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Characterization and utilization of newly identified resistance sources for sustainable clubroot control on canola
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Executive summary

Clubroot resistance (CR) has been reported previously in B. *rapa* (A genome), *B. oleracea* (C genome) and *B. napus* AC genome). This project built our earlier work that identified a number of resistant *Brassica rapa, B. nigra* (B genome), *B. napus* and *B. oleracea* cultivars/lines against clubroot. Most CR genes have been from European turnips *(B. rapa),* but the sources for the CR genes in Canadian canola cultivars have not been revealed. It appeared that most of the cultivars carry the CR gene used in the European winter *B. napus* rapeseed cv. Mendel which has broken down in several EU regions. It is expected that resistant cultivars in Canada will eventually be eroded with a shift in the pathogen population. Recent reports on the pathotype *5X* in Alberta showed that this race is virulent on all resistant commercial cultivars in the marketplace; clearly this is a serious concern. The aim of the project was to identify CR genes and develop resistant canola germplasms to support a long-term host resistance strategy by diversifying CR genetic background. This work includes the characterization of CR genes, development of molecular markers linked to the CR genes for marker-assisted selection (MAS), and efficient introgression of selected CR genes into canola breeding lines using MAS for technology transfer.

Initially, resistant candidates were tested against pathotypes 2, 3, *5,* 6 and 8 of *P brassica* (races found in Canada prior to 2013) to assess the range of effectiveness, and several candidates showed strong resistance to all the pathotypes. To establish resistant populations, three CR *B. rapa* lines were hybridized with clubroot susceptible (CS) double-haploid (DH) canola lines DH *B. rapa* line ACDC-10 (ACDC), DH *B. napus* line Topaz16516 (DHT) and DH *B.juncea* line J072-0l 904 (DHJ). One CR *B. nigra* line was crossed with the DH *B. carinata* line 080798EM-086 (DHC) and DHJ, respectively. When clubroot resistance segregated (IR:1S) in the F_1, the population would be used for genetic mapping immediately. When non-segregating (all R or 1:0), the F_1 would be back crossed with one of the susceptible DH lines to obtain segregating $BC_,$, BC_1F_1 or BC_2F_1 populations prior to mapping. AnAAFC microsatellite marker system was used initially to screen all populations on a Megabace sequencer and linkage analysis was performed using the software MAPMAKER® 3.0 to map the CR genes against phenotyping data.

The resistant oriental vegetable cv. Flower Nabana (FN, *B. rapa)* was the first studied. It was found to carry a single dominant CR gene. Using a small F_1 population and 318 microsatellite

markers distributed Qn l0_B. *raP_a* chromosomes (A genome). A total of 104 markers were found to be polymorphic·and were.then used to screen a larger population of 1,587 plants. The markers sN8591, sR12302I and sS2093 were linked to the CR gene in FN on one side of the linkage group A03, with the genetic distance at 0.54, 0.77 and 3.07cM, respectively. The markers sR6340I, sJ5076F and sB4889B were on the other side of the CR gene at 0.72, 4.41 and 5.62 cM. A linkage map including the CR gene ***Rcrl*** (previously ***Rpblor CROJ)*** was constructed with the CR gene located within a range of 1.31 cM flanked by the markers sN8591 and sR6340I. This region was between 23.43 Mb and 24.50 Mb with 158 genes annotated and five of them encoding TIR- NBS-LRR proteins (host defence related). The CAPS markers A3-020 and MS7-9 were developed later, and were closer to ***Rcrl*** (0.19 and 0.06 cM, respectively) than sR6340I. The interval flanked by sN8591 and A3-020 was about 0.76 cM, consisting of about 67 annotated genes. The fine mapping of ***Rcrl*** precisely located the CR gene on A03 and identified markers closely linked to the CR gene.

A similar approach was taken in developing other resistant populations and mapping of CR genes. A segregating BC_1 population with the CR gene from the Chinese cabbage cv. Jazz napa cabbage (JNC, *B. rapa)* consisted of 926 plants and screened with 69 markers on the A03 chromosome. The CR gene ***(Rcr2)*** was flanked by the markers sR9448 (23.43 Mb) and sJ1807 (23.52 Mb). Two populations (BC_1 and F_2) were established for the winter rape breeding line 96-6992 *(B. rapa*), consisting of 175 and 147 plants, respectively. A total of 190 markers distributed on 10 chromosomes of the A genome were used to screen the populations, and the CR gene ***(Rcr3)*** was located on A08 flanked by the markers sR6489R and sN13095/sN4145. This CR gene co-segregated with the marker sR02133. The F1 population of *B. carinata* (DHC) x *B. nigra* BRA also showed a resistance segregation ratio of 1:1. Fine mapping of the CR gene ***(Rcr4)*** is on going. Markers that flank ***Rcrl, Rcr2*** and *Rcr3* have been validated..

Since most of CR genes were identified from *B. rapa* sources and >90% of canola cultivars in western Canada are *B. napus,* interspecific hybridization was used to introgress CR genes from diploid *B. rapa* (AA) into amphidiploid *B. napus* (AACC). Early backcross generations typically consisted of allotetraploids carrying a varying number of univalent C chromosomes from *B. napus.* These individuals are not always distinguishable phenotypically and require a lengthy process of backcrossing before becoming stable. A genome-wide marker-assisted selection strategy was investigated a 6K SNP array technology to identify most suitable candidates for backcrossing. A total of 5,506 SNP markers were used to screen 113 BC1F1 [DHT x FN (F1), then *B. napus* SVl 1-17667 x $F_{1)}$ plants for genetic similarity to the *B. napus* and *B. rapa* parents. The plant (line) 66 was found to carry the CR gene ***Rcr1*** as well as a full complement of C chromosomes, and show the phenotypic characteristics and fatty-acid profile similar to those of the *B. napus* SVl 1-17667 line. This genome-wide marker-assisted selection speeded up the germplasm development during introgression of ***Rcrl*** from the vegetable *B. rapa* FN into *B.*

napus canola lines. This approach may be used for the introgression of other resistance genes via the interspecific hybridization.

Mapping of ***Rcr1, Rcr2*** and ***Rcr3,*** development of molecular markers linked to these CR genes, and use of the genome-wide SNP markers greatly facilitated the introgression of clubroot resistance into canola breeding lines. These CR genes have now been incorporated into elite AAFC *B. napus* and *B. rapa* canola lines and stable populations have been obtained for tech transfer. Preliminary testing also showed that lines carrying ***Rcrl*** might resist the newly-found pathotype 5X. This is very encouraging. Introgressing ***Rcr4*** into *B. carinata* canola has also been carried out, with a stable CR population developed.

Deliverables/achievements:

- The clubroot resistance genes ***Rcrl, Rcr2,*** and *Rcr3* have been mapped, with molecular markers developed and used for MAS.
- A genome-wide selection tool (6K SNP array) was used successfully in identifying the most suitable candidate carrying CR gene in early stages of backcrossing. - be a useful new tool.
- Introgression of ***Rcrl, Rcr2*** and *Rcr3* into *B. napus* or *B. rapa,* and ***Rcr4*** into *B. carinata* canola germsplasm has been completed with stable populations produced for tech transfer.
- Preliminary testing indicates that lines carrying ***Rcrlare*** also resistant to the newly-identified pathotype 5X.
- A total of 9 refereed papers, 18 conference proceeding papers/book chapters, 7 scientific abstracts, 1 Invention Disclosure, 45 other extension publications/presentations relating to this project have been generated.

Background

Clubroot, caused by the plasmodiophorid pathogen *Plasmodiophora brassicae* (Pb) can be a devastating disease to crops in Brassicaceae. In western Canada, the disease has been spreading on canola in Alberta, with an increased range of infestation reported yearly for past several years. Pb or clubroot disease has been found in isolated fields in Saskatchewan at low levels. Since 2009, resistant canola cultivars have been available in Canada but all of them are based on a single dominant *R* gene. There is a lack of genetic characterization of *R* genes in these canola cultivars, and it appears that different cultivars may share similar resistance genes. Clubroot resistance **(CR)** genes are generally race specific and the resistance can be eroded when the pathogen race structure shifts. Therefore, a single-gene based resistant cultivar would not be expected to be long lasting.

So far, there are only limited numbers of CR genes identified and most of them are from *Brassica rapa* (A genome), with the loci *Crrl, Crr2, Crr3, Crr4, CRa, CRb, CRc* and *CRk* rerported. *CRa* and *Crr1* have also been isolated recently. Another CR gene *(RPBJ)* was reported

from *Arabidopsis thaliana* ecotype Tsu-0, but there has been no further report on its orthlogs in other *Arabidopsis* ecotypes. There is no single CR gene that is effective against all Pb races. For effective long-term management of clubroot in western Canada, there is a need to broaden CR genetic arsenal. Since 2009, over 1,000 Brassica lines, mostly *B. rapa,* have been screened at AAFC Saskatoon, with 21 candidates from *B. rapa, B. nigra, B. napus* and *B. oleracea* found to be highly resistant. It is interesting to note that among these 21 lines, ten *B. rapa* and one *B. nigra* lines showed complete resistance against the predominant race of Pb (pathotype 3) found in Alberta. The current project aimed to build on these newly identified CR sources, characterize some of CR genes in these materials, and introgress useful CR genes into canola germplasms or breeding lines to contribute to the long-term clubroot management strategy. Properly tagged CR genes can be efficiently introgressed or stacked during canola breeding.

Brief literature review

Useful clubroot resistance has been reported in *Brassica rapa* (A genome) and *B. oleracea* (C genome), although more frequently from *B. rapa* (Voorrips et al.1997; Hirai 2006; Werner et al. 2008). Some of the most resistant sources have been found in European fodder turnips *(B. rapa* ssp. *rapifera)* with three independent dominant genes identified, each conferring resistance to different Pb races. This resistance was first incorporated into *B. napus* oilseed rape in Europe, with two cultivars (Mendel, Tosca) released in 2000 (Diederichsen et al. 2006, 2009). Each of the cultivars received only a single race-specific CR gene, while another *R-gene* was lost during backcrossing. Similar CR genes have also been introgressed into rutabaga *(B. napus,* ssp. *napobrassica)* in UK (Bredshaw et al. 1997), and likely into Chinese cabbage in Japan (Hirai 2006). Breakdown of CR has been reported with Mendel (Oxley 2007) and in our initial trials Tosca was not sufficiently resistant to the pathotype 3, the predominant Pb race on the prairies. A fair amount of research has been directed to mapping and identification of CR genes, especially in Chinese cabbage (Suwabe et al. 2006; Saito et al. 2006; Hayashida et al. 2008) and Arabidopsis (Suwabe et al. 2006; Jubault et al. 2008). Some useful markers have been developed for major R-genes (Voorrips et al.1997; Hirai et al. 2004; Matsumoto et al. 2005) and QTLs (Nomura et al. 2005; Sakamoto et al. 2008; Kamei et al. 2010; Nagaoka et al. 2010). However, CR sources are very limited (Hirai 2006) and most of the commercially available CR genes seem to derive from original fodder turnips.

Objectives

The goal of the project was to capitalize on a broad range of clubroot resistant materials obtained from an earlier study and identify useful CR genes and markers for the development of resistant canola germplasms.

- Analysis of newly identified CR materials through genetic mapping
- Characterize new *R* genes in comparison with the CR genes in existing cultivars
- Develop useful markers closely linked to new *R* genes for marker-assisted selection

- Evaluate the robustness of newly identified CR genes against different Pb pathotypes
- Develop resistant canola germplasms/lines via introgression of selected *R* genes

Research design and methodology

Development of clubroot-resistance segregating populations: Segregating populations were developed for three *B. rapa* lines, including pak choy, Chinese cabbage and turnip, and one *B. nigra* lines, by crosses between the susceptible DH lineACDC-10 *(B. rapa)* and the resistant *B. rapa* candidates, or a susceptible *B. carinata* DH line 080798EM-086 with the resistant *B. nigra* line. The DH *B. rapa* line (ACDC) was developed using microspore culture at AAFC Saskatoon; it is self-compatible and susceptible to pathotype 2 and 3 of *P. brassicae.* F_I plants were selfed to obtain F2 populations, or crossed withACDC or DH *B. carinata* to produce BC_1 population for mapping. Reciprocal crosses were made between the parents mentioned above. Five well developed buds per female plant were kept for crossing, and the other flowers and small buds were removed. Each bud that remained was opened and the anthers removed carefully with a pair of forceps. Anthers were collected from newly opened flowers of donor plants, and pollen grains were dusted to pistils of the female plants with a small paintbrush. Each pollenated plant was covered with a plastic crossing bag for 5 days.

Bioassa for clubroot test: A field population of *P. brassicae* (Leduc-AB-2010), consisting primarily of pathotype 3 of *P. brassicae,* was used for inoculation throughout the study. Mature clubroot galls filled with pathogen resting spores were dried at room temperature for 2 weeks and stored at -20°C until use. The inoculum was prepared as a resting-spore suspension using the method described by, with the concentration adjusted to I x10 ^{7}spores/mL. For inoculation, 5 mL of a resting-spore suspension were pipetted around the seed in each conetainer immediately after sowing to result in an inoculum dose of about 1x $I0^6$ spores/g growth medium. fuoculated conetainers were kept in the growth room and watered daily for 2 weeks to maintain a high level of soil moisture to facilitate infection. ACDC was used as a susceptible control in all inoculated trials. Non-inoculated plants would not develop any visible clubroot symptoms.

Seeds were sown in Sunshine #3 soil-less planting mix in tall plastic "conetainers" (5-cm diam, 20-cm tall), and large plants were transplanted later into the same growth medium in 15-cm-diam. pots (1 plant/pot) at 5 weeks after seeding. The parents and their progenies were inoculated as described above, and plants were assessed at 5 weeks after seeding for clubroot severity using a 0--3 scale. A rating of 0 was considered resistant (R) and 1-3 susceptible (S). Due to heterozygosity of CR donor, F_1 populations resulting from the reciprocal crosses between segregated for resistance and susceptibility. The goodness of fit for the segregation was analyzed using the Chi-square (X^2) Test. For fme mapping, only the F_1 populations of susceptible (female) x CR donor (male) crosses were used.

Resistance of selected CR candidates against 5 pathotypes of *P. brassicae* found in Canada

Ten CR lines with high resistance against pathotype 3 of Pb under high disease pressure were selected for this test to assess the effective against different pathotypes. Seeds were pre-germinated on moistened filter paper in Petri dishes, and I-wk-old seedlings were inoculated with Pb by dipping the entire root system in a resting spore suspension for 10 s prior to being transplanted individually to a Sunshine potting mix in 6-cm-diam plastic pots. Resting spores were extracted from frozen galls of each of the five single-spore Pb isolates (pathotypes 2, 3, 5, 6 and 8) using the protocol described earlier. Resting spore suspensions were adjusted to 1 x 10^7 spores mL^{-1} using a haemocytometer. The inoculated seedlings were placed in a greenhouse (20 ± 2 °C) for 6 weeks to allow disease symptoms to develop. The growth medium was saturated with tap water during the first week after inoculation and kept moist by regular watering. 'Granaat' was used as the CS control in these trials.

Resistance/susceptibility to each pathotype was determined individually for each accession. Two repetitions of the experiment were conducted, one at the University of Alberta, Edmonton and the other at the Crop Diversification Centre North, Alberta Agriculture and Rural Revitalization, Edmonton. The experiment was a randomized complete block design (RCBD) with three blocks in each repetition. Within a block, 12 plants of each accession and the CS control were inoculated.

Molecular mapping: Simple sequence repeat (SSR) markers were used for rough/fine mapping. Over 2,000 SSR markers, distributing on 19 linkage groups of *B. napus,* had been developed at AAFC Saskatoon Research Centre. Polymorphic SSR markers were identified and used initially for rough mapping of CR genes to specific chromosomes. Markers flanking CR gene were further used to screen segregating F_1 (testcross) populations of hundreds to up to 1,587 plants, each of which was also tested for clubroot reaction.

The MegaBACE 1000 DNA Analyser (GE Healthcare, Mississauga, CA), a capillary-array electrophoresis system with automated gel matrix replacement, sample injection, DNA separation and base calling, was used for SSR marker analysis. PCR products were amplified with selected polymorphic markers, and forward primers labeled by adding fluorescent phosphoramidite as HEX (yellow), TET (green) or 6-FAM (blue), and segregated on the MegaBACE. Laser excitation and confocal laser scanning are used to excite and detect fluorescent dye-labelled DNA fragments, respectively, as they migrate past a detection window. The fragment analysis was carried out using the Genetic Fragment Profiler Software Suit VI .2. DNA fragments from the F_1 population could be separated into three bands (1, 2 and 3) by some markers; the band 1 and 2 were from the heterozygous CR donors, and band 3 from homozygous DH susceptible line. Since we were more interested in the R allele (band 1), only two genotypes were grouped based on marker analysis; genotypes with the band 1 and 3 were scored as "h" and those with band 3 and 2 as ' a". The linkage analysis was performed using JoinMap 4.1. DNA

sequences identified within the region of the CR gene flanked by SSR markers were used to search for similar *B. rapa* genomic DNA sequences at http://brassicadb.org/brad/, and the information used to develop CAPS markers. PCR primers were designed using Primer3 (http://frodo.wi.mit.edu/).

DNA sample preparation and PCR conditions: DNA was extracted following the method described previously [43] with these slight modifications: Freeze-dried leaf samples were incubated with extraction buffer (2% CTAB; pH 8.0) at 65°C, followed by chloroform-isoamylalcohol (24:1, v/v) extraction and alcohol precipitation. RNA was eliminated by adding 1/10 volume of 10 mg/mLRNaseA. The DNA concentration was estimated using the NanoDrop ND-2000c (Thermo Scientific) and adjusted tol0 ng/μL with sterile Milli-Q water. APCR mixture containing 0.5μL each of forward and reverse primers (5μmoVL), 4 μL 10 ng/μL genomic DNA, 5 μL AmpliTaq Master Mix (Life technologies, Burlington, CA) was pipetted to a 384-well PCR plate. The reaction conditions were as follows: denaturation at 95°C for 10 min, followed by 8 cycles of 94°C for 15 s, 50°C for 15s, and 72°C for 30 s; then 27 cycles of 89°C for 15 s, 50°C for 15 s, 72°C for 30 s, and a final extension at 72°C for 10 min.

The resistant oriental vegetable cultivar Flower Nabana (FN, *B. rapa* ssp. *chinensis)* was the first CR candidate studied, due to its almost immunity to pathotype-3 of *P. brassicae.* The Chinese cabbage cv. Jazz Napa Cabbage (JNC, *B. rapa* ssp. *pekinesis),* the turnip line 96-6992 (6992, *B. rapa* ssp. *rapa)* and a black mustard line BRA.

Validation of selected markers for detection of *Rcrl:* Four markers, i.e., sN8591, sR6340I, sB4889B and sS2093, were examined to confirm the presence of *Rcrl* in resistance BC1 (BC1F1) progeny derived from backcrossing the canola DH lines BHl 1-17938 *(B. rapa)* and SVll-17667 *(B. napus*), respectively, with cv. FN. These two populations were different from that used for mapping and RNA-seq (ACDC x cv. FN); they were produced during introgression of *Rcr1* into AAFC canola breeding lines. The purpose of this experiment was to assess the selected markers for the presence of *Rcr1* during resistance introgression. To produce BC_1 progenies, about 20 plants were produced for each donor F_1 population *(B. rapa, B. napus),* with five resistant plants selected based on their clubroot reaction, assessed as described previously. The crosses of recurrent breeding lines (female) x resistant F1 (male) lines were made and BC_1 seeds were bulked in case some of the "resistance plants" were misidentified due to escape. A population of 176 plants were tested from each of the *B. rapa* and *B. napus* BC_1 populations. The clubroot reaction of each plant was assessed as described previously. Marker detection for ***Rcrl*** was performed on the MegaBACE and compared with phenotype data for each plant. Markers for other CR genes were validated similarly against phenotyping data.

Whole-genome-based selection using SNP array: Most CR genes were found from *B. rapa* (2n=20, AA) during the and most canola cultivars are *B. napus* (2n=38, AACC), an inter-specific

hybridization had to be used to transfer CR genes from the diploid into the tetraploid. As a result, most progenies in early backcross generations would be allotriploids (2n=29, AAC), plants with univalent C chromosomes randomly distributed, resulting in varying number of C chromosomes. This genome-based selection strategy aimed to identify plants with the least genetic background dragged from the CR donor (FN) while having a complement of all C chromosomes among BC1 plants carrying ***Rcrl.*** The technology used is a 6K Illumina Infinium SNP array, which may be used to estimate the similarity of the A and C genomes between the parents and hybridization progenies. This approach may speed up the introgression and germplasm development.

DNA was extracted from freeze-dried leaf samples (described above) incubated with the extraction buffer (2% CTAB; pH 8.0) at 65°C, followed by chloroform-isoamylalcohol (24:1, v/v) extraction and alcohol precipitation. RNA was eliminated by adding 1/10 volume of 10 mg/mL RNase A. The DNA concentration was estimated using the NanoDrop ND-2000c and adjusted tolO ng/μL with sterile Milli-Q water for the SSRs screening. DNA for the SNPs was quantified using the Appliskan multiplate reader with Quant-itTM PicoGreen® Assay, with the concentration adjusted to a minimum of 50 ng/μL.

The Infinium assay was performed following the manufacture's protocol (Illumina Inc.): SNP chips covering 6K SNP loci based on sequencing fourteen *B. napus,* three *B. rapa* and three *B. oleracea* accessions. The markers consisted of 2,876 SNPs in the A genome and 3,256 SNPs in C genome, with 275 markers from both A and C genomes. Among these markers, a total of 5,506 SNPs were developed at AAFC Saskatoon, and were included in four *B. napus* SNP chips. The allele calling for each locus was employed by the Genome Studio software (Illumina Inc.). Most of the genotype calls in a two-dimensional analysis produced only one cluster type, and clearly defined clusters would represent three possible genotypes (AA, AB and BB). The highlighted clusters displayed three genotypes with homozygous alleles AA (red) and BB (blue), heterozygous allele AB (purple). Allele calls that fall in lightly colored areas would be set to the "failed" category. The X axis on an SNP graph was normalized to theta values (Norm Theta), which would range from 0 to 1. The Y axis was also normalized call signal (Norm R).

Bulked segregant analysis was used to identify the chromosome location of the CR gene in a $BC1F_2$ population (113 plants). Genomic DNA of eight resistant and eight susceptible plants were pooled to generate resistant and susceptible bulks, respectively. The SSR markers sR6340I, sN8591 and sS2093 were evaluated in the mapping population using the method described by Chu et al. (2014). Briefly, 10 μL PCR mixture containing 0.5μL each of forward and reverse primers (5μmol/L), 4 μL 10 ng/μL genomic DNA, 5 μLAmpliTaq Master Mix was pipetted to a 384-well PCR plate. The reaction conditions were as follows: denaturation at 95°C for 10 min, followed by 8 cycles of 94°C for 15 s, 50°C for 15s, and 72°C for 30 s; then 27 cycles of 89°C for 15 s, 50°C for 15 s, 72°C for 30 s, and a final extension at 72°C for 10 min. Amplified products were pooled and diluted by 10 times with a 0.1% Tween® 20 solution. Genotyping was

performed on a MegaBACE 1000 DNA Analysis System with an Applied Biosystems ET ROX 550 size standard. The data were analyzed using the MegaBACE Genetic Fragment Profiler Software Suit V1.2 and the genetic distance determined with JoinMap 4.1.

Traits related to plant growth and morphological characteristics, including days to flowering, plant height, 1000-grain weight, the average final siliques per plant and the number of seeds per silique were assessed on a BC_1F_1 population (226 plants) under greenhouse conditions. At maturity, four parental and BC_1F_1 plants of each line were randomly selected and evaluated. The trait assessment was based on phenotypic descriptors of *B. brassica.*

Seed fatty acid: Samples were collected from individual lines with three biological replicates. Seed fatty acid profile was analyzed using gas chromatography following preparation of fatty acid methyl esters by base-catalyzed methanolysis. Ten seeds of each sample were placed in a 6.5 mL scintillation vial containing a steel rod. Then 0.25 mL of hexane was added to each vial before capped vials were put on an Eberbach reciprocating shaker for a 30 min (280 rpm, 37 mm stroke). After centrifugation at 2300 rcf for 15 min, 0.04 mL of supematants were transferred to 2 ml autosampler vials. Hexane (0.05 ml) and 2% sodium methoxide (0.1 ml) were added to each vial. After contents of vials were mixed and a 15-min stay at 20°C, 0.05 ml 0.2M phosphate buffer (27.3996 g $NaH_2PO_4 \cdot H_2O$ and 28.3920 g Na_2HPO_4 were dissolved with water to 250 ml) was added to each vial. Methanol was evaporated under a stream of air for less than 2 min. Heptane (0.5 ml) was added before 1 µl of samples was loaded to GC.

GC-FID analyses were performed on an Agilent 6890N gas chromatograph equipped with a flame ionization detector (FID), an Agilent INNOWAX fused silica capillary column (7.5 m x 250 µm diameter x 0.5 µm film thickness) and split injection. The injector/detector temperatures were set at 250°C and 300°C, respectively, with the initial oven temperature set at 190°C. After sample injection, the oven temperature was increased to 255°C at a rate of 40°C min^{-1} and maintained at 255°C for 1.6 min. The split ratio was 40:1. Data were analyzed using the Agilent ChemStation software. Peaks were identified by comparing their retention times with the fatty acid methyl ester standard mixture GLC-428. Individual fatty acids were reported as a percent of total fatty acid methyl esters by mass.

Introgression of CR genes into canola germplasm: Selected CR genes ***(Rcrl, Rcr2, Rcr3*** and ***Rcr4)*** were introgress into AAFC *B. napus* (SV11-17667), *B. rapa* (DH 03-8429) or *B. carinata* (DH 080798EM-086) canola breeding lines for tech transfer. Validated markers or flanking SSR markers were used to tag ***Rcr1*** and ***Rcr2*** during the introgression and the selection of resistant plants carrying ***Rcr2*** or ***Rcr4*** was based primarily on phenotyping. Backcrossing was carried out to normally BC_5 generations, and stable populations were phenotyped against pathotype 3 of Pb using the bioassay before being released to industry.

Results

Resistance against 5 patho types of *P. brassicae* found in Canada: Ten CR lines were tested against the five *P. brassicae* pathotypes (2, 3, 5, 6 and 8) found in Canada. Throughout the study, the universally susceptible control 'Granaat' (ECD-05) developed severe clubroot symptoms (DSI 2: 97.0%) after inoculation with each of the pathotypes (Table 1). FN, JNC, 'BRA 192/78', 'Kilaherb' and 'Tekila' were immune or highly resistant to each of these pathotypes, consistently reducing DSI by> 90% relative to the control. For each of these lines, there was little variation in resistance to the different pathotypes ($P < 0.05$, LSD). 'Emiko' and 'Bejo 2833' appeared slightly less resistant than the above-mentioned accessions, but their reaction to the different pathotypes did not differ ($P < 0.05$, LSD). 'Pl 219576', 'Purple Top' and 'Purple Top White Globe' turnips, however, were only moderately resistant to these pathotypes, reducing OSI by 44--65% (Table 1). These lines did show slight variation in reaction to different pathotypes; 'PI 219576' was slightly less resistant to pathotype 3 than to pathotypes 2 or 5, and 'Purple Top White Globe' was slightly less resistant to pathotype 3 than to pathotypes 2 or 8 ($P < 0.05$, LSD). In contrast, 'Purple Top' was more resistant to pathotypes 2 and 3 than to pathotype 8 ($P < 0.05$, LSD). There was no clear pattern of differential interaction against a specific pathotype between different *Brassica* species. The line 6992 was not included in this test due to its lateness in discovery, but the unique location of CR gene carried by this line warrants its inclusion in further studies. In recent testing against the newly identified pathotype 5X, lines carrying ***Rcrl*** appeared to be resistant while those carrying ***Rcr2*** or ***Rcr3*** were ineffective (data not shown). This result is encouraging and further testing/validation is underway.

Resistance to clubroot is conferred by a single dominant allele in FN, JNC, 6992 and BRA: For FN, almost all plants of the parent were resistant to pathotype 3 of *P. brassicae,* showing no clubroot symptom 5 weeks after inoculation, whereas all of the ACDC plants were susceptible (Figure 1). Analyses of F_1 populations from reciprocal crosses showed a segregation pattern that would fit a 1:1 ratio between Rand S plants ($\chi^2 = 2.98$, $P = 0.084$), indicating that the resistance in FN is conferred by a single dominant nuclear gene. This gene is designated as ***Rcrl*** *(Rpbl,* previously). The pattern of clubroot disease response in parental and F_1 populations (Figure 1) indicated that the ***Rcrl*** locus was likely heterozygous in the cv. FN. Similar segregation patterns were observed with JNC, 6992 and BRA, and the CR genes in these resistant parents were then designated as ***Rcr2, Rcr3*** and ***Rcr4,*** respectively.

Table 1 Disease severity index (%) on selected *Brassica* accessions in response to inoculation with single-spore isolates of the five *Plasmodiophora brassicae* pathotypes identified in Canada (Trial 3).

Type	Cultivar or accession	P-type 2[a]	P-type 3	P-type 5	P-type 6	P-type 8
Chinese cabbage	Granaat[b]	97.0 e	98.6 f	98.9 d	97.7 e	97.7 e
Black mustard	BRA 192/78	0 a	0 a	0 a	1.4 a	0 a
Black mustard	PI 219576	40.7 d	51.9 e	38.5 c	45.8 d	44.0 d
Cabbage	Kilaherb	0 a	0 a	0 a	0 a	2.3 ab
Cabbage	Tekila	0 a	1.4 ab	0.9 a	3.3 ab	0 a
Chinese cabbage	Bejo 2833	3.2 b	15.7 cd	17.6 b	16.2 bc	10.2 bc
Chinese cabbage	Emiko	16.7 c	18.1 d	25.0 b	25.5 c	19.0 c
Pak choy	Flower nabana	5.7 b	5.1 bc	1.4 a	1.9 a	4.2 ab
Chinese cabbage	Jazz napa cabbage	0.9 a	0.9 ab	2.3 a	0.9 a	2.3 ab
Turnip (forage)	Purple top	34.7 d	43.1 e	45.8 c	55.1 d	55.6 d
Turnip (vegetable)	P-T white globe [c]	43.1 d	53.7 e	49.1 c	47.7 d	39.8 d

[a]Means (n = 6) within a column followed by the same letter(s) do not differ ($P > 0.05$, Fisher's Protected LSD).

[b]Universally susceptible control.

c P-T White Globe: the turnip line –Pouple top while globe

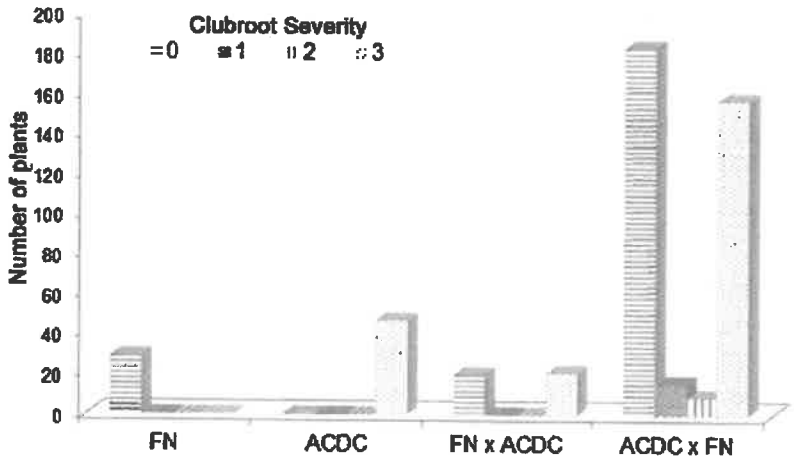

Figure 1 Segregation in clubroot resistance for parents (FN and ACDC) and F_1 populations derived from reciprocal crosses (FN × ACDC and ACDC × FN, respectively).

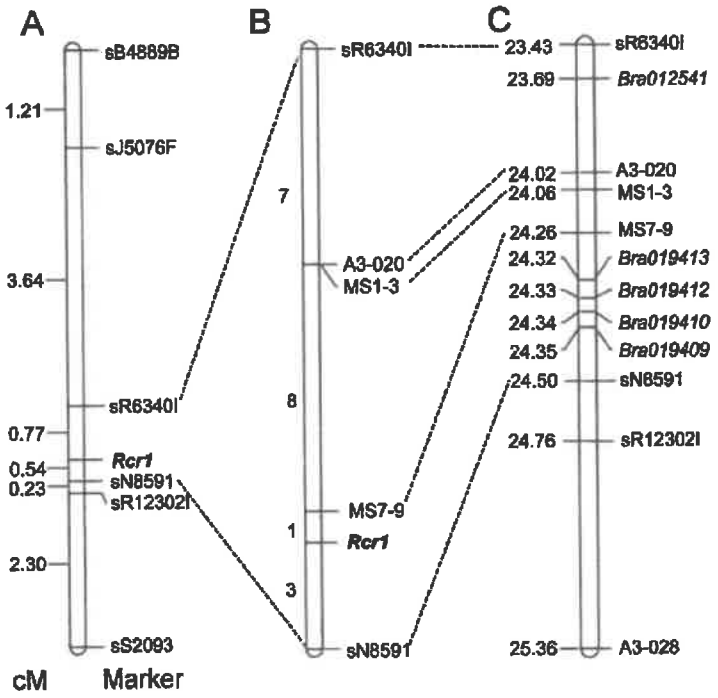

Figure 2 Linkage maps of the regions in which the *Rcr1* gene is located. Broken lines drawn regions defined by different molecular markers on *B. rapa* linkage group A03. A) Rough mapping of *Rcr1* based on a small F_1 population (300 plants) derived from ACDC × FN. The genetic distance is shown on the left. B) Fine mapping of *Rcr1* based on 1,587 F_1 plants. C) Physical locations in Mb (left) of the molecular markers and TIR-NBS-LRR genes in the region flanked by the markers sN8591 and sR6340I.

Mapping of the gene *Rcr1* and development of molecular markers: *Rcr1* was mapped initially to a range of 1.31 cM in the *B. rapa* linkage group A03 flanked by the markers sN8591 and sR6340I (Figure 2A) (Chu et al 2013), and fine mapping was based on testing additional 1,587 F_1 plants using pathotype 3 of *P. brassicae* and on analysis using additional markers (Figure 2B). The flanked segment is homologous to the region between 23.43 Mb and 24.50 Mb on the A03 (*B. rapa* reference genome sequence, Chromosome v1.2), with 158 genes annotated (http://brassicadb.org/brad) and five of them (*Bra012541, Bra019409, Bra019410, Bra019412* and *Bra019413*) were identified as encoding toll interleukin-1 receptor (TIR)- nucleotide-binding site (NBS)-leucine-rich repeat (LRR) class of proteins (Figure 2C). *Bra012541* is located close to 23.69 Mb and the rest were in a cluster located between 24.32 Mb and 24.35 Mb.

A total of 19 recombinants were identified via comparison of marker and phenotype data over the 1,587 F_1 plants (Figure 3), with 3 falling between sN8591 and ***Rcrl*** and 16 between *Rcrland* sR6340I. A CAPS marker (A3-020), homologous to *Bra038794* at 24.02 Mb, was developed for further analysis of the 19 recombinants, and showed an approximate distance of 0.57 cM from ***Rcrl,*** which was closer to the CR gene than sR6340I. The interval flanked by sN8591 and A3-020 was estimated at 0.76 cM, consisting of approximately 480 Kb with 67 genes annotated. The CAPS marker MS7-9 (5'-AGAGGCTTTCTCCATCAA-3', 5'-GACATAA GAATCCCACAA-3') was identified slightly later and appeared even closer to ***Rcrl*** thanA3-020 (Figure 2B). Based on the rate of recombination, the genetic distance of ***Rcr1*** was estimated at 0.19 cM from sN8591 and 0.06 cM from MS7-9, respectively. Four of the TIR-NBS-LRR genes are located within this interval (Table 2).

Table 2 The defense-related genes annotated within the fine mapped region flanked by the markers sN8591 andA3-020 in the *Brassicae rapa* linkage group A03 and their associated gene ontology (GO) terms.

Seq. ID	Seq. Description	GO term
Bra019409	tir-nbs-lrr class resistance protein	P: Defense response to bacterium; F: Adenyl ribonucleotide binding
Bra019410	disease resistance protein	P: Defense response to bacterium; F: Nucleotide binding
Bra019412	tir-nbs-lrr class resistance protein	F: Nucleoside-triphosphatase activity; P: Defense response; F: ADP binding; P: Signal transduction; C: Intracellular
Bra019413	tir-nbs-lrr class resistance protein	C: Golgi membrane; C: Endoplasmic reticulum membrane; F: Binding; P: Defense response to fungus, incompatible interaction; C: Plasma membrane; P: Response to oomycetes

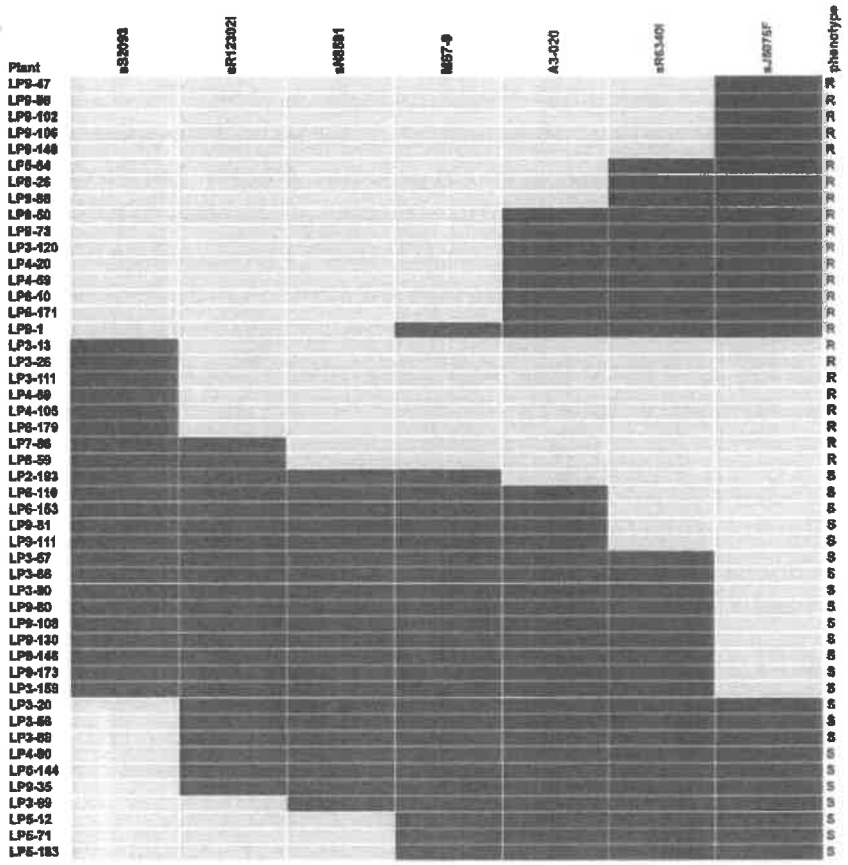

Figure 3 Genotypes and phenotypes of recombinants selected from the mapping population inoculated with pathotype 3 of *Plasmodiophora brassicae*. Line identifications and phenotypes (R for resistant, S for susceptible) are denoted on the left and right, respectively, with marker names at the top. Resistance alleles are denoted in light grey and susceptible alleles in black. The two markers in a grey shadow flank the narrowest interval containing the ***Rcr1*** gene

Mapping of CR genes in JNC, 6992 and BRA: Analyses of the BC_1 population of JNC (926 plants) derived from the cross with ACDC indicated also a single dominant allele involved in clubroot resistance, which was designated as ***Rcr2*** (also on A03) and flanked by the markers sR9448 and sJ1807. Additional 182 plants were analyzed further with 69 markers on the A03 and a linkage map was constructed using 15 polymorphic markers (Figure 4A). The marker sR0660 was used later to flank ***Rcr2*** with sJ1807, and the two markers were 1.7 cM and 13.7 cM from the CR gene, respectively. Mapping of the CR gene in 6992 used a total 190 microsatellite markers distributed on 10 chromosomes of the A genome over a BC_1 (175) and a F_2 (147) population, and located the gene on the chromosome A08 flanked by the markers sR6489R and sN13095/sN4145 (Figure 4B). This CR gene, which co-segregated with the marker sR02133, was designated as ***Rcr3***. Flanking markers for ***Rcr1***, ***Rcr2*** and ***Rcr3*** have been validated. An F_1 population of *B. carinata* (DHC) × *B. nigra* BRA has been developed for mapping.

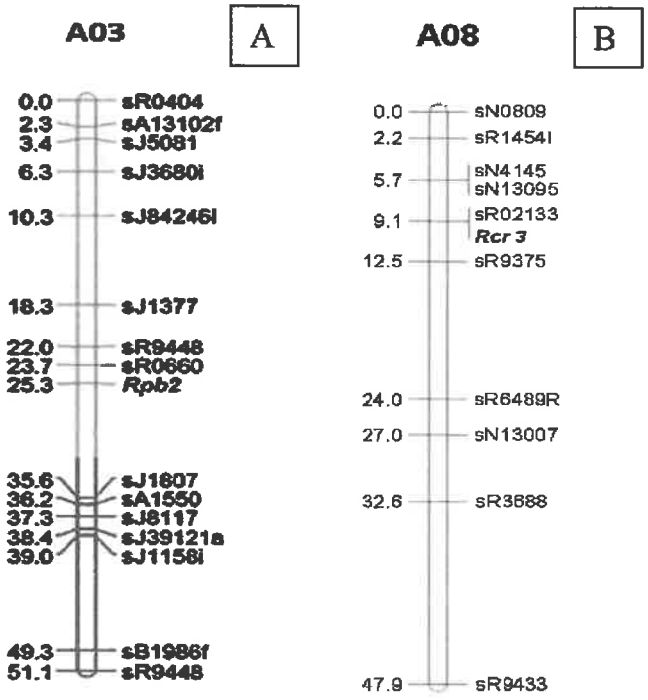

Figure 4 The location of ***Rcr2*** (A), formerly ***Rpb2***, and ***Rcr3*** (B) in the *B. rapa* linkage group A03 and A08, respectively. Markers are labelled on the right side and genetic distance (cM) on the left.

Validation of selected markers for detection of *Rcr1* in backcross populations: On the BC_1 *B. napus* population, sN8591 detected ***Rcr1*** in 99.8% of the resistant (R) and 0.2% of susceptible (S) plants, while sR6340I detected the CR gene in 95.9% of R and 4.1% of S plants, respectively (Table 3). On *B. rapa*, however, the accuracy was slightly poorer for both sN8591 (96.5% of R, 3.5% of S) and sR6340I (92.7% of R, 7.3% of S). The accuracy was much poorer for the markers sB4889B and sS2093 on both *B. napus* and *B. rapa*, with erroneous identification of ***Rcr1*** at >7.3%.

Table 3 Validation of flanking markers for detecting ***Rcr1*** (%) in BC_1 progenies.

	B. napus [a]		***B. rapa*** [a]	
Molecular markers	**Resistant** [b]	**Susceptible** [b]	**Resistant**	**Susceptible**
sN8591	99.8%	0.2%	96.5%	3.5%
sR6340I	95.9%	4.1%	92.7%	7.3%
sB4889B	79.4%	20.6%	87.8%	12.2%
sS2093	92.7%	7.3%	80.2%	19.8%

[a] The BC_1 populations were derived from crosses of a DH line of *B. napus* (SV11-17667) and *B. rapa* (BH11-17938), respectively, with cv. FN. Each BC_1 population use for the experiment consisted of 176 plants.

[b] "Resistance" and "Susceptible" are phenotypical reactions to pathotype 3 of *P. brassicae*. The percentage indicates the rate of *Rcr1* identification in plants using the marker.

Whole-genome-based selection using the SNP array technology: We explored a strategy to identify suitable candidates among CR plants in the BC_1 population using SSR markers, the 6K SNP array and the information based on fatty-acid analysis. For plants confirmed carrying ***Rcr1***, the SNP array technology was used to characterize the progenies during introgression of clubroot resistance from *B. rapa* into *B. napus*.

Initially, the BC_1F_1 population and the parents were tested against pathotype 3 of *P. brassicae*. The recurrent *B. napus* parent (SV11-17667) displayed susceptibility consistently while the donor parent FN (*B. rapa*) showed no clubroot symptom at 5 weeks after inoculation. The BC_1 plants yielded either no disease (0) or severe clubroot (3). The resistance segregation pattern against the pathotype 3 was investigated in a BC_1F_2 (81 plants) and BC_2F_1 (113 plants) population, and the results showed a ratio of 3:1 and 1:1, respectively. This further indicates that the resistance in these populations is controlled by a single dominant gene.

The F_1 population derived from *B. napus* DH Topaz 16516 (DHT) × *B. rapa* (FN) carried 9 unpaired C chromosomes (Figure 5). The fertility was generally poor, with limited number of seeds produced (data not shown). After the F_1 was crossed with the *B. napus* again, the fertility was improved substantially for the BC_1F_1 population and rare progenies may carry the full complement of C chromosomes. However, it is difficult to identify these plants in the BC_1F_1 population based on their phenotypes. Therefore, the 6K SNP array was used to assist the selection. All the SNP markers were designed according to the genomic databases of *B. rapa* and *B. oleracea*, and anchored at specific physical positions in the A and C genomes. Some markers may be located at the homologous regions of A or C genome. All BC_1F_1 plants had 9 univalent C chromosomes from the *B. napus* SV11-17667, but varying numbers of C chromosomes from the *B. napus* DHT (Figure 4). The SNPs were used to differentiate the individuals. Among 3,526 C-

genome SNPs, 1,782 markers showed different segregation patterns (AA or BB) in DHT or SV11-17667, and others showed a heterozygous pattern (AB) or no signal. 306 polymorphic markers between the two parents were used to screen the BC_1F_1 further. For most of the plants, some markers located on 1 or more C chromosomes were not detected (Figure 6), and this indicated that these plants would possess at least 1 unpaired C chromosome. Almost half of the BC_1F_1 population showed to carry the paired C-chromosome 5, and 42% carried the paired C-chromosome 4 (Table 4).

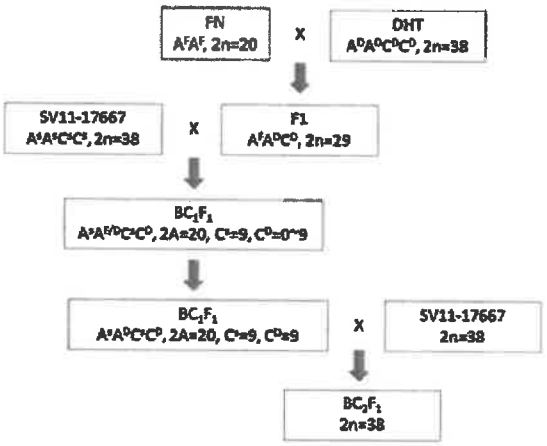

Figure 5 Crosses used in the whole-genome-based marker assisted selection. Clubroot resistance was selected using markers in each generation.

Table 4 Identification of the C chromosomes in the BC_1F_1 population using 6K SNP array

Chromosome	# markers	Bivalent	%	Univalent	%	Others	%
C1	21	140	61.9	82	36.3	4	1.8
C2	30	150	66.4	73	32.3	3	1.3
C3	54	154	68.1	66	29.2	6	2.7
C4	79	190	84.1	35	15.5	1	0.4
C5	19	116	51.3	106	46.9	4	1.8
C6	10	148	65.5	78	34.5	0	0.0
C7	44	164	72.6	58	25.7	4	1.8
C8	35	144	63.7	78	34.5	4	1.8
C9	14	150	66.4	72	31.9	4	1.8

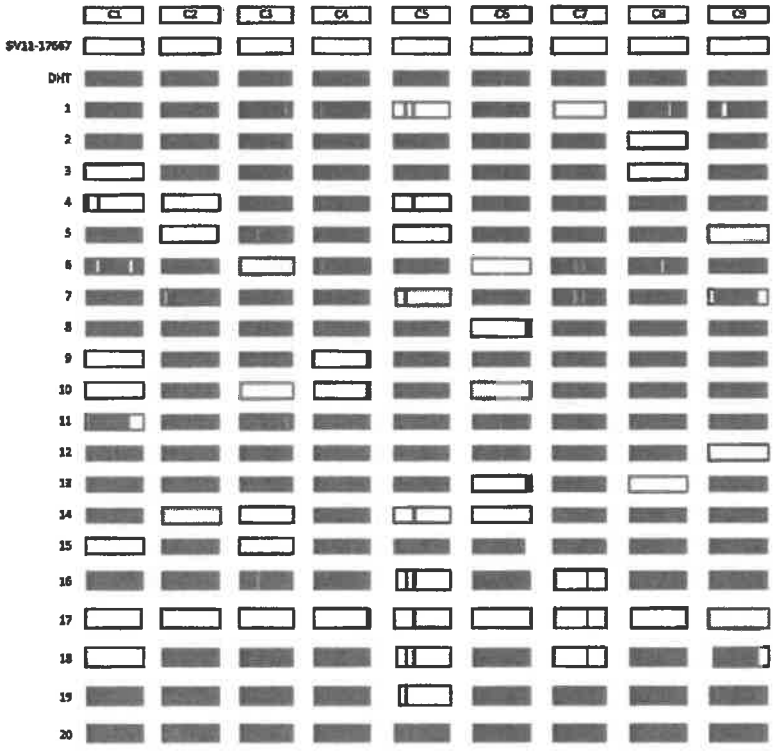

Figure 6 Screening of the BC_1F_1 population in comparison with *B. napus* parents using the SNP maker on the C chromosomes

In order to select resistant plants with most of the A chromosomes from *B. napus* (or least from FN *B. rapa*), markers showed the same pattern in three selected *B. rapa* cultivars or lines (FN, ACDC and "Jazz napa cabbage" –Chinese cabbage) but different pattern in three *B. napus* cultivars or lines (DHT, BH12-18996 and SV11-17667) were assigned to A-genome specific markers in *B. napus*. A total of 383 SNP markers on the A chromosomes (A01-A10) showing different segregation patterns were used to analyze the BC_1F_1 population, and 65.5% of the plants carried the chromosome A04 from the SV11-17667 genome (Table 5). In addition, the SSR markers sS2093, sR6340I were used to differentiate resistant and susceptible bulks in the BC_2F_1 population; the resistant bulk carried 152 bp of the FN allele and 157 bp of the DHT allele by sS2093 whereas the susceptible bulk carried only 157 bp of the DHT allele. A total of 185 bp of the FN allele were found by sR6340I in the resistant bulk whereas only 172 bp of the DHT allele were found with the susceptible bulk (Table 6). Together, the marker analysis placed the CR gene on the chromosome N03. An integrated linkage map was constructed that includes both SNP and SSR markers (Figure 7).

Table 5 Identification of A chromosomes in the BC_1F_1 population using the 6K SNP array

Chromosome	# markers	# plants	%
A01	45	68	60.2
A02	36	46	40.7
A03	27	51	45.1
A04	20	74	65.5
A05	32	68	60.2
A06	51	70	61.9
A07	26	50	44.2
A08	36	23	20.4
A09	80	34	30.1
A10	30	53	46.9

Table 6 The size (pb) of allele in parents linked to the clubroot resistance gene ***Rcr1*** estimated with SSR markers

Marker	FN	DHT	SV11-17667
sN8591	233	230	230
sR6340I	185	172	172
sS2093	152	157	157

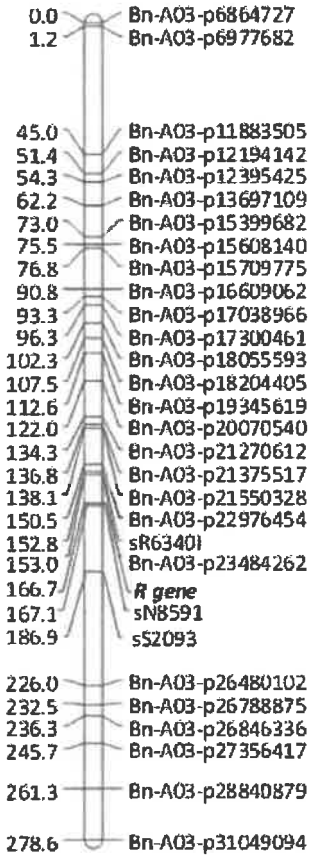

Figure 7 Linkage map of ***Rcr1*** constructed using SNP and SSR markers

Further assessment of the BC_1F_1 population based on plant growth and development showed that the height, 1000-grain weight and yield decreased with the number of paired C chromosomes (Table 7). Primary branching and flowering time were less affected by univalent C chromosome while the pod numbers per plant appeared more variable and was the lowest when 8 of the 9 C chromosomes were univalent. Variation in fatty-acid profile was observed among the BC_1F_1 lines; the 18:1fatty acid was lowest in the ***Rcr1*** donor FN (Table 8) but several lines showed similar levels of 18:1fatty acid to that of the canola line SV11-17667 (*B. napus*). The lines 13 and 154, with 6 and 8 univalent C chromosomes respectively, showed a lower proportion of 18:1 fatty acid. The line 66 stood out in the BC_1F_1 population; it showed a full complement of paired C chromosome, strong resistance to clubroot, similar growth/yield traits to those of the *B. napus* parent (SV11-17667), and a near-canola quality of fatty-acid profile. As a result, this line was selected for further clubroot resistance introgression into *B. napus* canola breeding lines.

Table 7 Growth and yield measurements for BC1F1 lines with varying number of C chromosomes

lines	Height (cm)	Flowering (Days) [a]	Primary branch #	Pods (#) [b]	1000-seed (g)	Yield (g) [b]	C chromosome missing [c]
FN	94.3	46.2	7.5	42.6	2.5	0.2	
SV11-17667	90.4	45.0	4.6	142.4	2.8	5.4	
66	117.1	48.2	4.8	177.8	2.6	5.1	0
117	111.5	43.5	3.5	102.8	2.8	5.3	1
162	102.2	53.4	3.2	93.8	2.7	3.6	2
44	93.2	51.0	3.0	124.3	2.8	3.5	5
127	99.8	49.0	4.5	85.8	1.3	2.5	4,5,9
13	79.2	50.4	5.1	123.6	1.5	1.7	1,3,4,5,6,9
154	95.4	56.1	5.5	58.3	1.4	0.6	2,3,4,5,6,7,8,9

[a] Days after seeding under growth-room conditions.

[b] Total pod # and grain yield from the plant

[c] Univalent C chromosomes.

Table 8 Fatty-acid profile of selected lines from BC_1F_1 relative to that in parents (n=4)

Lines	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0	24:1	Other	Saturated	Missing C chromosome
FN	3.54	0.42	1.98	14.43	8.90	9.10	1.03	8.81	1.37	44.78	0.67	1.75	1.43	8.07	
SV11-17667	5.01	0.36	1.58	60.61	18.37	10.03	0.97	1.42	0.39	0.04	0.32	0.25	0.76	8.01	
66	4.19	0.33	1.86	57.11	15.99	6.12	0.93	5.69	0.41	5.95	0.30	0.44	0.95	7.61	
117	5.15	0.34	2.11	63.33	15.68	9.02	0.67	1.49	0.45	0.04	0.26	0.23	1.42	8.76	1
162	5.25	0.39	2.04	62.50	15.10	9.63	0.62	1.48	0.49	0.05	0.29	0.27	1.35	8.94	2
44	4.54	0.42	1.55	65.89	15.03	8.36	0.53	1.55	0.42	0.07	0.31	0.24	0.84	7.51	5
127	4.74	0.36	1.52	62.40	16.43	10.52	0.54	1.51	0.38	0.05	0.23	0.23	0.85	7.56	4,5,9
13	4.57	0.39	1.77	47.09	18.09	8.96	0.58	7.35	0.32	8.03	0.25	0.55	0.86	7.68	1,3,4,5,6,9
154	5.79	0.52	2.49	47.83	14.31	7.73	0.75	8.52	0.41	8.83	0.39	0.79	1.08	10.09	2,3,4,5,6,7,8,9

Inter-specific hybridization and chromosomal engineering are common strategies for developing useful cultivars of *Brassica* crops with improved tolerance to biotic and abiotic stress

(Kaneko 2014), and have been used between *B. napus* and its wild relatives in the tribe Brassiceae to introduce resistance against important fungal diseases (Siemens et al. 2006). Analysis of genes, genomes and identification of gnetic recombinations are necessary for efficient development of new gene-introgression lines. Olson (2013) demonstrated that two stem-rust *R* genes could be introduced from the diploid D genome donor species *Aegilops tauschii* (2n=2x=14, DD) into the hexaploid wheat *Triticum aestivum* (2n=6x=42, AABBDD), and the *R* genes were located on the chromosome arm IDS using SSR markers based on the analysis of a BC_2F_1 population. However, the line selection among the BC_2F_1 progenies was done based on phenotypic traits. In the current study, the line selection was based on genetic background of the BC_1F_1 plants in comparison with that of both parents using 6K SNP markers, taking advantage of genetic insights into early progenies and making efficient choices on optimal candidates for further breeding steps. The line 66 is a true *B. napus* with a full complement of C chromosomes. Ideally, a bigger BC_1F_1 population should have been used to obtain more plants of the line 66 type for further genetic analysis on the A genome to select individuals with the most similar background to that of the parental *B. napus* (canola). This approach can effectively shorten the process of backcrossing during resistance introgression and production of stable populations of new lines.

When a major clubroot resistance gene was evaluated within the monosomic-addition line generated by intergeneric hybridization between radish *(Raphanus sativus,* 2n=18, RR) and rape *(Brassica napus,* 2n=38, AACC), the resistance allele became hemizygous and the stability of resistance against clubroot was a concern (Akalb 2009). In our study, ***Rcrl*** was detectable in all populations developed (data not shown), indicating that this resistance gene is highly inheritable. Although homologous recombinations between A and C chromosomes can occur, starting with meiosis in F_1 hybrids, its frequency is still not well understood (Lefton et al. 2006; Howell et al. 2008; Nicolas et al. 2009). Therefore, we did not take this aspect into consideration in this study and kept the A and C chromosomes separate. With the fast development of next-generation sequencing technologies, an increasing number of SNPs are being identified and developed via high-throughput SNP arrays (60K, Illumina), and can potentially offer a comprehensive tool for genotyping to be used in the whole genome-based selection. For genotyping *B. napus* progenies originating from interspecific hybridizations, the key consideration is being able to distinguish genome-specific SNPs within the complex allotetraploid genome. SNP arrays can be highly time and cost effective for this purpose, and its high heritability makes the technology an excellent indicator of genetic diversity, phylogeny and genomic inheritance. Studies have shown that *B. napus* a SNP can be identified from every 600 bp of the genome sequence (Edwards et al. 2007; Fourmann et al. 2002).

This study showed that the plant selection with the SNPs in the BC_1F_1 population derived from the cross between the diploid species *B. rapa* and ampidiploid species *B. napus* can be an efficient strategy to speed up the resistance introgression process. Since there were not enough

SNP markers close to ***Rcr1*** available, some SSR markers closely linked to the CR gene (Chu. et al. 2014) were still utilized during the analysis. For most part, the SSR markers appeared to be polymorphic between the parental lines. Other markers, including sR12302I and MS1-3, were monomorphic and the variation may be due to the different background of the current population relative to that reported previously (Chu et al. 2013, 2014). The CR gene was mapped on the chromosome N03 flanked by SSRs and SNPs, this indicates a dominant CR gene was transferred successfully from the diploid *B. rapa* to the amphidiploid *B. napus* breeding line (SV11-17667).

Introgression of *Rcr1*, *Rcr2*, *Rcr3* and *Rcr4* into canola germplasms: The plant 66 (BC_1F_1), carrying a full set of *C* chromosomes and the CR gene ***Rcr1***, was backcrossed with the elite *B. napus* breeding line SV11-17667 (AAFC) and stable BC_5 seeds have been obtained for tech transfer. Backcrosses with the *B. napus* DH line BH11-17938 (yellow-seed, AAFC) is still in progress and should be available soon. ***Rcr1*** has also been incorporated into an elite AAFC *B. rapa* canola line (DH 03-8429), and the population is also at stable BC_5 stage for tech transfer. ***Rcr2*** has been backcrossed into the same AAFC canola lines and BC_4 populations are being established. Introgression of ***Rcr3*** into *B. napus* (SV11-17667) has made significant progress due to use of marker-assisted selection (MAS). The introgression has reached the BC_3 stage and the population is stable for tech transfer. Pyramiding ***Rcr1*** (N03) and ***Rcr3*** (N08) into the *B. napus* SV11-17667 line using MAS has been attempted and lines carrying both CR genes have been obtained but require confirmation. The introgression of ***Rcr4*** into DH *B. juncea* (J072-01904) and DH *B. carinata* (080798EM-086) lines has been carried out, with greater successes seen with *B. carinata* and a stable BC_4 population has been obtained for tech transfer. Interspecific crossing between *B. nigra* and *B. juncea* has proved to be more difficult due to low fertility, and the same challenge has been observed with the interspecific hybridization between *B. rapa* (carrying ***Rcr1*** or ***Rcr2***) and *B. juncea*.

Overall, the project is completed on schedule and on budget. The clubroot resistance genes ***Rcr1***, ***Rcr2***, ***Rcr3*** and ***Rcr4*** have been mapped, with molecular markers developed and used for marker assisted selection during introgression of clubroot resistance into canola germplasms. A genome-wide selection tool (6K SNP array) was used to identify the most suitable candidate carrying the CR gene ***Rcr1*** in early stages of resistance introgression to speed up the process of germplasm development. This technology, with substantial improvements now involving 60K SNP markers, can be a useful selection tool during interspecific hybridization. Introgression of ***Rcr1***, ***Rcr2*** and ***Rcr3*** into *B. napus* and *B. rapa*, and ***Rcr4*** into *B. carinata* canola germsplasm has produced stable germplasm materials for tech transfer. Preliminary results indicate that lines carrying ***Rcr1*** are also resistant to the newly-identified pathotype 5X, and this is very encouraging. A total of 9 refereed papers, 18 conference proceeding papers/book chapters, 7 scientific abstracts, 1 Invention Disclosure and 45 other extension publications/presentation relating to this projects have been generated.

Additional professionals involved in the project

NSERC Post-doctoral Fellows

1. Dr. Lahlali R (2011-present): Biological-genetic resistance interaction, mechanisms of clubroot resistance –induced systemic resistance and synchrotron-based technologies.
2. Dr. Chu M (2011-2014): Mapping of clubroot resistance genes (***Rcr1***, ***Rcr3***) and marker development
3. Dr. Zhang X (2012-2014): Mapping of clubroot resistance gene (***Rcr2***) and marker development

Co-op Students

1. Terry Tran –Univ. Manitoba (Co-op): Clubroot-resistance populations (2011).
2. Jehn Francisco –Univ. Manitoba (Co-op): Clubroot-resistance populations (2011)
3. Chantal de Carmen –Univ. Manitoba (Co-op): Mapping of clubroot resistance (2011).
4. Nick Dimopoulos –Univ. Victoria (Co-op): Development of clubroot resistance (2012)
5. Laura Kessler –Univ. Saskatchewan (Summer Student): Development of clubroot resistance (2012, 2013)
6. Adrian Chang (2012-2013, now in M.Sc.): Mapping of clubroot resistance genes (***Rcr4***) and marker development

Productivity

Peer-reviewed papers

1. Chu M, Yu F, Falk KC, Liu X, Zhang X, Chang A, **Peng G**. 2013. Identification of the clubroot resistance gene *Rpb1* and introgression of the resistance gene into canola breeding lines using a marker-assisted selection approach. *Acta Horticulturae* 1005: 599-605.
2. **Peng G**, Pageau D, Strelkov SE, Lahlali R. Hwang SF, Hynes RK, Anderson K, McDonald MR, Gossen BD, Turkington KT, Falk, CK, Yu FQ, Boyetchko SM, McGregor L 2013. Assessment of crop rotation, cultivar resistance and *Bacillus subtilis* biofungicide for control of clubroot on canola. *Acta Horticulturae* 1005: 591-598.
3. Lahlali R, **Peng G**, Gossen BD, McGregor L, Yu FQ, Hynes RK, Hwang SF, McDonald MR, and Boyetchko SM. 2013. Evidence that the biofungicide serenade (*Bacillus subtilis*) suppresses clubroot on canola via antibiosis and induced host resistance. *Phytopathology* (Impact Factor: **2.75**) 103: 245-254 (***Editor's Pick***)
4. Rahman H, **Peng G**, Yu F, Falk KC, Kulkarni M, Selvaraj G. 2014. Genetics and breeding for clubroot resistance in Canadian spring canola (*Brassica napus* L.). *Can. J. Plant Pathol.* 36 (Suppl. 1): 122-134.
5. Lahlali R, **Peng G.** 2014. *Clonostachys rosea* confers suppression of clubroot on canola via antibiosis and induced host resistance. *Plant Pathology* (Impact Factor: **2.969**) 63:447–455.
6. Lahlali R, McGregor L, Song T, Gossen BD, Narisawa1 K, **Peng G**. 2014. *Heteroconium chaetospira* induces resistance to clubroot via upregulation of host genes involved in

jasmonic acid, ethylene, and auxin biosynthesis. *PLoS One* (Impact Factor: **3.534**) **9**:4 e94144.

7. **Peng G**, Lahlali R, Hwang SF, Pageau D, Hynes RK, McDonald MR, Gossen BD, Strelkov SE. 2013. Crop rotation, cultivar resistance, and fungicides/biofungicides for managing clubroot (*Plasmodiophora brassicae*) on canola. *Can. J. Plant Pathol.* 36 (Suppl. 1): 99-112.
8. **Peng G**, Falk KC, Gugel RK, Franke C, Yu FQ, James B, Strelkov SE, Hwang SF and McGregor L. 2014. Sources of resistance to *Plasmodiophora brassicae* (clubroot) pathotypes virulent on canola. *Can. J. Plant Pathol.* 36: 89–99.
9. Chu M, Song T, Falk KC, Zhang X, Liu X, Chang A, Lahlali R, McGregor L, Gossen BD, Yu F, **Peng G**. 2015. Fine mapping of ***Rcr1*** and analyses of its effect on transcriptome patterns during early infection by *Plasmodiophora brassicae*. *BMC Genomics* (Impact Factor: **4.041**). 15:1166 (http://www.biomedcentral.com/1471-2164/15/1166).

Conference proceedings, presentations & book chapters

1. **Peng G**, Lahlali R, Hwang SF, Hynes RK, McDonald MR, Pageau D, Gossen BD, Strelkov SE, McGregor L. 2011. Control of clubroot on canola using the biofungicide Serenade plus cultivar resistance. In *Proc. 13th Int. Rapeseed Congress*, June 5–9, 2011, Prague, Czech Republic. Pp.1134–1137.
2. Strelkov SE, Hwang SF, Howard RJ, **Peng G**. 2011. Clubroot in the Canadian canola (*Brassica napus*) crop: challenges and successes in dealing with an emerging disease. In *Proc. 13th Int. Rapeseed Congress*, June 5–9, 2011, Prague, Czech Republic. Pp. 1126-1127.
3. Hwang SF, Ahmed HU, Strelkov SE, Gossen BD, **Peng G**, Turnbull GD. 2011. Clubroot development in resistant and susceptible canola cultivars affects soil populations of *Plasmodiophora brassicae*. *In 13th Int. Rapeseed Congress*, Prague, Czech Republic, June 5–9, 2011. Pp. 1122-1125.
4. Lahlali R, **Peng G**, McGregor L, McDonald MR, Yu F, Gossen BD, Hwang SF, Hynes RK, Boyetchko SM, Hupka D, Geissler J. 2012. Induced host defense responses are involved in suppressing clubroot on canola with *Bacillus subtilis*, *Clonostachys rosea* and *Heteroconium chaetospira*. Poster and proceedings of Ann. Metng of CPS, June 26-29, Niagara Falls, ON.
5. Lahlali R, **Peng G**, McGregor L, Yu F, Zhang W, Parkin I, McDonald MR, Gossen BD. 2012. Transcriptome changes of canola in response to a treatment with *Heteroconium chaetospira*, an endophytic biocontrol fungus against clubroot. Poster and proceedings of the Saskatchewan-Alberta 4th Joint Plant Pathology Conference, November, 5-7, 2012, Lloydminster, SK.
6. **Peng G**. Presentation at of the Saskatchewan-Alberta 4th Joint Plant Pathology Conference on "Mapping and cloning of clubroot resistance genes in Brassica species". Nov. 7, 2012, Lloydminster, SK.
7. Chu M, Yu F, Falk KC, Liu X, Zhang X, Chang A, **Peng G**. 2012. Identification and mapping of clubroot resistance genes. Poster and proceedings of the Saskatchewan-Alberta 4th Joint Plant Pathology Conference, November, 5-7, 2012, Lloydminster, SK.
8. **Peng G**. Presentation at of the Sask-Alberta 4th Joint Plant Pathology Conference on "Control of clubroot with cultivar resistance crop rotation". Nov. 7, 2012, Lloydminster, SK.

9. Chu M, Yu F, Falk KC, Liu X, Zhang X, Chang A, **Peng G**. 2012. Identification of the clubroot resistance gene *Rpb1* and development of molecular markers to facilitate introgression of the resistance gene into canola germplasm. Poster at 6th Int. Symposium on Brassica and 18th Crucifer Genomics Workshop. Nov. 12-16, 2012, Catania, Italy.

10. **Peng G**, Lahlali1R, Hynes RK, Boyetchko SM, Gossen, BD, Hwang SF, Pageau D, McDonald MR, Strelkov SE. 2013. *Plasmodiophora brassicae* Woronin, clubroot of crucifers (Plasmodiophoraceae). In Mason P and Gillespie D (eds.), *Biological Control in Canada 2001-2010*, Chapter 74, *pp* 429-237, CABI Publishing, Wallingford, UK.

11. Chu M, Yu, F, Falk KC, **Peng G**. 2013. Identification and mapping of clubroot resistance genes. Poster at *Int. Clubroot Workshop*. June 19-21, 2013, Edmonton, AB.

12. **Peng, G**., Lahlali, R., Hwang, S.F., Pageau, D., Hynes, R.K., McDonald, M.R., Gossen, B.D., and Strelkov, S.E. Integrated management of clubroot on canola with crop rotation, cultivar resistance, and fungicides /biofungicides. Pg. 7 in *Proc. Int. Clubroot Workshop*, June 20, 2013, Edmonton, AB.

13. **Peng G**, Pageau D, Hwang SF, Lahlali R, Hynes RK, Anderson K, Strelkov SE, McDonald MR, Gossen BD, Turkington TK,Yu F, Falk KC, Boyetchko SM, McGregor L. 2013. Assessment of crop rotation in combination with cultivar resistance or a biofungicide for control of clubroot on canola. Oral presentation at *10th International Cong. Plant Pathology* (O39.013), Aug. 25-30, 2013, Beijing, China. *Acta Phytopathol. Sinica* 43: (Supple) 513-514.

14. Yu F, **Peng G**, Falk KC, Chu M, Liu X, Zhang X. 2014. Identification and genetic mapping of clubroot resistance genes in Brassica species for introgression of resistance into canola. *Proc. 19th Int. Crucifer Genetics Workshop*. Mar 30-Apr 2, 2014, Wuhan, China (Oral).

15. Song T, Lahlali R, Chu M, Karunakaran C, Yu F, **Peng G** . 2014. Understanding the mechanism of clubroot resistance gene ***Rpb1*** based on transcriptome, metabolome and fourier transform infrared (FT-IR) analyses. *Proc. 11th Conference Eur Foundation for Plant Pathology (*S11), 8-13 September 2014, Cracow, Poland. *p*145 (Oral).

16. Yu F, Zhang X, Huang Zhen, Song T, Chu M, Falk KC, Gossen BD, Deora DA, McDonald MR, **Peng G**, 2014. Developing gene-specific SNP markers for introgression of ***CR01*** resistance into canola based on the analysis of RNA-seq data. *Proc. 34th Annual Meeting of the Plant Pathology Society of Alberta*, Oct 18-20, 2014, Brooks, AB (Poster).

17. Zhang Y, **Peng G**, Gossen BD, Vail S, Strelkov SE, Hwan SH, Yu F. 2014. Developing near-isogenic lines of spring type *Brassica napus* lines for resistance to clubroot through introgression of resistance genes from *B. rapa*. *Proc. Sask-CPS Regional Meeting*, Dec. 12, 2014. Saskatoon, SK. *p13-14*.

18. Yu F, Huang Z, Chu M, Song T, Chen Q, Falk KC, **Peng G**. 2014. SNP markers for the clubroot resistance gene CR01 based on RNA sequencing. *Proc. Sask-CPS Regional Meeting*, Dec. 12, 2014. Saskatoon, SK. *p*13.

Scientific abstracts

1. Lahlali, R, **Peng G**, McGrego, L, McDonald MR, Yu F, Gossen BD, Hwang SF, Hynes RK, Boyetchko SM, Geissler J. 2013. Induced host defense responses are involved in suppressing

clubroot on canola with *Bacillus subtilis*, *Clonostachys rosea* and *Heteroconium chaetospira*. *Can. J. Plant Pathol*: 35:117.

2. **Peng, G.**, Lahlali, R., Pageau, D., Hwang, S.F., Hynes, R.K., Anderson, K., McDonald, M.R., Gossen, B.D., Strelkov, S.E., Turkington, K.T., Yu, F.Q., Falk, K.C., Boyetchko, S.M., McGregor, L., Hupka, D., and Geissler, J. 2013. Assessment of crop rotation, resistance and *Bacillus subtilis* for management of clubroot on canola. *Can. J. Plant Pathol*: 35:107.
3. Yu F, Chu M, Falk KC, **Peng G**. 2013. Identification and mapping of clubroot resistance genes in *Brassica* species. *Can. J. Plant Pathol*: 35:130-131.
4. Chu M, Yu F, Falk KC, Liu X, Zhang X, Chang A. and **Peng G**. 2013. Identification and mapping of a novel clubroot resistance gene (*Rpb1*) in *Brassica rapa*. Can. J. Plant Pathol. 35: http://dx.doi.org/10.1080/07060661. 2013.850783
5. **Peng, G.**, Pageau, D., Strelkov, S.E., Lahlali, R., Gossen, B.D., Anderson, K., Hwang, S.F., McDonald, M.R., Yu, F.Q., Falk, C.K., Turkington, K.T., Hynes, R.K., Boyetchko, S.M., and McGregor, L. 2013. Management of clubroot disease on canola with crop rotation combined with host resistance or biofungicide seed dressing. Can. J. Plant Pathol. 36: http://dx.doi.org/10.1080/07060661.2013.850783.
6. Gossen BD, McDonald MR, Sharma K, Deora AD, Peng G. 2014. Metabolic cost of resistance to *Plasmodiophora brassicae* when inoculum pressure is high. *Can. J. Plant Pathol* 36:265.
7. Song T, Lahlali R, Yu F, Gossen BD, **Peng G**. 2014. Transcriptome analysis of the clubroot-resistance gene *Rpb1* using high-throughput RNA sequencing. *Can. J. Plant Pathol* 36:289.

Tech transfer

Patents and invention disclosure

1. **Peng G**, Yu FQ, Falk KC. 2012. Identification of three clubroot resistance genes (*Rpb1, Rpb2*, and *Rpb3*) and introgression of resistance using marker-assisted selection (AAFC Invention Disclosure).

Extension publications

1. **Peng G.** 2011. Clubroot on canola in western Canada –Medium Line IBD #130961, April 27, 2012, 3pp.
2. **Peng G.** 2011. *Plasmodiophora brassicae* found in Saskatchewan – Media Line, IBD #168322, Nov 4, 2011. 3pp.
3. **Peng G.** 2013. Canola disease –Clubroot. Media Pitch. AAFC/AAC #3860221.
4. **Peng G.** 2013. Clubroot. AAFC/AAC factsheet #4065141

Workshops, seminars and media interviews

1. **Peng G.** Interviewed by CJWW on "Clubroot in Saskatchewan", Dec. 2nd, 2011. Saskatoon, SK.
2. **Peng G.** Presentation at AgraQuest Inc on "Strategies for managing three important diseases on canola in western Canada". Oct. 18, 2011, Davis, CA.

3. Yu, F.Q., Falk, K.C., and **Peng, G.** 2012. Genetic Control of Clubroot, Clubroot Summit 2012, Edmonton, AB, Canada, March 6, 2012. (talk).
4. **Peng G.** Oral presentation at the Soil and Crops 2012 on "Mitigating the risk of blackleg disease of canola using fungicide strategies". Mar. 13, 2012. Saskatoon, SK.
5. Gossen, B.D., **Peng, G.**, and McDonald, M.R. Biology/Pathology, Invited talk to SARM reps and SK Agric. and Food agronomy specialists, Moose Jaw, SK, April 2012.
6. **Peng G.** Interviewed by Western Producer on "Key messages for clubroot control in Saskatchewan" Dec. 10, 2012.
7. **Peng G.** Interviewed by Western Producer and published on December 20, 2012 paper: "How far can Canadian farmers push their Cinderella crop? –Short canola rotations pay out now, but collect from you later", pp32-33.
8. **Peng G.** Presentation at 6th Int. Symposium on Brassica and 18th Crucifer Genomics Workshop on "Crop rotation, cultivar resistance, and biofungicide seed dressing for clubroot control on canola" Nov. 15, 2012, Catania, Italy.
9. **Peng G.** Presentation at the Saskatchewan Disease Diagnostic Workshop on "Clubroot of canola, disease symptoms and distribution". Nov. 5, 2012. Lloydminster, SK.
10. Kutcher HR and **Peng G.** Presentation at Saskatoon Coop Agro Seed Launch on "Disease issues with intensive canola rotations". Nov. 4th, 2012, Saskatoon, SK.
11. **Peng G.** Presentation at Western Forum for Pest management on "Blackleg of canola in western Canada – current status and control". Oct 16, 2012. Regina, SK.
12. **Peng G.** Interviewed by Top Crop Manager on "Clubroot-resistant varieties and longer rotation reduce disease impact". Sept. 11, 2012, Saskatoon, SK. Article was published in the December Issue (West), 20-22.
13. **Peng G.** Presentation at the Melfort Filed Day on "Canola diseases and canola pathology trials on the AAFC Melfort Research Farm" July 18, 2012, Melfort, SK.
14. **Peng, G.**, Lahlali, R., Pageau, D., Hwang, S.F., Hynes, R.K., Anderson, K., McDonald, M.R., Gossen, B.D., Strelkov, S.E., Turkington, K.T., Yu, F.Q., Falk, K.C., Boyetchko, S.M., McGregor, L., Hupka, D., and Geissler, J. 2012. An oral presentation at CPS Annual Meeting on "Assessment of crop rotation, cultivar resistance and biofungicides for control of clubroot on canola". June 28, 2012, Niagara Fall, ON.
15. **Peng G**, Lahlali R, Pageau D, Hwang SF, Hynes RK, McDonaldMR, Gossen BD, Strelkov SE. A presentation at the Clubroot Summit 2012 on "Cultivar resistance, crop rotation, and biofungicides for control of clubroot on canola". March 7, 2012. Edmonton, AB
16. Yu F, Falk KC, **Peng G**. Presentation at the Clubroot Summit 2012 on "Identification of new clubroot-resistance sources and development of clubroot resistance canola germplasm" March 7, 2012. Edmonton, AB.
17. **Peng G**, Gossen BD. 2013. Clubroot – A threat to canola production in western Canada. AAFC – STB booth at the Crop Production Show, January 6-9, 2013.

18. **Peng G.** Interviewed by Medicine Hat News on clubroot issues. The article was written by Stacey Lee (reporter) and published under the title "Three-year Rotation Suggested for Managing Clubroot" on April 10, 2013.

19. **Peng G.** An invited talk at Saskatchewan Municipal Hail Insurance Adjusters' Annual Mtg. entitled "Managing disease issues on canola", June 5, 2013, Regina, SK.

20. Fernando WGD, **Peng G**, and Jurke C. Industry meeting: Potential *R* gene labelling for commercial canola cultivars in Canada. Presenter and Facilitator. June 18, 2013, Edmonton, AB.

21. **Peng G.** An invited talk at Agronomists Training seminars "Fungicide application and timing for control of canola diseases". July 10, 2013, Price Albert, SK.

22. **Peng G.** Presentation at the AAFC Melfort Field Day –Managing blackleg of canola. July 24, 2013, Melfort, SK.

23. **Peng G**, Yu F, Falk KC, Vail S, Gossen BD. 2013. Presentation at Clubroot Research Planning meeting on "Progresses in clubroot research –developing resistant canola germplasm, understanding resistance mechanisms, and value of crop rotation with *R* canola cultivars", Dec. 5-6, 2013, Edmonton, AB.

24. Hwang, S.F., Strelkov, S.E., Gossen, B.D., **Peng, G.**, and Howard, R.J. Progress in canola clubroot management in Alberta, Canada. Invited presentation, CRMI Planning Meeting, Edmonton, December 6, 2012.

25. Gossen, B.D., Deora, A., **Peng, G.**, and McDonald, M.R. Update on environment and clubroot risk 2013. Invited presentation, Planning Meeting, Clubroot in GF2, Edmonton, AB. Dec. 5, 2013.

26. Hwang, S.F., Strelkov, S.E., Howard, R.J., Gossen, B.D. and **Peng, G.** Management of clubroot on canola in western Canada. Invited presentation, Planning Meeting, Clubroot in GF2, Edmonton, December 5, 2013.

27. **Peng G**, Yu F, Falk KC, Gossen BD. An invited talk at Saskatchewan Disease Subcommittee Annual Meeting on "Managing clubroot on canola –what's new in the research pipeline?" Dec. 10, 2013, Saskatoon, SK.

28. **Peng G**, Fernando WGD, Lange R, Kutcher HR. An invited talk at Agronomy Research Update on "Blackleg of canola –what's new in the research pipeline?" Sponsored by the Saskatchewan Institute of Agrologists, Dec.11, 2013, Saskatoon, SK.

29. Turkington KT, Xi K, Kutcher HR, **Peng G.** A talk entitled "Spray it and forget it" might not be the best approach to getting the most out of a fungicide application. Manitoba Agronomist Conference, Dec. 12, 2013, Winnipeg, MB.

30. **Peng G.** Interviewed by Real Agriculture (Debra Murphy, Reporter) on "Managing blackleg of canola –Crop rotation, cultivar resistance, and fungicides" www.realagriculture.com. Dec. 12, 2013.

31. Invited Seminar (*international*): Clubroot on canola – a case for integrated control. April 2, 2012, Anhui Agricultural University, Hefei, China (expenses paid by host).

32. Invited Speaker (*international*): Crop rotation, cultivar resistance, fungicides & biofungicides for managing clubroot on canola. *International Clubroot Workshop*, June 20, 2013, Edmonton, AB

33. Invited Speaker (*industry*): Clubroot disease on canola –what's new in the research pipeline? Canola Industry Meeting, December 4, 2013, Saskatoon, SK (complementary registration).

34. Invited Seminar (*international*): Developing resistant germplasm for sustainable management of clubroot on canola. April 1-4, 2013, Zhejiang University, Hangzhou, China.

35. **Peng G**, Gossen BD, Yu F. 2013. Clubroot –a threat to canola production in western Canada. Poster at Crop Production Show, Jan 6-9, 2013, Saskatoon, SK.

36. **Peng G**. "Canola rotation: Risk assessment" Canola Watch (Mar. 5, 2014), Issue 3.

37. **Peng G**. Interviewed by CJVRFM/CK750AM Melfort, SK on "Crop rotations relative to blackleg, clubroot and sclerotinia", Mar. 27, 2014.

38. **Peng G**, Yu F, Fernando WGD, Turkington TK, Buchwaldt L. Genetic resistance for control of blackleg, clubroot and sclerotinia on canola. An invited talk at Topnotch Farm, Mar. 27, 2014, Melfort, SK.

39. Lahlali R, Song T, Karunakaran C, **Peng G**. Understanding the mechanisms of clubroot resistance via the analysis of transcriptome, metabolome and fourier transform infrared technologies. An invited talk at the workshop "*The application of synchrotron imaging for Crop Improvement*". June 11, 2014, Saskatoon, SK.

40. **Peng G**. "Emerging" disease issues with canola production –clubroot and blackleg. Invited talk at Canola Discovery Forum 2014, Oct. 22, 2014, Saskatoon, SK.

41. **Peng G**. Clubroot of canola -What we know/don't know, and how to manage it? Invited talks at "Fertility of Minds". Nov 18 & 19, 2014, Calgary & Edmonton, AB.

Financial Statement

Overall, the project stayed on budget. The deficit on M&S was due to higher cost of supplies used for mapping CR genes. The lower amount of pay to PDF was due to a delay in finding a Post-doctoral Fellow. As a result, more Co-op students were used to jump start the development of resistance populations to be use for genetic mapping (by PDFs). The $-22,725 is the final payment owing.

Characterization and utilization of newly identified resistance sources for sustainable

AAFC Project # A08189

Final Financial Statement prepared January 5, 2015

Dr. G. Peng

Approved Budget	163,415.00				
Funds Received	140,691.00	86%			
	Budget	**Funds Received**	**Expended**	**Committed**	**Balance**
Materials & supplies	30,060.00	25,879.00	49,304.36	0.00	-23,425.36
PDF	85,321.00	73,456.00	57,411.74	0.00	16,044.26
Students/Salary	26,720.00	23,004.00	35,383.90	0.00	-12,379.90
Administrative Overhead	21,315.00	18,351.00	21,315.00	0.00	-2,964.00
Total	**163,416.00**	**140,690.00**	**163,415.00**	**0.00**	-22,725.00

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Bradshaw, J. E., Gemmell, D. J. and Wilson, R. N. 1997. Transfer of resistance to clubroot (Plasmodiophora brassicae) to swedes (Brassica napus L. var. napobrassica Peterm) from B. rapa. Ann appl Biol 130,337-348

Chu M, Yu F, Falk KC, Liu X, Zhang X, Chang A, Peng G. 2013. Identification of the clubroot resistance gene Rpb1 and introgression of the resistance gene into canola breeding lines using a marker-assisted selection approach. Acta Hortic.1005: 599–605.

Chu, M., Song, T., Falk, K.C., Zhang, X., Liu, X., Chang, A., Lahlali, R., McGregor, L., Gossen, B.D., Yu, F., Peng, G. 2015. Fine mapping of *Rcr1* and analyses of its effect on transcriptome patterns during early infection by *Plasmodiophora brassicae*. BMC Genomics. 15:1166 (http://www.biomedcentral.com/1471-2164/15/1166).

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Edwards D, Batley J, Cogan NOI, Forster JW, Chagné D (2007) Single Nucleotide Polymorphism discovery, in: NC Oraguzie, et al. (Eds.), Association mapping in plants, Springer, New York. PP53-76

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Jubault, M., Lariagon, C., Simon, M., Delourme, R. and Manzanares-Dauleux, M. J. 2008b. Identification of quantitative trait loci controlling partial clubroot resistance in new mapping populations of Arabidopsis thaliana. Theoretical and Applied Genetics 117, 191-202.

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