: 'AC Morgan', 'Bingo', and 'Kasztan'. 'AC Morgan' is a high yielding Canadian cultivar that lacks any major Pc genes (Kebede et al., 2019). Bingo and Kasztan both are Polish cultivars used as *Puccinia coronata* f.sp. *avenae* P. Syd. & Syd. (Pca) susceptible checks in Poland (Paczos-Grzęda and Sowa, 2019). The F5:6 family of *Pc40* x 'AC Morgan' is comprised of 210 recombinant inbred lines (RILs), the F2 populations derived from *Pc40* x 'Bingo' and *Pc40* x 'Kasztan' consisted of 155 and 156 lines, respectively, and their F2:3 families contained 154 and 150 lines. These populations were tested to determine the number of resistance genes by evaluating the resistant/segregating/susceptible segregation ratio. Pca isolate CR257 (BRBG-97) was used for evaluation of the populations. Segregation ratios in all five populations indicated the presence of a single gene (i.e. *Pc40*) which governs resistance to crown rust (Table 2).

Table 2. Disease reaction data for five *Pc40* populations evaluated with the Pca CR257 isolate.

Population	Generation	Resistant	Segregating	Susceptible	Ratio	P-Value
Pc40 x AC Morgan	F5:6	114	–	96	1:1	0.21
Pc40 x Bingo	F2	120	–	35	3:1	0.58
	F2:3	41	77	36	1:2:1	0.85
Pc40 x Kasztan	F2	116	–	40	3:1	0.85
	F2:3	42	68	41	1:2:1	0.52

DNA was extracted from young leaves (one plant/line) of the *Pc40* x 'AC Morgan' population (and the parents) and was genotyped with the Oat 6K SNP Array at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND). After filtering of the 6K SNP dataset a total of 733 high quality markers were used for QTL mapping, of which 656 markers were placed on the *Pc40* x 'AC Morgan' linkage map which consisted of 27 linkage groups and a total map length of 1,091 centimorgans (cM) (Table 3). The average density across the entire map was 0.60 markers per cM. The Mrg01, Mrg03, Mrg09, Mrg11, Mrg12 and Mrg20 consensus linkage groups, as defined by Chaffin et al. (2016), were each represented by two linkage groups in the current map (Table 3).

One major QTL, named *Pc40* (Mrg17_109.7-110.4 cM), was identified on chromosome Mrg17 (Table 4) and the allele responsible for resistance was contributed by the *Pc40* parent. The *Pc40* QTL explained 68.7% of the phenotypic variation within a QTL interval of 1.3 cM (Table 4) and the additive effect of one *Pc40* allele was to reduce the phenotypic score by 1.6 points.

Ten markers in the region of the *Pc40* QTL were converted to KASP assays and screened on all three populations. Seven of the markers were polymorphic on the *Pc40* x 'AC Morgan' and *Pc40* x 'Bingo' populations, while six were polymorphic on the *Pc40* x 'Kasztan' population (Figure 1). Good conservation of marker order was observed in all three populations (Figure 1) with markers avgbs_122060 and avgbs_213353 being the closest markers to the *Pc40* gene, both being located within 2-5 cM depending on the population (Figure 1). A common marker, GMI_ES05_c20576_219, was consistently found on the opposite side of the *Pc40* gene at a distance ranging from 16-20 cM. The avgbs_122060 or avgbs_213353 marker would be best suited to select for this gene, with use of the GMI_ES05_c20576_219 marker less helpful, but still able to provide some guidance for presence of the gene (region) when incorporating into oat breeding lines or for map-based cloning efforts.

Table 3. Summary information for *Pc40* x 'AC Morgan' population genetic linkage map.

Linkage Group	Mapped Markers	Length (cM)	Marker Density (markers/cM)	Largest Gap (cM)
Mrg01.1	26	13.3	1.95	5.9
Mrg01.2	62	50.5	1.23	8.5
Mrg02	48	61.2	0.78	12.2
Mrg03.1	5	2.3	2.11	0.8
Mrg03.2	17	34.3	0.49	21.8
Mrg04	29	30.9	0.94	6.7
Mrg05	18	11.7	1.54	3.7
Mrg06	29	40.5	0.72	7.4
Mrg08	21	116.1	0.18	24.1
Mrg09.1	19	76.7	0.25	31.3
Mrg09.2	9	48.9	0.18	15.5
Mrg11.1	14	51.5	0.27	24.4
Mrg11.2	12	19.2	0.62	11.6
Mrg12.1	37	81.2	0.46	11.6
Mrg12.2	15	14.2	1.05	6.1
Mrg13	22	18.8	1.17	3.2
Mrg15	10	40.7	0.25	18.4
Mrg17	50	88.1	0.57	17.4
Mrg18	40	84.0	0.48	21.2
Mrg19	7	26.7	0.26	14.3
Mrg20.1	9	23.1	0.39	14.5
Mrg20.2	25	31.7	0.79	17.8

Mrg21	42	40.8	1.03	5.7
Mrg23	34	16.6	2.04	7.0
Mrg24	34	29.9	1.14	4.1
Mrg28	8	4.8	1.66	2.0
Mrg33	14	45.4	0.31	21.6
Total	656	1090.9	0.60	31.3

Table 4. Summary information for the crown rust resistance QTL identified in the *Pc40* x 'AC Morgan' population.

QTL	Chrom.	Consensus Map Location (cM)	Flanking Markers	LOD[a]	Variation Explained (%)[b]	Additive[c]
Pc40	Mrg17	109.7-110.4	GMI_ES03_c5940_422 GMI_ES01_c15612_54	54.9	68.7	-1.6

[a] indicates significance of the QTL, a LOD score of 3.0 was established to declare a QTL significant.
[b] indicates the percentage of phenotypic variation explained by the QTL.
[c] indicates the direction (in this case negative) and magnitude of the effect of substituting one *Pc40* parental allele for one 'AC Morgan' parental allele on the overall phenotypic score.

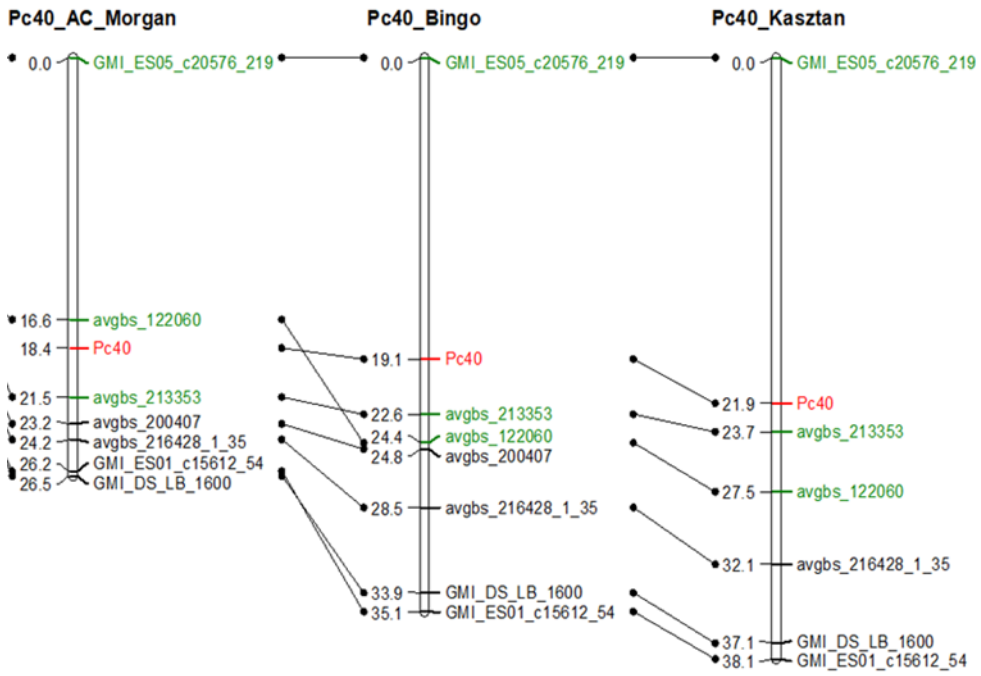

Figure 1. Linkage maps for the three *Pc40* mapping populations created using the KASP markers. Mapping populations are indicated above each linkage group, marker names are indicated to the right of each linkage group while genetic distance is indicated in cM to the left of each linkage group. The *Pc40* gene is indicated in red while the three closest KASP markers are indicated in green.

Pc46

The *Pc46* differential line (Pendek*4/CAV 5115) (Chong et al., 2000) was crossed with three crown rust susceptible cultivars: 'AC Morgan', 'Kasztan' and 'Ajay'. 'Ajay' is a short, high yielding variety that is susceptible to crown rust and has been used as a susceptible parent in bi-parental crown rust mapping studies (Abdullah et al. 2023). The F6 family of *Pc46* x 'AC Morgan' is comprised of 202 RILs, the F2 population and F2:3 families derived from *Pc46* x 'Kasztan' consisted of 199 lines and 189 families, respectively, while the F2:3 families derived from *Pc46* x 'Ajay' consisted of 200 families. These

populations were tested to determine the number of resistance genes by evaluating the resistant/segregating/susceptible segregation ratio. Pca isolate CR254 (LRBG) was used for evaluation of the populations. As shown in Table 5, the segregation ratio for the *Pc46* x 'AC Morgan" F5:6 population best fit a 3:1 ratio, indicative of two independent resistance genes being present in the *Pc46* parent. In the *Pc46* x 'Kasztan' F2 population is it unclear as to the number of genes. The observed ratio was tested against both a dominant and recessive form of resistance with neither fitting. However, the F2:3 population clearly indicated the presence of a single gene (i.e. *Pc46*) which governs resistance to crown rust (as assessed with Pca isolate CR254). In the F2 population there were a large number of plants scored as a rating of 3 (i.e. susceptible). As this is close to the cut-off point between declaring a line to be resistant or susceptible, it may be that some of these plants could have been alternatively rated as a score of 2. The F2:3 population is a more reliable means to assess gene number as the data is based on 24 plants per family instead of a single F2 plant, and thus there is an opportunity to observe and discern the true interaction between the pathogen and the host. The 200 F2:3 families from the *Pc46* x 'Ajay' population were screened with a crown rust isolate belonging to the LBLB race and segregation was also consistent with a single gene controlling resistance (Table 5).

Table 5. Disease reaction data for five *Pc46* populations evaluated with the Pca CR254 isolate.

Population	Generation	Resistant	Segregating	Susceptible	Ratio	P-Value
Pc46 x 'AC Morgan'	F5	140	–	62	1:1	<0.0001
					3:1	0.07
Pc46 x 'Kasztan'	F2	88	–	111	3:1	<0.0001
					1:3	<0.0001
	F2:3	55	96	38	1:2:1	0.21
Pc46 x 'Ajay'	F2:3	50	105	45	1:2:1	0.25

DNA was extracted from young leaves (one plant/line) of the *Pc46* x 'AC Morgan' population (and the parents) and was genotyped with the Oat 6K SNP Array at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND). After filtering of the 6K SNP dataset a total of 739 high quality markers were used for QTL mapping, of which 623 markers were mapped on the *Pc46* x 'AC Morgan' map which included 29 linkage groups and a total map length of 1,073 cM (Table 6). The average density across the entire map was 0.58 markers per cM. The Mrg01, Mrg03, Mrg08, Mrg09, Mrg11, Mrg12, Mrg20 and Mrg24 consensus linkage groups, as defined by Chaffin et al. (2016), were each represented by two linkage groups in the current map (Table 6).

Despite the indications from the *Pc46* x 'AC Morgan' F5 population that potentially two resistance genes were present in this population, but consistent with the data from the *Pc46* x 'Kasztan' and *Pc46* x 'Ajay' F2:3 populations, only one major QTL, named *Pc46* (Mrg19_71.3-75.7 cM), was identified on chromosome Mrg19 (Table 7) and the allele responsible for resistance was contributed by the *Pc46* parent. The *Pc46* QTL explained 97.2% of the phenotypic variation within a QTL interval of 6.7 cM (Table 7) and the additive effect of one *Pc46* allele was to reduce the phenotypic score by 1.7 points. One could explain the difference between the genetic segregation ratio, indicating 2 genes, and the QTL mapping, indicating 1 gene, by the possibility that there was inadequate genome coverage with the 6K SNP marker data set, such that the second resistance gene was not identified due to the absence of markers in the region harboring the second gene. However, the fact that the one QTL identified explained nearly all the phenotypic variation would more strongly indicate that there was only a single gene responsible for resistance and that the population used for the mapping study was sampled by chance in such a manner as to be skewed toward lines with resistance.

Table 6. Summary information for *Pc46* x 'AC Morgan' population genetic linkage map.

Linkage Group	Mapped Markers	Length (cM)	Marker Density (marker/cM)	Largest Gap (cM)
Mrg01.1	52	47.2	1.10	7.2
Mrg01.2	25	13.2	1.89	6.1
Mrg02	15	100.5	0.15	26.2
Mrg03.1	5	1.5	3.25	1.2
Mrg03.2	17	16.2	1.05	5.9
Mrg04	28	27.2	1.03	10.4
Mrg05	12	18.6	0.64	8.2
Mrg06	19	28.9	0.66	14.6
Mrg08.1	40	50.8	0.79	18.1
Mrg08.2	6	35.2	0.17	17.4
Mrg09.1	16	42.2	0.38	15.6
Mrg09.2	8	7.9	1.00	2.6
Mrg11.1	21	10.5	2.00	7.5
Mrg11.2	8	72.7	0.11	17.4
Mrg12.1	19	18.1	1.05	10.6
Mrg12.2	15	22.8	0.66	14.6
Mrg13	25	31.9	0.78	12.5
Mrg15	8	30.3	0.26	14.2
Mrg17	51	112.6	0.45	25.3
Mrg18	23	87.1	0.26	21.4
Mrg19	27	104.9	0.26	30.5
Mrg20.1	6	17.1	0.35	15.2
Mrg20.2	31	41.2	0.75	14.9
Mrg21	60	50.2	1.19	8.6
Mrg23	30	12.1	2.47	4.6
Mrg24.1	21	31.8	0.66	21.9
Mrg24.2	14	1.0	13.59	0.3
Mrg28	8	5.0	1.57	3.4
Mrg33	13	32.9	0.39	10.0
Total	623	1072.8	0.58	30.5

Table 7. Summary information for the crown rust resistance QTL identified in the *Pc46* x 'AC Morgan' population.

QTL	Chrom.	Consensus Map Location (cM)	Flanking Markers	LOD[a]	Variation Explained (%)[b]	Additive[c]
Pc46	Mrg19	71.3-75.7	GMI_DS_LB_4867 GMI_GBS_35330	103.4	97.2	-1.7

[a] indicates significance of the QTL, a LOD score of 3.0 was established to declare a QTL significant.
[b] indicates the percentage of phenotypic variation explained by the QTL.
[c] indicates the direction (in this case negative) and magnitude of the effect of substituting one *Pc46* parental allele for one 'AC Morgan' parental allele on the overall phenotypic score.

Twenty markers were selected for design of KASP genotyping assays from the Mrg19 region spanning 70.3-92.2 cM. Among the twenty KASP assays, eight were polymorphic between the *Pc46* and 'AC Morgan' parents and only one was polymorphic between the *Pc46* and 'Kasztan' parents (Figure 2). There was generally good conservation of marker order observed between the two mapping populations and the consensus map for Mrg19, with some minor rearrangements distal to the *Pc46* gene (Figure 2). Markers avgbs_9827 and avgbs_200022 were the closest flanking markers to the *Pc46* gene, being 1.8 cM and 5.4 cM away, respectively (Figure 2). Unfortunately, neither marker was polymorphic in the *Pc46* x 'Kasztan' population, but the marker avgbs_67655 was polymorphic in both and was located 4.3-12.4 cM from *Pc46*, depending on the population (Figure 2).

To determine how accurately the KASP markers flanking the *Pc46* gene would select for the presence of this gene, the susceptible genotypes within the CORE diversity panel were screened. In the subset of 53 genotypes susceptible to the Pca CR254 isolate, avgbs_9827 and avgbs_200022 marker combination had collective prediction accuracy of 72%, while the avbgs_9827 and avbgs_122535 combination was 97% accurate. Similarly, using the avbgs_122525 and avgbs_67655 markers (avgbs_67655 was polymorphic in both mapping populations) produced a prediction accuracy of 98% among the susceptible subset of genotypes. Currently, markers avbgs_9827 and avbgs_122535 are the most useful pair of markers to incorporate the *Pc46* gene into breeding lines. We are currently attempting to identify additional flanking markers in the *Pc46* x 'Kazstan' population, using the OT3098 genome assembly, that will improve the efficiency and accuracy of incorporating *Pc46* into breeding lines.

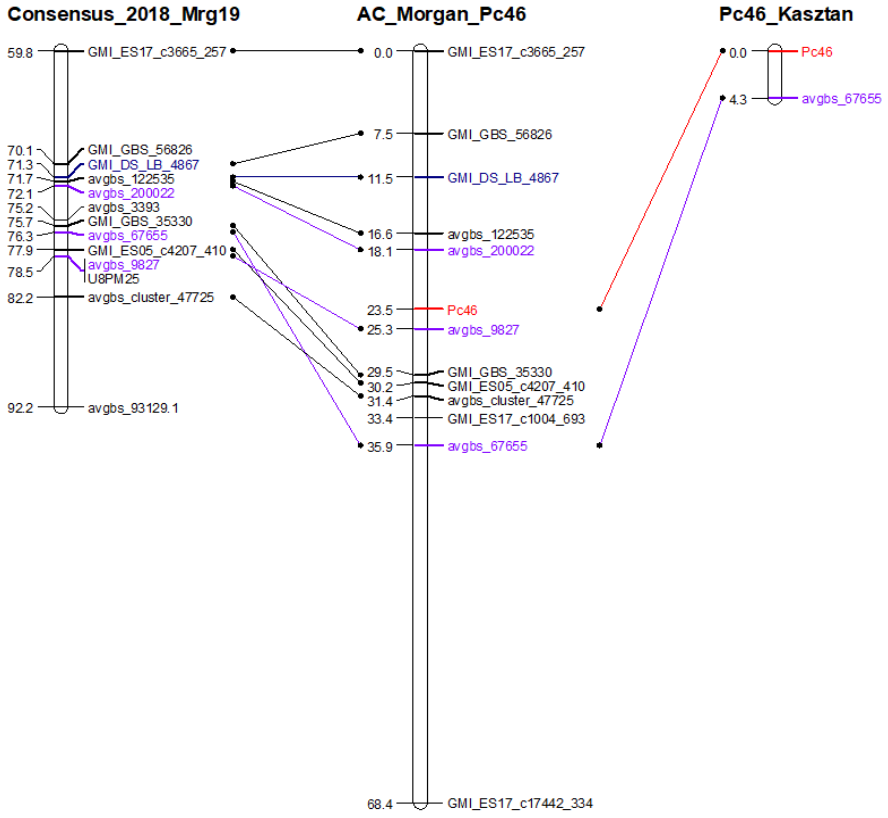

Figure 2. Linkage maps for the two *Pc46* mapping populations created using KASP markers in comparison to the Mrg19 consensus map. Mapping populations are indicated above each linkage group, marker names are indicated to the right of each linkage group while genetic distance is indicated in cM to the left of each linkage group. The *Pc46* gene is indicated in red while the three closest KASP markers are indicated in purple.

Pc62

The *Pc62* differential line (Fraser*4/CAV 4274) (Chong et al., 2000) was crossed with two crown rust susceptible cultivars: 'AC Morgan' and 'Kasztan'. The F5:6 family of *Pc62* x 'AC Morgan' is comprised of 198 recombinant inbred lines (RILs), the F2 population derived from *Pc62* x 'Kasztan' consisted of 143 lines. These populations were tested to determine the number of resistance genes by evaluating the resistant/segregating/susceptible segregation ratio. Pca isolate CR254 (LRBG) was used for evaluation of the populations. As shown in Table 8, the segregation ratio for the *Pc62* x 'AC Morgan' F5:6 population did not fit either a 1:1 or 3:1 ratio, indicative of a one or two independent resistance genes, respectively. However, the *Pc62* x 'Kasztan' F2 lines and F2:3 families were both consistent with a single gene model for resistance derived from the *Pc62* parent (Table 8).

Table 8. Disease reaction data for three *Pc62* populations evaluated with the Pca CR254 isolate.

Population	Generation	Resistant	Segregating	Susceptible	Ratio	P-Value
Pc62 x 'AC Morgan'	F5:6	125	–	73	1:1	<0.0001
					3:1	<0.0001
Pc62 x 'Kasztan'	F2	108	–	35	3:1	0.29
	F2:3	38	82	35	1:2:1	0.68

DNA was extracted from young leaves (one plant/line) of the *Pc62* x 'AC Morgan' population (and the parents) and was genotyped with the Oat 6K SNP Array at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND). After filtering of the 6K SNP dataset a total of 692 high quality markers were used for QTL mapping, of which 619 markers were mapped on the *Pc62* x 'AC Morgan' map which included 24 linkage groups and a total map length of 1,120 cM (Table 9). The average density across the entire map was 0.55 markers per cM. The Mrg03, Mrg09, Mrg11, Mrg23 and Mrg24 consensus linkage groups, as defined by Chaffin et al. (2016), were each represented by two linkage groups in the current map, and markers of Mrg01 and Mrg17 were mapped together into one LG (Table 9).

Despite no clear indication from the *Pc62* x 'AC Morgan' F5:6 population regarding the number of resistance genes segregating in the population, but consistent with the data from the *Pc62* x 'Kasztan' populations, one major QTL, named *Pc62* (Mrg5_14.4-21.1 cM), was identified on chromosome Mrg5 (Table 10) and the allele responsible for resistance was contributed by the *Pc62* parent. The *Pc62* QTL explained 97.9% of the phenotypic variation within a QTL interval of 6.7 cM (Table 10) and the additive effect of one *Pc62* allele was to reduce the phenotypic score by 1.8 points. As with the disparity between the genetic segregation results and the QTL mapping results for *Pc46*, the fact that the one QTL identified explained nearly all the phenotypic variation for *Pc62* would strongly indicate that there was only a single gene responsible for resistance and that the population used for the mapping study was sampled by chance in such a manner as to be skewed toward lines with resistance.

Table 9. Summary information for *Pc62* x 'AC Morgan' population genetic linkage map.

Linkage Group	Mapped Markers	Length (cM)	Marker Density (marker/cM)	Largest Gap (cM)
Mrg01/17	111	106.1	1.05	9.8
Mrg02	10	26.1	0.38	10.3
Mrg03.1	20	50.1	0.40	12.0
Mrg03.2	13	35.6	0.36	9.6
Mrg04	33	13.4	2.46	4.7
Mrg05	33	94.1	0.35	33.4
Mrg06	17	2.7	6.12	1.3
Mrg08	45	93.1	0.48	19.3
Mrg09.1	11	52.4	0.21	17.6

Mrg09.2	9	11.4	0.79	5.1
Mrg11.1	8	7.6	1.04	6.4
Mrg11.2	63	42.9	1.47	4.5
Mrg12	25	67.3	0.37	17.3
Mrg13	11	24.1	0.45	20.9
Mrg15	19	82.1	0.23	19.4
Mrg18	28	56.4	0.50	13.0
Mrg20	47	83.9	0.56	19.6
Mrg21	27	56.5	0.48	15.9
Mrg23.1	14	4.9	2.86	2.0
Mrg23.2	18	36.5	0.49	25.8
Mrg24.1	6	10.4	0.57	4.2
Mrg24.2	13	40.0	0.32	21.6
Mrg28	16	58.9	0.27	14.6
Mrg33	22	62.9	0.35	16.0
Total	619	1120.2	0.55	33.4

Table 10. Summary information for the crown rust resistance QTL identified in the *Pc62* x 'AC Morgan' population.

QTL	Chrom.	Consensus Map Location (cM)	Flanking Markers	LOD[a]	Variation Explained (%)[b]	Additive[c]
Pc62	Mrg05	14.4-21.1	GMI_ES14_c11707_426 GMI_ES17_c11370_658	99.1	97.9	-1.8

[a] indicates significance of the QTL, a LOD score of 3.0 was established to declare a QTL significant.
[b] indicates the percentage of phenotypic variation explained by the QTL.
[c] indicates the direction (in this case negative) and magnitude of the effect of substituting one *Pc62* parental allele for one 'AC Morgan' parental allele on the overall phenotypic score.

Twenty-three markers were selected for design of KASP genotyping assays from the Mrg02 region spanning 87.3-112.7 cM. Among the twenty-three KASP assays, seven were polymorphic between the *Pc62* and 'AC Morgan' parents and five polymorphic between the *Pc62* and 'Kasztan' parents (Figure 3). There was again good conservation of marker order observed between the two mapping populations and the consensus map for Mrg02 (Figure 3). Markers avgbs_8039 was the closest marker to the *Pc62* gene, being 3.2-4.7 cM away (Figure 3). Unfortunately, there was no flanking marker identified for *Pc62* which decreases the accuracy of selecting for this gene. We are continuing the search for a suitable flanking marker using the OT3098 genome assembly that will improve the efficiency and accuracy of incorporating *Pc62* into breeding lines.

To determine how accurately the KASP markers flanking the *Pc62* gene would select for the presence of this gene, the susceptible genotypes within the CORE diversity panel were screened. In the subset of 53 genotypes susceptible to the Pca CR254 isolate, the avgbs_8039 marker had a prediction accuracy of 68%, while the GMI_ES17_c12203_326 marker (located further from the gene) had a higher accuracy of 73%. When the two markers are used in combination accuracy improved to 81%. These are currently the two best markers to use when selecting for the *Pc62* gene.

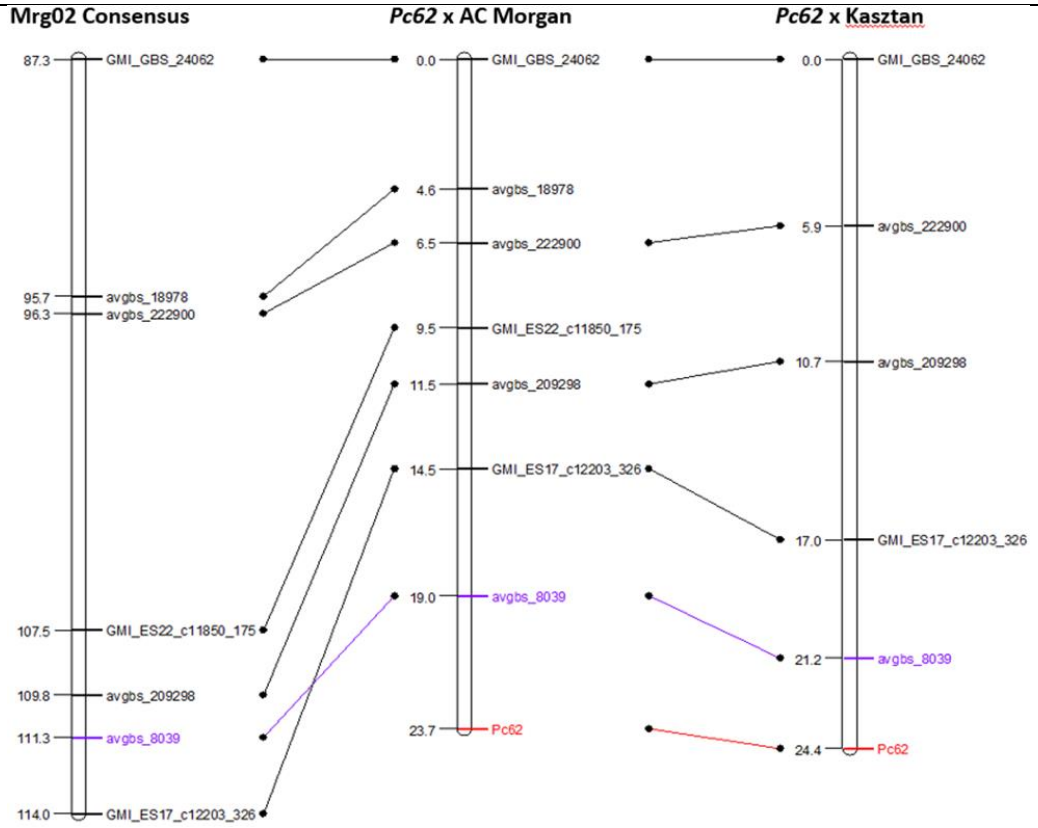

Figure 3. Linkage maps for the two *Pc62* mapping populations created using KASP markers in comparison to the Mrg02 consensus map. Mapping populations are indicated above each linkage group, marker names are indicated to the right of each linkage group while genetic distance is indicated in cM to the left of each linkage group. The *Pc62* gene is indicated in red while the closest KASP markers are indicated in purple.

Pc63

The *Pc63* differential line ('Fraser'/CAV 4540) was developed by backcrossing the resistance gene from the originating *A. sterilis* (CAV 4540) line into the susceptible cultivar 'Fraser' (Harder et al., 1980). In this study, the Pc63 differntial line was crossed with the susceptible cultivar 'Bingo'. The resulting F2 population consisted of 155 lines from which 151 F2:3 families were derived. Pca isolate CR223 (race NGCB-94) was used to evaluate the population and the segregation ratio for the *Pc63* x 'Bingo' F2 and F2:3 populations clearly indicated the presence of a single, dominant gene (i.e. *Pc63*) which governs resistance to crown rust (Table 11).

Table 11. Disease reaction data for two *Pc63* populations evaluated with the Pca CR223 isolate.

Population	Generation	Resistant	Segregating	Susceptible	Ratio	P-Value
Pc63 x 'Bingo'	F2	119	–	36	3:1	0.58
	F2:3	30	81	40	1:2:1	0.54

DNA was extracted from young leaves (one plant/line) of the *Pc63* x 'Bingo' F2 population (and the parents) and was sent for genotyping with the SoyWheOatBar 3K SNP Array at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND). After filtering of the 3K SNP dataset a total of 482 high quality markers were used for QTL mapping of which 380 markers were placed on the *Pc63* x 'Bingo' map which included 21 linkage groups and a total map length of 1,403 cM (Table 12). The average density across the entire map was 0.31 markers per cM. The Mrg04, Mrg17, Mrg18 and Mrg20 consensus

linkage groups, as defined by Chaffin et al. (2016), were each represented by two linkage groups in the current map (Table 12).

Consistent with the *Pc63* x 'Bingo' F2 and F2:3 populations regarding the number of resistance genes segregating in the population, one major QTL named *Pc63* (Mrg02_85.2 cM), was identified on chromosome Mrg2 (Table 13) and the allele responsible for resistance was contributed by the *Pc62* parent. The *Pc62* QTL explained 97.7% of the phenotypic variation and the additive effect of one *Pc63* allele was to reduce the phenotypic score by 1.9 points (Table 13). The QTL was located at the end of a linkage group in the *Pc63* x 'Bingo' genetic map, so markers were located on only one side of the QTL. Currently, a flanking marker is being searched for on the opposing side of the QTL to make introgression and selection for this gene more reliable.

Table 12. Summary information for *Pc63* x 'Bingo' population genetic linkage map.

Linkage Group	Mapped Markers	Length (cM)	Marker Density (marker/cM)	Largest Gap (cM)
Mrg01	9	56.7	0.16	16.5
Mrg02	23	53.5	0.43	13.5
Mrg03	34	80.2	0.42	10.2
Mrg04_1	7	16.0	0.44	10.7
Mrg04_2	6	7.2	0.83	2.7
Mrg05	27	91.8	0.29	23.4
Mrg06	12	46.5	0.26	19.8
Mrg08	16	84.5	0.19	33.7
Mrg09	22	139.9	0.16	36.7
Mrg11	33	80.8	0.41	17.6
Mrg13	13	76.9	0.17	35.9
Mrg15	18	68.3	0.26	29.4
Mrg17_1	32	83.4	0.38	19.2
Mrg17_2	12	27.2	0.44	16.5
Mrg18_1	4	22.1	0.18	12.3
Mrg18_2	3	6.0	0.50	4.4
Mrg20_1	5	33.4	0.15	27.2
Mrg20_2	12	59.0	0.20	25.5
Mrg21	26	106.0	0.25	25.7
Mrg23	17	62.1	0.27	30.4
Mrg24	27	55.6	0.48	17.3
Mrg28	11	50.3	0.22	27.2
Mrg33	11	95.5	0.12	35.3
Total	380	1403.8	0.31	36.7

Table 13. Summary information for the crown rust resistance QTL identified in the *Pc63* x 'Bingo' population.

QTL	Chrom.	Consensus Map Location (cM)	Flanking Markers	LOD[a]	Variation Explained (%)[b]	Additive[c]
Pc63	Mrg02	85.2	GMI_ES01_c13079_243	90.3	97.7	-1.9

[a] indicates significance of the QTL, a LOD score of 3.0 was established to declare a QTL significant.
[b] indicates the percentage of phenotypic variation explained by the QTL.
[c] indicates the direction (in this case negative) and magnitude of the effect of substituting one *Pc63* parental allele for one 'AC Morgan' parental allele on the overall phenotypic score.

Pc67

The *Pc67* differential line (Harder and Chong, 1983) was crossed with crown rust susceptible cultivar 'AC Morgan.' The F6 population of *Pc67* x 'AC Morgan' was comprised 220 RILs. A second population consisting of 154 F2 and 150 F2:3 families derived from the cross *Pc67* x 'Bingo' was created to serve as the confirmation population. Initial evaluation of the *Pc67* differential line with 10 crown rust isolates failed to identify an isolate to which the *Pc67* gene was effective. It was discovered that by lowering the temperature during inoculation to 18°C day/15°C night and inoculating with isolate CR249 (race DQGB-94) that effective disease could be developed on the susceptible parents while the *Pc67* differential line remained disease-free. It therefore appears that this gene is temperature sensitive, information that was not previously reported about this gene. The segregation ratio for the *Pc67* x 'AC Morgan' F6 population and the *Pc67* x 'Bingo' F2 and F2:3 populations clearly indicated the presence of a single gene (i.e. *Pc67*) which governs resistance to crown rust (Table 14). Interestingly segregation data also indicated that the gene is recessive in nature.

Table 14. Disease reaction data for four *Pc67* populations evaluated with the Pca CR249 isolate.

Population	Generation	Resistant	Segregating	Susceptible	Ratio	P-Value
Pc67 x 'AC Morgan'	F6	121	–	99	1:1	0.14
Pc67 x 'Bingo'	F2	35	–	121	1:3	0.76
	F2:3	40	67	43	1:2:1	0.49

DNA was extracted from young leaves (one plant/line) of the *Pc67* x 'AC Morgan' and was sent for genotyping with the Oat 6K SNP Array at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND). After filtering of the 6K SNP dataset a total of 542 high quality markers were used for QTL mapping of which 431 markers were placed on the *Pc67* x 'AC Morgan' map which included 16 linkage groups and a total map length of only 453 cM (Table 15). The average density across the entire map was 1.6 markers per cM. The Mrg09 consensus linkage group, as defined by Chaffin et al. (2016), was represented by two linkage groups in the current map, and markers of Mrg05 and Mrg13 were mapped together into one LG (Table 15).

Consistent with the *Pc67* x 'AC Morgan' and *Pc67* x 'Bingo' populations regarding the number of resistance genes segregating in the population, one major QTL named *Pc67* (Mrg21_82.7-113.2 cM), was identified on chromosome Mrg21 (Table 16) and the allele responsible for resistance was contributed by the *Pc67* parent. The *Pc67* QTL explained only 10.7% of the phenotypic variation and the additive effect of one *Pc67* allele was to reduce the phenotypic score by 0.4 points (Table 16). The amount of variation explained by the QTL is smaller than expected for a single gene trait. Among all the Pc genes evaluated in this project, this population had the lowest number of mapped markers and the smallest overall genetic map in terms of length. It is therefore possible that the true *Pc67* locus resides in a region of the genome that was not spanned with markers and was thus not identified. Future work will focus on identifying additional markers in this population using other genotyping platforms like DArTseq.

Table 15. Summary information for *Pc67* x 'AC Morgan' population genetic linkage map.

Linkage Group	Mapped Markers	Length (cM)	Marker Density (marker/cM)	Largest Gap (cM)
Mrg01	64	71.8	0.89	7.1
Mrg02	28	20.7	1.35	6.8
Mrg03	19	25.9	0.73	12.7
Mrg04	27	3.8	7.01	3.4
Mrg05/13	10	53.6	0.19	28.8
Mrg08	23	21.2	1.08	9.6
Mrg09_1	29	34.5	0.84	15.3
Mrg09_2	11	6.9	1.57	1.8
Mrg11	22	7.0	3.12	4.6

Mrg12	10	30.2	0.33	9.7
Mrg15	45	36.3	1.24	18.3
Mrg17	50	48.4	1.03	6.8
Mrg18	9	3.4	2.59	1.0
Mrg21	52	59.3	0.88	14.1
Mrg23	17	15.0	1.13	4.9
Mrg24	15	14.9	1.00	5.0
Total	431	453.6	1.56	28.8

Table 16. Summary information for the crown rust resistance QTL identified in the *Pc67* x 'AC Morgan' population.

QTL	Chrom.	Consensus Map Location (cM)	Flanking Markers	LOD[a]	Variation Explained (%)[b]	Additive[c]
Pc67	Mrg21	82.7-113.2	GMI_ES02_lrc12740_554 GMI_ES02_c9205_86	4.9	10.7	-0.4

[a] indicates significance of the QTL, a LOD score of 3.0 was established to declare a QTL significant.
[b] indicates the percentage of phenotypic variation explained by the QTL.
[c] indicates the direction (in this case negative) and magnitude of the effect of substituting one *Pc67* parental allele for one 'AC Morgan' parental allele on the overall phenotypic score.

Pc98

The *Pc98* differential line (Harmon*2/CAV 1979) (Paczos-Grzeda and Sowa 2019) was crossed with two crown rust susceptible cultivars: 'Bingo' and 'Kasztan'. The F2 populations derived from *Pc40* x 'Bingo' and *Pc40* x 'Kasztan' consisted of 168 and 154 lines, respectively, and their F2:3 families contained 168 and 152 lines. These populations were tested to determine the number of resistance genes by evaluating the resistant/segregating/susceptible segregation ratio. Pca isolate CR241 (DSGB) was used for evaluation of the populations. Segregation ratios in all four populations indicate the presence of a single gene (i.e. *Pc98*) which governs resistance to crown rust (Table 17).

Table 17. Disease reaction data for five *Pc98* populations evaluated with the Pca CR241 isolate.

Population	Generation	Resistant	Segregating	Susceptible	Ratio	P-Value
Pc98 x Bingo	F2	124	–	22	3:1	0.72
	F2:3	45	79	44	1:2:1	0.74
Pc98 x Kasztan	F2	111	–	43	3:1	0.40
	F2:3	38	73	41	1:2:1	0.36

DNA was extracted from young F2 plant leaves from which each F2:3 family was derived. A subset of 10 resistant and 10 susceptible F2 lines were genotyped with the Oat 6K SNP Array at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND) to identify markers linked to the *Pc98* gene. Twenty Oat 6K SNP Array and 12 GBS-derived SNPs linked to the *Pc98* locus (as determined by their co-segregation within the resistant or susceptible bulks) were selected for KASP assay design and tested on the entire mapping populations. Among the 32 markers, 19 were polymorphic in the *Pc98* x 'Bingo' population and 17 were polymorphic in the *Pc98* x "Kasztan' population. There was very good conservation of marker order observed between the two mapping populations and the consensus map for Mrg20, with some minor rearrangements noted (Figure 4). The closest flanking markers to *Pc98* were GMI_ES17_c6059_221_kom397 and GMI_ES22_c3052_382_kom399 on one side, and GMI_ES14_lrc18344_662_kom398 on the other, with the entire region spanning only 1.2 cM (Figure 4). Given the tight linkage of these three markers to the *Pc98* locus they should allow efficient selection of this gene in breeding programs with very little chance of false positives occurring (i.e. expecting to have the gene present but in fact it is not). The work is described in Zhao et al. 2020.

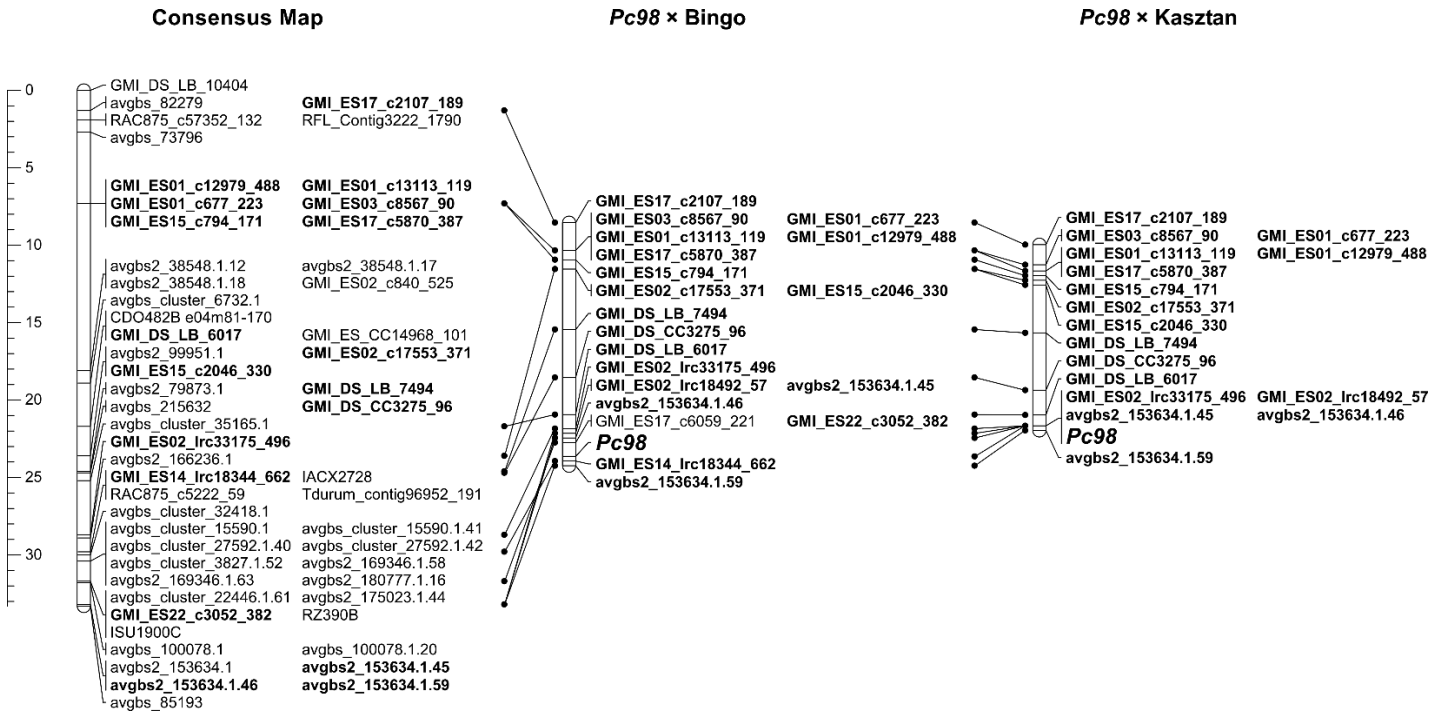

Figure 4. Linkage maps for the two *Pc98* mapping populations created using KASP markers in comparison to the Mrg20 consensus map. Mapping populations are indicated above each linkage group, marker names are indicated to the right of each linkage group while genetic distance is indicated in cM to the left of all linkage groups. The *Pc98* gene is indicated in bold text.

Pc101

The *Pc101* differential line ('Harmon'/PI334961) was developed from an *A. sterilis* line (PI334961) collected from Israel which was previously described to contain two dominant resistant genes (Paczos-Grzęda and Sowa, 2019). *Pc101* was crossed with two crown rust susceptible cultivars: 'AC Morgan' and 'Kasztan'. A population of 156 F2 plants and 151 F2:3 families were developed from both *Pc101* x 'Kazstan' and *Pc101* x 'AC Morgan'. Pca isolate CR254 (race LRBG) was used to evaluate the populations and the segregation ratio for both the *Pc101* x 'Kasztan' and *Pc101* x 'AC Morgan'F2 populations and the F2:3 families clearly indicated the presence of two dominant genes (i.e. *Pc101*) which governs resistance to crown rust (Table 18).

Table 18. Disease reaction data for four *Pc101* populations evaluated with the Pca CR254 isolate.

Population	Generation	Resistant	Segregating	Susceptible	Ratio	P-Value
Pc101 x 'Kazstan'	F2	145	–	11	15:1	0.74
	F2:3	86	57	8	7:8:1	0.10
Pc101 x 'AC Morgan'	F2	141	–	15	15:1	0.30
	F2:3	88	57	6	7:8:1	0.05

DNA from the *Pc101* x 'AC Morgan' F2 population (and the parents) was extracted from young leaves (one plant/line) and was sent for genotyping with the SoyWheOatBar 3K SNP Array at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND). After filtering of the 3K SNP dataset a total of 619 high quality markers were used for QTL mapping, of which 548 markers were placed on the *Pc101* x 'AC Morgan' map which included 23 linkage groups and a total map length of 1,440 cM (Table 19). The average density across the entire map was 0.70 markers per cM. The markers of Mrg08 and Mrg18 consensus linkage groups, as defined by Chaffin et al. (2016), were mapped together into one LG (Table 19).

Table 19. Summary information for *Pc101* x 'AC Morgan' population genetic linkage map.

Linkage Group	Mapped Markers	Length (cM)	Marker Density (markers/cM)	Largest Gap (cM)
Mrg01	40	123.3	0.32	23.1
Mrg02	25	7.5	3.32	1.8
Mrg03	42	95.4	0.44	10.0
Mrg04	17	51.1	0.33	11.7
Mrg05	23	45.4	0.51	10.1
Mrg06	18	103.1	0.17	37.4
Mrg08	34	82.7	0.41	11.2
Mrg08/18	16	46.3	0.35	11.2
Mrg09	23	70.3	0.33	21.0
Mrg09	16	34.8	0.46	9.6
Mrg11	44	101.9	0.43	8.4
Mrg12	18	48.0	0.37	15.8
Mrg13	25	47.8	0.52	4.7
Mrg15	37	62.8	0.59	5.6
Mrg17	43	106.0	0.41	21.0
Mrg18	6	22.1	0.27	9.0
Mrg19	6	17.4	0.34	10.9
Mrg21	36	147.3	0.24	22.1
Mrg21	20	74.3	0.27	24.6
Mrg23	12	10.4	1.15	4.7
Mrg24	12	2.7	4.33	1.6
Mrg28	14	47.6	0.29	16.0
Mrg33	21	90.3	0.23	13.0
Total	548	1439.6	0.70	37.4

Consistent with the *Pc101* x 'AC Morgan' and *Pc101* x 'Kazstan' F2 and F2:3 populations regarding the number of resistance genes segregating in the population, two major QTL named *Pc101_1* (Mrg08_47.5-62.4 cM) and *Pc101_2* (Mrg08_131.3-142.0 cM), were identified on chromosome Mrg08 (Table 20) and the alleles responsible for resistance was contributed by the *Pc101* parent. The *Pc101* QTLs explained 18.1% and 75.1% of the phenotypic variation and the additive effect of one *Pc63* allele was to reduce the phenotypic score by 1.3 and 1.7 points (Table 20). Currently, KASP assays are being

developed for the flanking markers associated with these two QTL in order to make introgression and selection of these loci more efficient.

Table 20. Summary information for the crown rust resistance QTL identified in the *Pc101* x 'AC Morgan' population.

QTL	Chrom.	Consensus Map Location (cM)	Flanking Markers	LOD[a]	Variation Explained (%)[b]	Additive[c]
Pc101_1	Mrg08	47.5-62.4	GMI_ES14_c5917_794 GMI_ES17_c20660_396	46.9	75.1	-1.7
Pc101_2	Mrg08	131.3-142.0	GMI_ES01_c14013_124 avgbs_7505	24.3	18.1	-1.3

[a] indicates significance of the QTL, a LOD score of 3.0 was established to declare a QTL significant.
[b] indicates the percentage of phenotypic variation explained by the QTL.
[c] indicates the direction (in this case negative) and magnitude of the effect of substituting one *Pc101* parental allele for one 'AC Morgan' parental allele on the overall phenotypic score.

'CDC Dancer' Adult Plant Resistance (APR)

The 'CDC Dancer' x 'AC Morgan' F4:7 population consisted of 163 lines and was grown from 2018-22 in the crown rust field nurseries located at AAFC-Morden (Morden, MB) and the University of Saskatchewan (USask, Saskatoon, SK). No data was collected from 2019 or 2021 at USask or 2021 at AAFC-Morden due to hot and dry conditions which prevented the development of disease. Summary statistics for the crown rust coefficient of infection (CI) collected on the population in the remaining years is provided in Table 21. 'CDC Dancer' which harbored the APR had a consistently lower CI than the susceptible 'AC Morgan'. The mean CI for the population resided between the two parents at the USask nursery across all years, but was consistently outside the range of the two parents at the AAFC-Morden nursery in each year except 2022. At each site year transgressive segregation was observed in the population for CI.

Table 21. Descriptive statistics associated with crown rust coefficient of infection (CI) for the 'CDC Dancer' x 'AC Morgan' RIL population grown at the University of Saskatchewan (USask) and AAFC-Morden crown rust field nurseries from 2018-22.

			RIL Population			
Nursery & Year	**CDC Dancer**	**AC Morgan**	**Mean**	**Std. Dev.**	**Minimum**	**Maximum**
USask						
2018	7.2	17.1	8.6	6.5	0.4	31.0
2020	20.6	42.9	29.8	29.8	6.3	68.0
2022	49.1	78.0	43.3	18.2	8.1	95.0
Average	25.6	46.0	27.2	9.9	7.0	53.0
AAFC-Morden						
2018	8.0	20.0	5.0	5.2	0.4	30.0
2019	20.0	25.0	6.7	8.0	0.4	50.0
2020	1.6	5.0	12.4	16.2	0.4	80.0
2022	0.4	4.0	2.7	6.6	0.2	62.0
Average	7.5	13.5	6.7	6.0	0.6	37.5

After filtering the 6K SNP dataset a total of 729 high quality markers were used for mapping, of which 720 markers were placed onto the 'CDC Dancer' x 'AC Morgan' map which included 31 linkage groups and a total map length of 1,042 cM (Table 22). The average density across the entire map was 0.69 markers per cM. The Mrg01 and Mrg08 consensus linkage groups, as defined by Chaffin et al. (2016), were each represented by three linkage groups, while Mrg06, Mrg12, Mrg18, Mrg19, Mrg23, and Mrg24 were each represent by two linkage groups (Table 22).

Table 22. Summary information for 'CDC Dancer' x 'AC Morgan' population genetic linkage map.

Linkage Group	Mapped Markers	Length (cM)	Marker Density (marker/cM)	Largest Gap (cM)
Mrg01.1	49	61.7	0.79	14.0
Mrg01.2	18	10.2	1.76	2.7
Mrg01.3	7	11.1	0.63	7.0
Mrg02	48	52.2	0.92	14.6
Mrg03	3	5.3	0.56	5.3
Mrg04	20	26.9	0.74	16.3
Mrg05	60	42.9	1.40	8.9
Mrg06.1	18	19.9	0.90	13.7
Mrg06.2	27	16.2	1.67	4.9
Mrg08.1	31	73.8	0.42	20.4
Mrg08.2	20	56.7	0.35	22.1
Mrg08.3	8	29.2	0.27	13.2
Mrg09	31	55.2	0.56	20.0
Mrg11	21	33.9	0.62	20.1
Mrg12.1	9	23.2	0.39	13.2
Mrg12.2	5	15.6	0.32	11.2
Mrg13	4	16.9	0.24	14.0
Mrg15	27	59.0	0.46	33.2
Mrg17	82	98.4	0.83	23.2
Mrg18.1	31	11.1	2.77	7.6
Mrg18.2	17	14.7	1.15	5.6
Mrg19.1	13	7.4	1.74	1.8
Mrg19.2	13	35.2	0.37	23.4
Mrg20	31	51.4	0.60	9.3
Mrg21	12	8.5	1.41	4.3
Mrg23.1	10	9.9	1.01	8.0
Mrg23.2	8	14.3	0.56	4.3
Mrg24.1	19	44.5	0.43	19.6
Mrg24.2	7	13.1	0.53	4.8
Mrg28	24	76.7	0.31	16.0
Mrg33	47	46.4	1.01	13.3
Total	720	1042.8	0.69	33.2

QTL mapping using data from individual site years identified loci significantly associated with APR in each site year except 2018 USask, 2018 AAFC-Morden and 2019 AAFC-Morden (Table 23). In addition, QTL were identified for average CI values at both USask and AAFC-Morden (Table 23). Of the 12 QTL associated with average CI at the USask and AAFC-Morden nurseries, eight were identified at both locations (Table 23). Of particular note, QTL identified on Mrg15 and Mrg33 from the average USask CI data were also identified in each of the individual site years at USask (Table 23). Similarly, the QTL on Mrg01.1, Mrg01.2, Mrg05 and Mrg06.1 from the average AAFC-Morden CI data were also identified in each of the individual site years (Table 23). This would suggest that these QTL are stable and thus good targets for selection. The effect of each QTL associated with average CI at both USask and AAFC-Morden showed that the 'CDC Dancer' allele consistently decreased the CI value and the variation explained ranged from 8.4-17.0% at USask and 13.0-18.2% at AAFC-Morden (Table 24). Total variation explained by all QTL identified at each nursery exceeded 100%. The location of the QTL were located within the OT3098 genome assembly to determine if some QTL represented common locations, but this was found not to be the case. Therefore, the QTL do not act in an additive fashion.

To account for any seedling resistance genes potentially carried by 'CDC Dancer', crown rust isolates with known virulence patterns against most known Pc genes were screened against 'CDC Dancer' to identify those which are unable to cause disease (i.e. 'CDC Dancer' displays resistance). This would allow us to understand which genes 'CDC Dancer' may carry. Three isolates, CR13, CR223 and CR258, were unable to cause disease on 'CDC Dancer' and based on their virulence spectrums against Pc genes, it was determined that 'CDC Dancer' could potentially carry *Pc35, Pc50, Pc51, Pc58, Pc62, Pc64, Pc91, Pc97* and *Pc98* (Supplemental Figure 1 in Appendix). However, *Pc91*, *Pc97* and *Pc98* can be ruled out since they were introduced to breeders following the release of 'CDC Dancer'. Among the remaining genes, only *Pc35* and *Pc50* have assigned map positions. *Pc50* was mapped to chromosome 6A which does not coincide with any of the QTL identified in this project, while *Pc35* maps to the same chromosome as QTLs as represented by Mrg02 (38.7 cM) and Mrg 33 (43.2 cM), but likely to a different location. While the remaining genes cannot be ruled out definitely, the use of a large number of races when inoculating the nursery would likely defeat the remaining older Pc genes. Thus, the QTL identified likely represent APR loci that differ from known seedling Pc resistance genes.

Table 23. Summary of QTL associated with APR in the University of Saskatchewan (USask) and AAFC-Morden crown rust field nurseries from 2018-22. 'x' indicates the identification of a QTL.

		USask			AAFC-Morden		
Linkage Group	**Map Position (cM)**	**Average**	**2020**	**2022**	**Average**	**2020**	**2022**
Mrg01.1	44.0	x			x	x	x
Mrg01.2	5.7	x			x	x	x
Mrg02	24.3				x		
Mrg02	38.7	x		x			
Mrg05	35.5	x		x	x	x	x
Mrg06.1	18.7	x			x	x	x
Mrg08.3	14.2	x			x	x	
Mrg09	1.2				x	x	
Mrg09	50.5	x					
Mrg15	1.3	x	x	x	x		
Mrg17	30.3	x		x	x	x	
Mrg33	43.2	x	x	x	x	x	

Table 24. Detail of the effects for each QTL associated with APR in the University of Saskatchewan (USask) and AAFC-Morden crown rust field nurseries averaged across 2018-22.

Linkage Group & Map Position (cM)	Nursery	Peak Marker	LOD[a]	Variation Explained (%)[b]	Additive[c]
Mrg01.1 (44.0)					
	USask	GMI_ES17_c793_567	3.4	8.4	-2.8
	AAFC-Morden	GMI_ES17_c793_567	7.6	18.2	-2.6
Mrg01.2 (5.7)					
	USask	GMI_GBS_89713	4.5	12.1	-3.4
	AAFC-Morden	GMI_GBS_89713	5.9	15.2	-2.4
Mrg02 (24.3)					
	AAFC-Morden	GMI_ES03_c6926_224	4.8	17.9	-2.6
Mrg02 (38.7)					
	USask	GMI_ES17_c3660_713	6.1	17.0	-4.1
Mrg05 (35.5)					
	USask	GMI_ES15_c9667_138	4.8	12.9	-3.7
	AAFC-Morden	GMI_ES15_c9667_138	5.4	13.0	-2.2
Mrg06.1 (18.7)					
	USask	GMI_ES17_c3397_167	3.6	9.5	-3.0
	AAFC-Morden	GMI_ES17_c3397_167	5.6	14.8	-2.4
Mrg08.3 (14.2)					
	USask	GMI_ES17_c24_198	3.7	10.4	-3.2
	AAFC-Morden	GMI_ES17_c24_198	6.5	16.8	-2.5
Mrg09 (1.2)					
	AAFC-Morden	GMI_ES01_c513_769	8.5	20.3	-2.7
Mrg09 (50.5)					
	USask	GMI_ES17_c2308_1026	3.6	10.8	-3.2
Mrg15 (1.3)					
	USask	GMI_GBS_73795	5.6	15.4	-3.9
Mrg17 (30.3)					
	USask	GMI_ES15_c6161_133	5.5	13.6	-3.7
	AAFC-Morden	GMI_ES15_c6161_133	6.9	14.5	-2.4
Mrg33 (43.2)					
	USask	GMI_ES03_c13331_202	6.2	16.4	-4.0
	AAFC-Morden	GMI_ES03_c13331_202	7.0	14.8	-2.4

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J. Chong, K.J. Leonard, J.J. Salmeron, 2000. A North American system of nomenclature for *Puccinia coronata* f. sp. *avenae*. Plant Dis., 84:580-585.

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Conclusions and Recommendations *(maximum 500 words)*

Highlight significant conclusions based on the findings of this project, with emphasis on the project objectives specified above. Provide recommendations for the application and adoption of the project findings.

A significant amount of new information pertaining to seven Pc seedling resistance genes and an APR derived from 'CDC Dancer was generated over the course of this project. The genomic location for six of the seven Pc seedling resistance genes (*Pc40*, *Pc46*, *Pc62*, *Pc63*, *Pc98*, *Pc101*) was definitively established, with only the location of the *Pc67* gene remaining in question. For four of these genes KASP assays have been developed which will permit efficient incorporation of these genes within breeding programs. By assessing these KASP assays on a diverse collection of oat lines (via the CORE panel) there is good evidence that the assays are quite specific to their related Pc genes which should, in combination with isolate testing, minimize the occurrence of false positives (i.e. thinking the gene is present when actuality it is not). KASP assays will also be available within the next year for *Pc63* and *Pc101*. It was also determined that each of these genes resides at different locations within the oat genome which would allow them to be pyramided together in any number of combinations, thus potentially providing more effective resistance. The *Pc67* gene was also determined to be recessively inherited, which is important information should a breeder decide to do early generation selection of segregating populations within disease nurseries. In addition, the gene appears to be temperature sensitive, specifically, it does not appear to function effectively at higher temperatures which again may impact the decision of a breeder to utilize this gene. The APR loci associated with 'CDC Dancer' resistance did not appear to be associated with seedling Pc genes and some of the APR QTL were detected across multiple site-years, indicating that they are stable and good targets for selection. Overall, the information derived from this project will enable oat breeders to more effectively incorporate crown rust resistance into future varieties resulting in value to the oat industry through maintenance of both yield and quality.

Follow-up Research

Please identify if there is a need to conduct further research. Detail any further research, development and/or communication needs arising from this project.

There are several areas of research that will continue following from the work done over the course of this project. These are as follows:

1) screen the susceptible genotypes within the CORE diversity panel to determine how accurately the KASP markers flanking the *Pc40, Pc63* and *Pc101* genes would select for the presence of these genes,

2) identify additional flanking markers using the OT3098 genome assembly, that will improve the efficiency and accuracy of incorporating *Pc46*, *Pc62* and *Pc63* into breeding lines,

3) identifying additional markers in the *Pc67* mapping population using genotyping platforms like DArTseq to help identify the major QTL associated with this resistance gene.

Patents/ IP generated/ Commercialized Products

List any products developed from this research.

None.

Sustainable Canadian Agricultural Partnership (Sustainable CAP) Performance Indicators

a) List of performance indicators for the entire lifespan of the project

Sustainable CAP Indicator	Total Number
Scientific publications from this project (List the publications under section b)	
• Published	1
• Accepted for publication	0
Highly Qualified Personnel (HQP) trained during this project	
• Master's students	0
• PhD students	0
• Post docs	1
Knowledge transfer products developed based on this project (presentations, brochures, factsheets, flyers, guides, extension articles, podcasts, videos)[1]	5 presentations

[1] Please only include the number of unique knowledge transfer products.

b) List of scientific journal articles published/accepted for publication from this project. Please ensure that each line includes the following: **Title, Author(s), Journal, Date Published or Accepted for Publication and Link to Article (if available).** *Add additional lines as needed*.

1. Chromosomal location of the crown rust resistance gene *Pc98* in cultivated oat (*Avena sativa* L.). J. Zhao, A.Z. Kebede1, J.G. Menzies, E. Paczos-Grzęda, J. Chong, J.W. Mitchell Fetch, A.D. Beattie, Y.-Y. Peng, C.A. McCartney. Theoretical and Applied Genetics, 133:1109-1122 (2020).

Technology Transfer Activities

List any technology transfer activities. Include presentations to conferences, producer groups or articles published in magazines except scientific journals.

<u>Conference Presentations</u> X.M. Zhang, M. Colin, E. Paczos-Grzeda, J.G. Menzies, A.D. Beattie, 2024. Genetic mapping and validation of *Avena sterilis*-derived crown rust resistance genes *Pc40* and *Pc46* in cultivated oat. American Oat Workers Conference, Saskatoon, SK, July 21-24, 2024.

Field Tours

MGM Seeds Oat Field Tour (Saskatoon, SK, July 12, 2023).
Grain Millers Oat Field Tour (Saskatoon, SK, July 19, 2023).
FP Genetics Oat Field Tour (Saskatoon, SK, July 19, 2023).
CDC Oat Industry Field Day (Saskatoon, SK, July 19, 2022).

Media

Oat Scoop, Pam Yule, November 2022, pp. 4 “On-going Crown Rust Resistance Project”
Oat Scoop, Pam Yule, Fall 2021, “Ousting Crown Rust from the Throne”

Producer/Industry Presentations

A.D. Beattie, 2022. Update on CDC Oat Breeding and Research Activities, Prairie Oat Growers Association AGM, Saskatoon, SK, November 29, 2022.

A.D. Beattie, 2022. CDC Annual Forage Varieties: What’s New, in the Pipeline and Good for Producers. 11th Annual Dairy Info Day, Warman, SK, February 24, 2022

A.D. Beattie, 2021. Canadian Oat Quality. Prairie Oat Growers Association Presentation to Japanese Oat Industry, Webinar, February 24, 2021.

A.D. Beattie, 2020. CDC Forage Barley and Oat Breeding: How it all Works. Saskatchewan Ministry of Agriculture Regional Services Branch, Saskatoon, SK, December 11, 2020.

Contributions and Support

List any industry contributions or support received.

Co-funding of this project was received from the Western Grains Research Foundation and the Prairie Oat Growers Association.

Acknowledgements

Include actions taken to acknowledge support by the Ministry of Agriculture, the Canadian Agriculture Partnership (for projects approved between 2017 and 2023) and the Sustainable Canadian Agriculture Partnership (for projects approved between 2023 and 2028).

Acknowledgements were made to the funders of this project during the technology transfer activities listed in that section.

Appendices

Identify any changes expected to industry contributions, in-kind support, collaborations or other resources.

Supplemental Table 1. Disease reaction data for the 114 CORE genotypes evaluated with four crown rust isolates.

	Pca Isolate & Screening Temperature					
Genotype	**CR223 21/17°C**	**CR249H 21/17°C**	**CR249L 18/15°C**	**CR254 21/17°C**	**CR257 21/17°C**	**Average**
Sun II-1	4.0	4.0	3.3	4.0	4.0	3.9
Hurdal	4.0	3.0	2.3	3.3	3.4	3.2
Morgan_AC	2.5	3.7	3.7	4.0	4.0	3.6
Marion (Canada)	1.0	2.4	0.0	0.6	0.0	0.8
Goslin	1.4	3.5	2.8	1.6	1.8	2.2
Asencao	2.5	1.5	2.3	2.4	4.0	2.5
Ajay	1.0	3.3	3.2	3.0	3.0	2.7
Ogle	1.0	1.5	3.3	2.8	2.8	2.3
Rigodon_AC	1.0	1.3	2.8	3.0	2.7	2.1
Gem	0.3	0.3	0.3	0.3	0.3	0.3
Morton	1.0	0.4	0.3	0.3	0.0	0.4
Marie_AC	2.8	2.0	3.0	2.2	2.5	2.5
Dancer_CDC	1.0	2.4	3.0	0.8	2.3	1.9
Assiniboia/S42	1.0	2.3	3.5	0.3	2.3	1.9
Buckskin	0.3	0.6	1.0	3.6	0.0	1.1
HiFi	0.3	0.2	0.2	0.3	0.1	0.2
TAM O-301	4.0	1.0	2.6	0.6	2.2	2.1
Coker 227	1.2	1.0	2.3	2.0	1.3	1.6
Kanota	2.2	1.7	4.0	3.0	2.5	2.7
Kangaroo	3.0	0.5	1.3	0.9	0.4	1.2
CI 4706-2	3.0	2.0	3.7	2.0	2.0	2.5
Tardis	1.0	1.3	2.3	1.3	1.8	1.6
Buffalo	2.0	1.3	3.4	3.0	2.5	2.4
Maverick	1.2	0.5	2.2	1.6	1.0	1.3
Calibre	2.4	1.1	4.0	1.0	1.6	2.0
Dane	0.3	0.6	3.7	2.8	1.6	1.8
Florida 501	2.0	0.9	0.7	2.0	0.8	1.3
Jay	1.0	0.3	0.3	0.3	0.3	0.4
Jerry	1.0	0.7	4.0	3.5	2.0	2.2
Ronald_AC	0.2	1.0	4.0	2.8	2.0	2.0
Sesqui	0.1	1.0	0.3	0.3	0.0	0.3
Sol-Fi_CDC	2.0	0.5	4.0	4.0	2.8	2.7
TAM O-397	0.1	0.1	0.0	0.3	0.0	0.1
Triple Crown	0.6	0.2	0.0	1.0	0.3	0.4
Troy	1.0	0.3	2.3	2.0	1.0	1.3
Aarre	2.0	1.0	3.0	4.0	0.8	2.2

Baler_CDC	2.3	1.0	2.0	1.0	1.4	1.5
Blaze	0.6	0.3	0.6	0.6	1.0	0.6
Boyer_CDC	0.8	1.3	4.0	0.3	2.3	1.7
Drummond	1.0	1.0	4.0	2.8	3.0	2.4
Furlong	0.3	1.3	4.0	4.0	4.0	2.7
IL86-5698-3	0.3	0.3	2.3	3.5	0.7	1.4
Kaufman	0.2	1.2	4.0	3.6	3.3	2.5
MAM 17-5	0.9	1.0	3.0	2.7	2.0	1.9
OT380	0.3	1.1	4.0	3.5	2.3	2.2
ProFi_CDC	1.0	0.6	1.0	2.6	1.0	1.2
SO-1	0.8	1.0	2.2	2.3	2.0	1.7
Vista	0.4	0.3	0.0	0.3	0.1	0.2
MN841801-1	2.0	0.6	0.0	2.0	0.1	0.9
Mortlock	2.6	1.5	0.2	4.0	0.4	1.7
Noble-2	2.8	2.3	2.8	3.0	NA	2.7
Maldwyn	4.0	1.3	2.2	3.5	1.6	2.5
Gehl	1.1	3.3	3.5	4.0	3.3	3.0
Coker 234	0.6	1.6	2.5	3.4	3.2	2.3
WAOAT2132	2.8	0.5	0.6	2.0	1.4	1.4
OA1063-8	0.1	1.5	3.5	4.0	2.2	2.3
Prescott	0.4	0.3	0.0	0.4	0.0	0.2
Robust	0.6	0.4	0.1	0.2	0.0	0.3
Shadow	1.6	2.6	3.8	2.8	1.0	2.3
Belinda	2.6	0.7	3.7	3.8	1.2	2.4
Dominik (Bauer)	2.6	1.1	4.0	3.3	1.0	2.4
Matilda	2.5	0.5	1.3	2.6	1.0	1.6
Sang	2.7	3.6	3.0	3.7	1.3	2.8
SW Betania	0.8	3.6	4.0	4.0	1.5	2.8
Pg11	0.3	3.5	3.4	1.6	3.0	2.4
Pg16	3.3	3.2	3.3	3.0	3.1	3.2
Ajax	3.4	3.2	3.3	3.0	4.0	3.4
Cherokee	3.6	3.8	4.0	3.0	2.8	3.4
Z615-4	1.0	3.2	2.8	2.8	2.6	2.5
Boudrias	0.3	3.8	3.0	2.7	2.8	2.5
UFRGS 8	2.3	3.5	3.8	0.8	2.4	2.6
UFRGS 881971	0.3	0.1	0.1	0.1	0.0	0.1
UFRGS 930605	0.0	0.2	0.1	0.6	0.0	0.2
Centennial	0.3	0.2	0.3	4.0	0.0	0.9
Dal	2.3	3.2	4.0	3.6	3.2	3.3
Exeter	1.6	2.7	4.0	3.8	2.8	3.0
OT586	0.0	0.2	0.1	0.0	0.0	0.1
Pinnacle_AC	0.0	2.5	3.8	3.8	2.6	2.5
MF9522-523	0.1	0.3	1.0	1.0	0.3	0.5
Freddy	4.0	1.8	3.0	4.0	2.0	3.0

Melys	4.0	2.2	3.0	3.5	3.0	3.1
Ranch	3.5	1.6	3.7	3.6	3.7	3.2
Bountiful	3.0	2.5	4.0	4.0	2.7	3.2
Clav 6209	4.0	2.4	3.0	2.8	2.7	3.0
Ford Early Giant	3.3	2.4	3.8	2.8	2.8	3.0
Novojatkovo	3.0	1.5	3.0	3.0	1.3	2.4
Pusa Hybrid G	3.5	2.0	3.4	2.5	2.0	2.7
Akiyutaka	3.8	1.9	4.0	3.0	1.8	2.9
Provena	3.5	2.6	4.0	3.0	1.1	2.8
Chaps	0.1	0.8	1.4	3.0	0.3	1.1
Flaemingsnova	4.0	2.0	4.0	3.4	1.1	2.9
Fulghum	4.0	2.5	4.0	3.8	4.0	3.7
Lang	4.0	2.2	3.6	3.6	2.8	3.2
Lutz	4.0	2.0	4.0	4.0	3.8	3.6
Otana	3.8	2.4	4.0	3.6	1.8	3.1
Pacer_CDC	4.0	0.9	4.0	3.3	2.2	2.9
Salomon	4.0	2.4	4.0	3.8	3.5	3.5
Urano	0.1	4.0	4.0	3.8	3.3	3.0
Chernigovskij 27B	2.4	1.3	3.5	4.0	2.8	2.8
Clinton	2.5	4.0	4.0	4.0	3.4	3.6
H927-1-6-1-x-x-24	1.0	2.5	3.2	2.8	4.0	2.7
Hazel	0.3	0.3	0.3	2.3	0.4	0.7
MN 811045	4.0	0.8	1.1	2.4	0.3	1.7
Red Rustproof	3.6	3.5	4.0	2.3	3.8	3.4
Russell	3.7	3.3	4.0	3.8	3.8	3.7
Stout	2.5	2.0	3.3	2.8	3.3	2.8
Ukraine reselection	1.0	2.0	4.0	2.3	1.3	2.1
Victoria	0.6	2.4	3.0	2.5	2.0	2.1
Bia	0.1	1.1	3.7	2.7	2.0	1.9
Maverick	1.8	1.8	2.6	3.0	2.0	2.2
TAMO-406	0.0	0.1	0.1	0.3	0.0	0.1
Leggett	0.1	2.3	3.0	0.2	3.2	1.7
Rodgers	0.1	0.3	0.3	3.0	0.3	0.8
Horizon270	0.1	0.1	0.1	0.0	0.0	0.1

Supplemental Figure 1. Reaction of three crown rust isolates against a panel of seedling Pc genes. 'S' indicates the ability of that isolate to cause disease on plants harboring a specific Pc gene. Green highlighting indicates possible Pc genes carried by 'CDC Dancer'.

	Pc gene																																					
CR Isolate	14	35	38	39	40	45	46	47	48	50	51	52	54	55	56	58	59	60	61	62	63	64	67	68	70	71	91	94	96	97	98	99-1	99-2	101	102	103	104
13	S			S	S	S	S	S	S			S	S	S				S								S			S			S	S	S	S	S	S
223				S	S		S							S			S											S										
254			S	S										S	S			S	S		S		S	S	S	S												S