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PROJECT FINAL REPORT

Instructions:

- **Please note that making changes to the project without prior written consent from the funder(s) could constitute sufficient grounds for termination of funding.**
- This report must be a stand-alone report, *i.e.,* must be complete in and of itself. Scientific articles or other publications cannot be substituted for the report.
- A signed electronic copy of this report must be forwarded to the funders' representative on or before the due date, as per the investment agreement.
- A detailed, signed statement of revenues received and expenses incurred during the entire funding period of the project must be submitted along with this report, as per the investment agreement.
- For any questions regarding the preparation and submission of this report, please contact the funders' representative.

Section A: Project overview

1. Project number: 2018F111R
2. Project title: Re-synthesizing *Brassica napus* with clubroot resistance from C-genome
3. Abbreviations: Define ALL abbreviations used.
4. Project start date: (2018/03/01)
5. Project completion date: (2022/05/01)
6. Final report submission date: (2022/05/31)

7. Research and development team data	
a) Principal Investigator: (Requires personal data sheet (refer to Section 14) only if Principal Investigator has changed since last report.)	
Name	**Institution**
Fengqun Yu	Saskatoon Research and Development Centre, AAFC
b) Research team members (List all team members. For each new team member, *i.e.*, joined since the last report, include a personal data sheet. Additional rows may be added if necessary.)	
Name	**Institution**
Md Masud Karim	Saskatoon Research and Development Centre, AAFC

Section B: Non-technical summary (max 1 page)

Provide a summary of the project results which could be used by the funders for communication to industry stakeholders (*e.g.*, producers, processors, retailers, extension personnel, etc.) and/or the general public. This summary should give a brief background as to why the project was carried out, what were the principal outcomes and key messages, how these outcomes and key messages will advance the agriculture industry, how they will impact industry stakeholders and/or consumers, and what are the economic benefits for the industry. This summary should be in plain, non-scientific language.

Clubroot disease on canola (*B. napus*), caused by *Plasmodiophora brassicae*, continues to spread on the Canadian prairies, especially in Alberta. Genetic resistance is considered the most efficient method for disease control. Canola originated from hybridization between *B. rapa* and *B. oleracea*. Sources of resistance in A-genome derived from *B. rapa* have been extensively identified and are being used for developing canola resistant cultivars in western Canada. However, the sources of resistance to clubroot in C-genome from *B. olerace* with quantitative resistance have not been used for development of canola cultivars for resistance to clubroot in Canada so far. By screening a large collection of *B. oleracea* lines, we had identified two *B. oleracea* lines ECD11 and JL04 highly resistant to new strains identified in Alberta. We therefore proposed to re-synthesize *B. napus* lines using the *B. oleracea* lines crossed with a *B. rapa* line (T19) that carries three clubroot resistance genes. This will enable the rapid incorporation of a variety of clubroot resistance genes especially quantitative trait loci from the vegetable type brassica species into canola for durable resistance. A total of 12 re-synthesized *B. napus* have been developed through interspecific crosses, embryo rescue and conventional breeding methods. The presence of clubroot resistance genes/QTLs in the A-genome and C-genome has been confirmed using DNA markers. Among the lines, seven lines produced enough seeds for testing clubroot resistance with eight important races of *P. brsassicae* collected in western Canada. Our results show that the lines were highly resistant to all the races tested. One line was distributed to canola breeders in breeding companies. A Plant Variety/Germplasm Disclosure Form for the re-synthesized *B. napus* lines developed from the project was submitted.

Section C: Project details

1. **Background (max 1 page)**
   Describe the project background and include the relevant scientific and development work providing the impetus for the current project.

Clubroot disease, caused by *Plasmodiophora brassicae*, continues to spread on the Canadian prairies, where it poses a serious long-term threat to canola production. Five pathotypes of *P. brassicae* (pathotypes 2, 3, 5, 6 and 8) had previously been identified based on the differential system of Williams (1966), with pathotype 3 the most prevalent on canola in the prairie region (Strelkov et al., 2007). The first clubroot-resistant canola cultivar in western Canada was released in 2009, and was followed by the release of other first generation of resistant cultivars from various breeding companies starting in 2010. These cultivars exhibited strong resistance to these old pathotypes of *P. brassicae* present in Canada. However, resistance in Canadian canola cultivars was soon overcome by new strains of *P. brassicae* identified in canola field. Strains of *P. brassicae* collected in Canada have been classified into more than 30 pathotypes based on the reactions on the Canadian Clubroot Differential (CCD) set (Strelkov et al., 2018; Hollman et al., 2021). This rapid breakdown of resistance shows the vulnerability of qualitative resistance controlled by major genes, and we believe that the more durable resistance can be achieved by use of new sources of resistance with both quantitative resistance and qualitative resistance.

Genetic mapping of clubroot resistance (CR) genes has been extensively carried out in *Brassica* species. Our group at the Saskatoon Research and Development Centre (SRDC), Agriculture and Agri-Food Canada (AAFC) has identified major resistance genes (*Rcr1* to *Rcr10*) against the old pathotypes and some major new pathotypes such as 3A, 3D and 5X in *Brassica* species and developed robust SNP markers tightly linked to each of the resistance genes (Chu et al., 2014; Huang et al. 2017; Yu et al. 2016, 2017; Dakouri et al., 2018; Huang et al., 2019; Karim et al., 2020; Yu et al. 2021). Some of the genes have been transferred into canola though the conventional pedigree breeding method with marker assisted selection. The germplasms and molecular markers developed in our group have been distributed to canola breeding companies for developing canola cultivars for resistance to clubroot. Identifying major genes for resistance to Canadian pathotypes in *Brassica* species had been also carried out in Dr. Habibur Rahman's group at the University of Alberta (Fredua-Agyeman et al., 2016; Hasan et al., 2016) , Dr. Genyi Li's group at University of Manitoba (Gao et al., 2014) and Dr. Sheau-Fang Hwang's group (Zhang et al., 2016). However, these major genes are usually considered as race specific and could be overcome by the pathogen rapidly.

B. napus originating from hybridization between *B. rapa* and *B. oleracea* is the most important canola species worldwide especially in Canada. Sources of resistance in A-genome derived from *B. rapa* have been extensively identified and are being used for developing canola resistant cultivars in western Canada. However, sources of resistance to clubroot in C-genome from *B. olerace* had not been used in Canada when we initiated the studies for this project. One of the reasons for this is that it is difficult to transfer resistance from *B. oleracea* into

B. napus by directly interspecific crosses due to reproductive barrier. In addition, there are limited sources of clubroot resistance in *B. oleracea* were available. Genetic analysis of the CR genes in *B. oleracea* indicates that they are quantitative traits mainly controlled by quantitative trait loci (QTL) (Piao et al. 2009), which could provide more durable clubroot resistance. By screening a large collection of *B. oleracea* lines, we had identified two *B. oleracea* lines ECD11 and JL04 highly resistant to strains of pathotype 5X when we initiated this project. In addition, ECD11 was resistant to many pathotypes of *P. brassicae* based on the CCD set (Strelkov et al., 2018; Hollman et al., 2021), indicating that it may carry genes with broad spectrum of resistance. In this proposal, we proposed to re-synthesize *B. napus* lines using the *B. oleracea* lines crossed with a *B. rapa* line (T19) that carry three major resistance genes *Rcr4*, *Rcr8* and *Rcr9* (Yu et al, 2017). *Brassica* lines containing *Rcr4* were resistant to the old pathotypes while the lines with *Rcr8* and *Rcr9* were resistant to new strains of pathotype 5X. Under support by the current Canola Cluster funding, we recently identified two race non-specific QTLs in ECD11. We developed *B. napus* germplasms with both quantitative resistance and qualitative resistance to multiple races of *P. brsassicae* in this project.

2. Objectives and deliverables (max 1 page)

State what the original objective(s) and expected deliverable(s) of the project were. Also describe any modifications to the objective(s) and deliverable(s) which occurred over the course of the project.

1. Developing *B. napus* germplasms with quantitative resistance from C-genome species *B. oleracea* and qualitative resistance from A-genome species *B. rapa*.
2. Characterizing the germplasms for resistance to new pathotypes of *P. brassicae* identified in western Canada.

3. Research design and methodology (max 4 pages)

Describe and summarise the project design, methodology and methods of laboratory/field and statistical analysis that were actually used to carry out the project. Please provide sufficient detail to determine the experimental and statistical validity of the work and give reference to relevant literature where appropriate. For ease of evaluation, please structure this section according to the objectives cited above.

Materials

Plant materials and the spectrum of resistance[1]

Pathotype[2]		2, 3, 5, 6, 8	5X	A, C-L, N-R	B, M
B. rapa	T19	R	R	NT	NT
B. oleracea	JL04	NT	R	NT	NT
B. oleracea	ECD11	S	R	R	S

[1] R:resistant, S:susceptible, NT: not tested

[2] Pathotypes 2,3, 5, 6, 8 based on Williams' differential and the rest on the CCD

Pathogen strains

Strains and races of *Plasmodiophora brassicae*

Pathotype/Strain	Origin	Race[1]
3H	Alberta	*AvrM-1-8.2-9.3-9.4*
3A-2	Alberta	*Avr3-9.3*
5G-1	Alberta	*Avr1M-1-9.3-9.4*
5X-LG2	Alberta	*Avr8.2*
8J	Alberta	*Avr3-8.2-9.2-9.3*
8P	Alberta	*Avr3-9.3-9.4-10*
SK29	Saskatchewan	*Avr3-8.2*
PSI11	Manitoba	*Avr8.2-9.3-9.4*

[1]Races were determined based on a set of near isogenic lines developed by our group.

Methodology

a) Developing the re-synthesized *B. napus* germplasms

Reciprocal crosses was made between the *B. rapa* and the two *B. oleracea* lines. Two to five days prior to anthesis, buds on plants were emasculated and pollinated with pollen. The embryo rescue technique was adapted from that described by Crouch et al (1994) with some modifications. Siliques were collected at 16-20 days after pollination, surface sterilized with a 10% solution of sodium hypochlorite for ten minutes and wash twice in sterile water. They were cultured on MS agar medium supplemented with 1% sucrose at 20°C under 16 hr photoperiod for 20-30 days. Embryos were dissected out of the siliques and plated on the MS agar medium. After 10 – 20 days, regenerating embryos were transferred to basal MS agar medium for root and shoot development.

When regenerated plantlets were 4-5 centimeters tall, they were removed from culture and transferred into the AAFC greenhouse. Chromosomes were doubled as described previously (Coventry et al 1998). Plantlet roots were submerged in a 3.4g/L solution of colchicine for 1.5hr. Amphidiploids were identified by the development of fully formed stamens. The re-synthesized *B. napus* lines were self-incompatible. To obtain selfed seeds, plants with open flowers and unopened buds were enclosed in an air-tight plastic bag to be pumped into CO_2 to raise the internal concentration. Three to four hours later the bag were removed and replaced with a pollinating bag. Seeds were increased for further experiments.

b) Evaluating the newly re-synthesized *B. napus* and the parental lines for resistance to clubroot

Our group has developed a set of near isogenic lines carrying eight single CR genes. After testing the lines with 36 strains collected on canola in western Canada, the strains were classified into 28 races (unpublished data). The newly re-synthesized lines and the parental lines were tested for clubroot resistance with selected races of *P. brassicae* in an AAFC PPC1 facility. Plant growth conditions, preparation of *P. brassicae* inoculum and plant inoculations followed protocols described previously (Yu et al 2021). Seeds of the lines were sown into Sunshine #3 soilless mix (Sun Gro Horticulture Canada Ltd.; Seba Beach, AB) with Osmocote (Everris NA Inc.; Dublin, OH, USA) in 32-pot inserts held by trays (The HC Companies; Twinsburg, OH, USA). About 4 L water was added to each tray to soak the soilless mix overnight. Seven days after planting, inoculation

was performed by adding 15 ml of inoculum (1 x 10^7 spores/ml) into each pot with 6–9 seedlings of each line with two replications. The inoculated plants were grown in a growth chamber set at 22/18°C day/night temperature with a 16-h photoperiod. Six weeks after inoculation, plants were pulled and the roots were examined for clubroot symptoms. Clubroot severity was evaluated on a 0 to 3 scale, where 0 = no clubbing, 1 = a few small clubs, 2 = moderate clubbing, and 3 = severe clubbing. A disease severity index (DSI) was calculated for each line using the method of Horiuchi and Hori (1980) (Horiuchi and Hori, 1980):

$$\mathrm{DSI} = \frac{\sum (\text{rating class}) \times (\#\text{ plants in rating class})}{\text{total }\#\text{ plants in treatment} \times 3} \times 100$$

c) Kompetitive Allele Specific PCR (KASP) analysis

The presence of *Rcr4*, *Rcr8* and *Rcr9* in the *B. rapa* T19(Yu et al., 2017) and the QTLs in the *B. oleracea* ECD11 (Karim et al. unpublished data) in the newly re-synthesized *B. napus* lines was confirmed through analysis of SNPs markers using the KASP method (http://www.lgcgroup.com/) following the manufacturer's instructions. Polymerase chain reactions were performed in a StepOne Plus Real Time PCR System (Applied Biosystem, Mississauga, ON).

4. Results, discussion and conclusions (max 8 pages)

Present the project results and discuss their implications. Discuss any variance between expected targets and those achieved. Highlight the innovative, unique nature of the new knowledge generated. Describe implications of this knowledge for the advancement of agricultural science. For ease of evaluation, please structure this section according to the objectives cited above.

NB: Tables, graphs, manuscripts, etc., may be included as appendices to this report.

Results

Development of the re-synthesized *B. napus* germplasms

Reciprocal crosses were initiated between *Brassica rapa* (AA) T19 and *B. oleracea* (CC) ECD11. Six plants of T19 and 3 plants of ECD11 were selected and kept in a cold room for 2 to 3 months vernalization, and then transferred into a greenhouse at the SRDC, AAFC. Crosses were performed using 4 plants of T19 and 2 plants of ECD11, and the remaining plants were used for increasing seeds. Siliques were collected 16-20 days after pollination and cultured on the MS medium to produce hybrid plantlets (Figure 1). Strong F1 hybrid seedlings were obtained by sub-culturing the plantlets on the MS medium (Figure 2) and then transplanted into soil (Figure 3). The cross set for T19 (AA) × ECD11 (CC) resulted in 137 ovaries out of 221 flowers pollinated for an ovary setting rate of 61.9%. Thirteen hybrid plants germinated on the MS medium, 10 plants survived after transplanting into the soil and 4 hybrid F1 amphidiploid (AACC) plants (Re-4, Re-10, Re11A, Re-B) produced seeds after colchicine treatment. In the reciprocal cross set of ECD11 × T19, 26 ovaries were obtained from 57 pollinated flowers for an ovary setting rate of 45.6%. Three hybrid plants germinated on the MS medium, only one plant survived after transplanting into the soil but could not produce seed after colchicine treatment. A higher success silique set rate found using female parent *B. rapa* (61.9%) than *B. oleracea* (45.6%), so crosses with *B. rapa* as female only were performed between T19 and JL04. Crosses were performed using 10 plants of T19 and 6 plants of JL04. The cross set for T19 (AA) × JL04 (CC) resulted in 230 ovaries out of

269 flowers pollinated for an ovary setting rate of 85.5%. Thirty five hybrid plants germinated on the MS medium, 34 plants survived after transplanting into the soil and 4 hybrid F1 amphidiploid (AACC) plants (TJ1-B, TJ-E, TJ1-G, TJ4) produced seeds after colchicine treatment. A total 50 hybrid plants were germinated from which 8 hybrid plants successfully produced seeds from a total of 547 pollinations from the three cross combination (Table 1). Cross ability of these three cross combination (T19 × ECD11, ECD11 × T19, T19 × JL04) were 1.8 , 0.0, 1.5%.

Producing seeds from the newly re-synthesized plants

Hybridity of F1 plants was ensured by their morphological characteristics such as vigorous growth, plant height, leaf size and shapes, flower size and pollen sterility. Chromosome doubling was initiated soaking root into the colchicine solution or axillary floral buds of the F1 hybrids induced fertile flowers and by self-pollination F2 seeds were successfully produced from 8 hybrid plants. These plants considered as amphidiploid (AACC) *B. napus* (Figure 4). Most of the amphidiploid plants were self-incompatible. To obtain self seed, plants with open flowers and unopened buds were enclosed in an air-tight plastic bag and pumped into CO_2 to raise the internal concentration. Selfing was continued up to F6 generation, about 10 plants/line of 4 resynthesized lines from T19 × ECD11; Re-10, Re-11A, Re-11B produced higher seed set in advanced generations but Re-4 lines continued poor seed set in all 6 generations. Among four lines of T19 × JL04 cross combination; TJ1-B, TJ1-E and TJ1-G improved higher seed set in advanced generations (Table 2). As most of the amphidiploid plants were highly self-incompatible, more seeds were obtained from semi-resynthesized F1 plants, crossing with a *B. napus* doubled haploid canola (*B. napus*) line DH16516 (DHT) and re-synthesized *B. napus* lines (Table 2).

Characterization of the newly re-synthesized *B. napus* lines with selected races of *P. brassicae* identified in western Canada

Seven re-synthesized lines of different generations which produced good amount of seeds (Table 2) and their parental lines were selected for inoculation with selected races of *P. brassice*. The universally susceptible line, DHT, originating from the *B. napus* cultivar 'Topas', which was provided by Dr. G. Séguin-Swartz at AAFC, SRDC, was included as a susceptible check. A DH line, NRC11-24 (N1), developed by Nutrien Ag Solutions (Saskatoon, SK) and with known resistance to pathotype 3H, but susceptible to some new pathotypes, was used as a second susceptible check to verify the pathogenicity of new pathotypes characterized by Dr. Strelkov lab , and as a resistant check for 3H. Four lines of F4 generation of Re-10, Re-11A, TJ1-B1, TJ1-G1, two lines of F5 generation of Re-11A, Re-11B and one line of F6 generation of Re-11B were selected for inoculation test. Our group has identified 28 races of *P. brassicae* collected from canola fields in western Canada. Eight strains 3H, 3A-2, 5G-1, 5X-LG2, 8J, 8P, SK29 and PSI11, representing 8 races of *P. brassicae* (See **Research design and methodology**) were chosen for the characterization of the re-synthesized *B. napus*. About 8 plants/line were tested for each strain with two replications. A total 1498 plants were tested against 8 representative races of *P. brassicae*. All re-synthesized lines showed highly resistance against all the 8 strains (Figures 5 and 6); 3A-2 (%DSI = 0.0 – 8.3), 3H (%DSI = 0.0 – 11.1), 5G-1(%DSI = 0.0), 5X(%DSI = 0.0), 8J (%DSI = 0.0 – 4.2), 8P (%DSI = 0.0), SK29(%DSI = 0.0 – 4.8), PSI11 = (%DSI = 0.0 – 4.2). Test cross lines also showed resistance against all the strains tested; 3A-2 (%DSI = 0.0 – 27.8), 3H (%DSI = 0.0 – 14.6), 5G-1(%DSI = 4.2 – 28.2), 5X(%DSI = 0.0 – 12.5), 8J (%DSI = 0.0 – 25.0), 8P (%DSI = 0.0 – 13.3), SK29(%DSI = 0.0 –13.3), PSI11

= (%DSI = 0.0 – 8.3). The *B. rapa* parent (T19) was highly resistant against all the 8 pathotypes (%DSI = 0.0) but resistance of ECD11 and JL04 varied. The ECD11 showed highly resistance against 3A-2(%DSI = 2.8), 5X(%DSI = 4.2), 8J(%DSI = 4.8), 8P(%DSI = 6.7), SK29(%DSI = 9.5), moderately resistance against 3H (%DSI = 37.0), 5G-1(%DSI = 36.1) and susceptible against PSI11 (%DSI = 61.9). Another C-genome parent Jl04 showed highly resistance against 3A-2, 5G-1, 5X, 8J (%DSI = 0.0), partial resistance against 8P(%DSI = 33.3), SK29(%DSI = 20.0), PSI11 = (%DSI = 27.8), susceptible against 3H (%DSI = 100). Control DHT were highly susceptible against all 8 pathotypes (%DSI = 68.8 - 100) but N1 showed highly resistance against 3H, 5G-1, 8J, PSI11(%DSI = 0) and susceptible against 3A-2 (%DSI = 92.9), 5X (%DSI = 54.2), 8P (%DSI = 93.8), SK29(%DSI = 100).

Confirmation of the presence of clubroot resistance genes or QTLs in the newly re-synthesized *B. napus* lines

A total of 21 resynthesized lines with 3 lines each of 7 re-synthesized pedigrees were chosen for confirming the presence of identified clubroot resistance genes and QTLs (Table 3). As mentioned above, the *B. rapa* parental line T19 carried race specific resistance genes *Rcr4*, *Rcr8* and *Rcr9* (Yu et al., 2017). Recently, our group has identified two QTLs *Rcr_ECD11_C3, Rcr_ECD11_C8*, which confer race non-specific resistance to ten races of *P. brassicae* in the cabbage cultivar ECD11 (Karim et al. unpublished data). SNP markers linked to the race specific genes from T19 and race non-specific QTLs have been developed in our group (Table 4). The SNP markers associated with the resistance alleles (*Rcr4*, *Rcr8*, *Rc9, Rcr_ECD11_C3-1, Rcr_ECD11_C8-1*) were identified in the re-synthesized lines from both crosses T19 x ECD11 and T19 x JL04 (Figure 7-13), confirming the presence of the resistance genes and QTLs in the re-synthesized *B. napus* lines.

Discussion

Hybrid embryos terminate their growth due to lack of appropriate endosperm (Chen *et al.* 1988, Inomata 1993, Nishi *et al.* 1959, Olsson 1960, Takeshita *et al.* 1980). Therefore, embryo, ovule and ovary culture are widely used embryo rescue techniques in interspecific hybridization of *Brassica* species. Previous studies reported that ovary culture is effective when *B. rapa* used as a female parent, *B. rapa* (♀) × *B. oleracea* (♂), but inadequate in the reciprocal cross of *B. oleracea* (♀) × *B. rapa* (♂) (Hossain *et al.* 1989, Inomata1977, 1978, Takeshita *et al.* 1980, Karim et al. 2014). However, few exceptions were also reported. Re-synthesized *B. napus* was successfully produced in both crossing directions by using a *B. oleracea* line, CRGC 3-1 as a parent (Song *et al.*1993, Karim et al. 2014). In this study, we found that *B. rapa* line T19 had higher cross ability when used as a female parent.

Wide variation of seed set observed in the developed re-synthesized *B. napus* lines. Earlier generation preformed poor seed set and similar poor seed set was also reported in early generations of re-synthesized Brassica lines (Srivastava *et al.* 2004, Karim et al 2014, Hasan and Rahman 2018).

In this study our goal was to combine qualitative and quantitative resistance from *B. rapa* and *B. oleracea* in the *B. napus* re-synthesized lines. We developed the lines which showed broad range

of resistance to the representative races of *P. brassicae*. Variation of quantitative traits (flowering time) was observed due to structural rearrangement of chromosomes in re-synthesized *B. napus* (Pires *et al.* 2004). Stability of qualitative traits, self-incompatibility (Rahman 2005) and clubroot resistance (Diederichsen and Sacristan 1996), has often been seen in self-pollinated progeny of re-synthesized *B*. *napus* plant. Loss of genomic region carrying CR resistance (6-13%) was reported during development of self pollination (S0 to S1 families) of re-synthesized line due to meiotic anomalies (Hasan et al 2018). In the current study, all homozygous re-synthesized lines were highly resistance against all eight strains, all re-synthesized plants were rated as 0, very few are 1, no plants rated with 2 or 3, so we did not observe any CR resistance loss. We observed some susceptible plants in semi-re-synthesized *B. napus* lines, which were developed by crossing with re-synthesized lines and the clubroot susceptible line DHT. The reason for this is to be determined.

Due to meiotic anomalies and homoeologous pairing of chromosomes in the early generations, re-synthesized Brassica allopolyploids can consequence in some structural rearrangements with loss or gain of chromosomes (Gaeta et al. 2007, Gaeta and Pires 2010, Szadkowski et al. 2010, Udall et al. 2005, Xiong et al. 2011). European winter CR canola cultivar 'Mendel' was developed from a re-synthesized *B. napus* line (Diederichsen and Sacristán 1996). 'Mendel' was expected to have three dominant CR genes but genetic mapping discovered that 'Mendel' had only one dominant CR gene and other two dominant genes were loss during breeding process. Loss of CR genes has also been reported in the breeding of rutabaga line (Bradshaw *et al.* 1997). Our recent study indicates that there are two dominant genes in 'Mendel' (Rahaman et al. unpublished data). Alternatively, two *B. rapa* CR loci (*Crr1* and *Crr2*) were transferred efficiently through developing re-synthesized *B. napus* lines and introduced properly into the recurrent parental lines by using CR loci-linked markers (Kawasaki et al. 2021). In the current study, all targeted loci *Rcr4*, *Rcr8* and *Rcr9* in the re-synthesized *B. napus* lines were confirmed with SNP markers tightly linked to the genes. QTLs from C-genome resistance from ECD11 were also confirmed with tightly linked markers with two QTLs, *Rcr_ECD11_C3, Rcr_ECD11_C8* in the re-synthesized lines.

We have competed the identification of QTLs in ECD11 and development of SNP markers linked to the QTLs under support of the current Canola Cluster funding. Two race no-specific QTLs have been identified. However, studies on genetic mapping for identification of QTLs in JL04 has not been carried out so far. It is interesting that some of the linked SNP markers for the QTLs in ECD11 were identified in the re-synthesized lines from T19 x JL04. Further research on genetic mapping for confirming the result and identification of novel QTLs in JL04 is needed.

Conclusion

In this current study, we successfully developed re-synthesized *B. napus* lines from T19 and ECD11 and JL04, as well achieved broad spectrum of resistance from qualitative and quantitate resistance background. All targeted loci were confirmed in the re-synthesized line with tightly linked markers. The germplasms and the information obtained from the project are available to canola breeders for rapid incorporation into their canola variety development programs. The results expected under the action plan for the project have been fully achieved.

Figures

Figure 1. Silique culture on MS medium

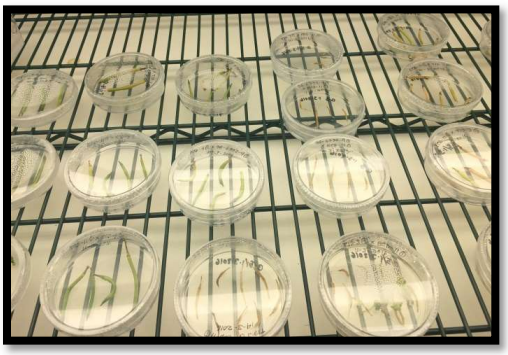


Figure 2. Sub-culture of plantlets

Figure 3 . Transplanting plants into soil

Figure 4. Resynthesized *Brassica napus* (AACC) generation from interspecific hybridization between *Brassica rapa* (AA) parental line T19 and *Brassica oleracea* (CC) parental lines ECD11 and JL04

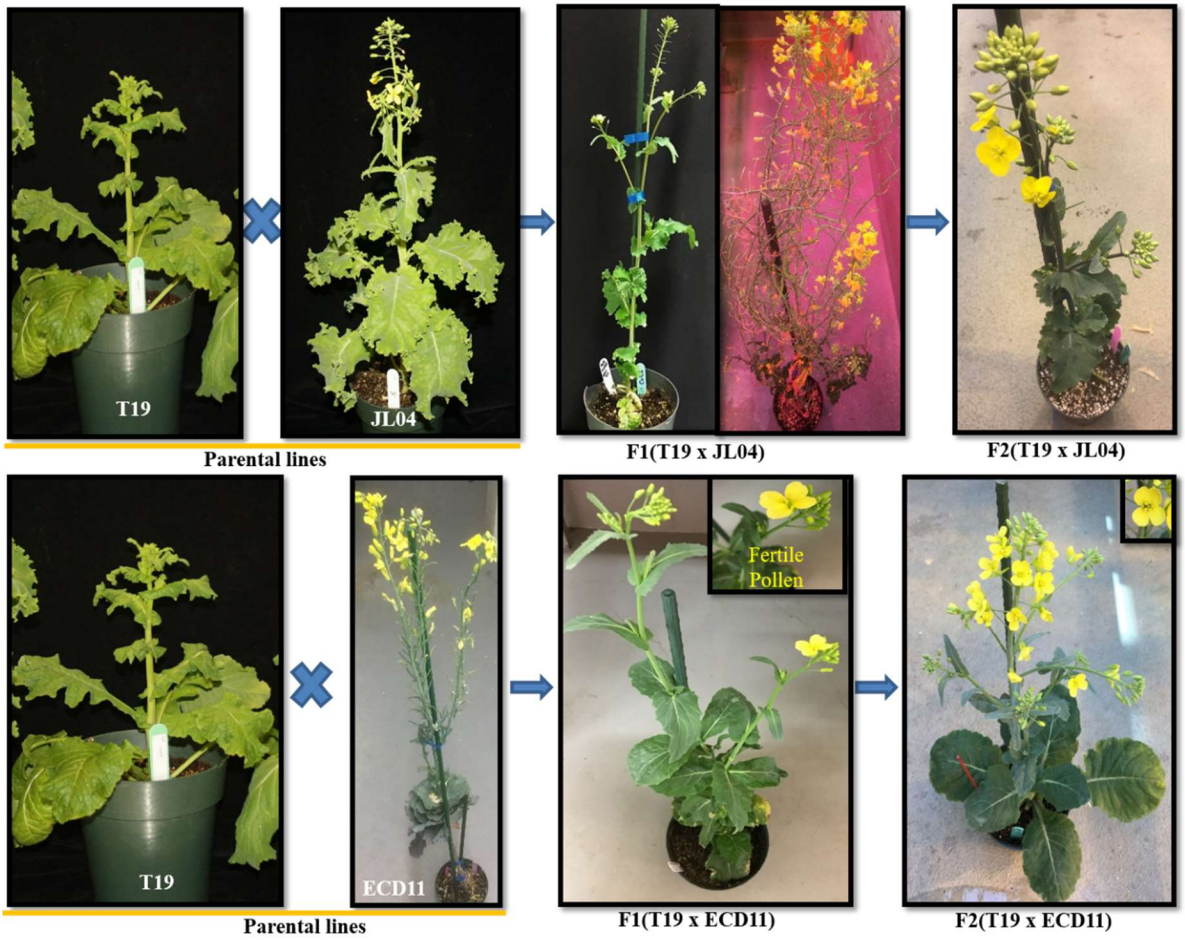


Figure 5. Evaluation of re-synthesized *Brassica napus* (AACC) lines of different generations; Re-10-4 (F4), Re-11A-5-1(F4), Re-11A-5-1-2(F5), Re-11B-4-3-3(F5), Re-11B-4-3-3-6(F6) lines were derived from the cross T19 × ECD11; lines TJ1-B1(F4) and TJ1-G1(F4) were derived from the cross T19 × JL04; four test lines derived from DHT × (T19 × ECD11). Distribution of disease severity indexes (DSIs) of re-synthesized lines, test cross, parental lines (T19, ECD11, JL04) and susceptible (S) check (DHT and N1) against 8 strains, 3H, 3A-2, 5G-1, 5X-LG2, 8J, 8P, SK29 and PSI11, representing 8 races of *P. brassicae* .

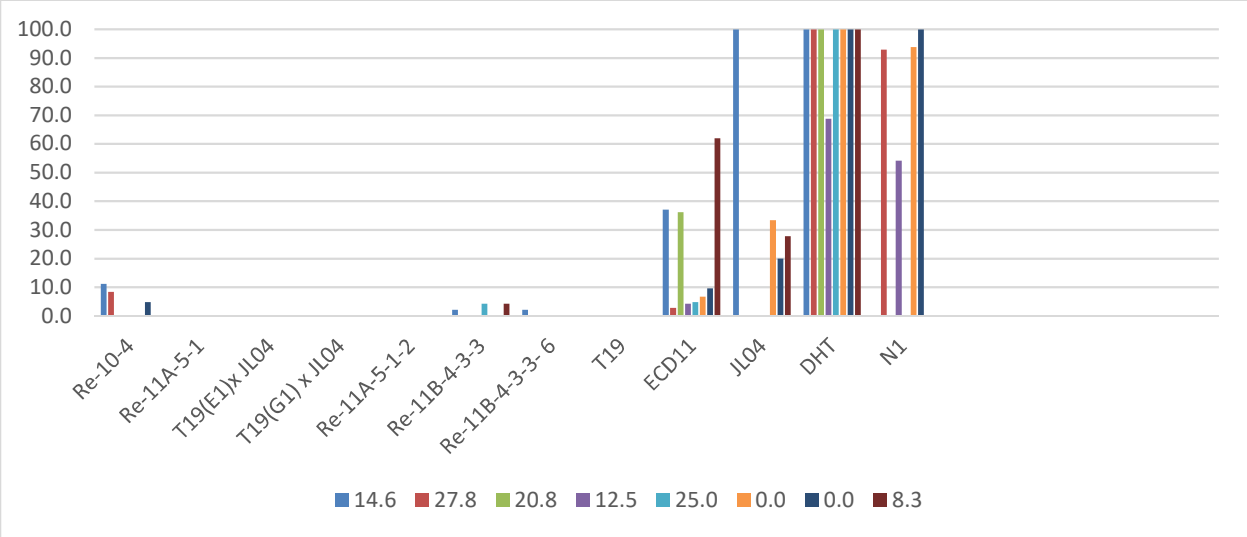


Figure 6. Evaluation of re-synthesized *Brassica napus* (AACC) lines derived from two crosses T19 × ECD11 and T19 × JL04; and test cross derived from DHT × (T19 × ECD11) against 8 strains of *Plasmodiophora brassicae*. Phenotypes of re-synthesized lines, test crosses, parental lines (T19, ECD11, JL04) and susceptible (S) check (DHT and N1) of pathotypes 5X-LG2, 8J, SK29 and PSI11

Figure 7. Allelic discrimination plot of re-synthesized *Brassica napus* (AACC) lines from the crosses T19 × ECD11 and T19 × JL04; *Rcr4* linked SNP marker A3-12 was used for KASP analysis, ACDC was used as susceptible check.

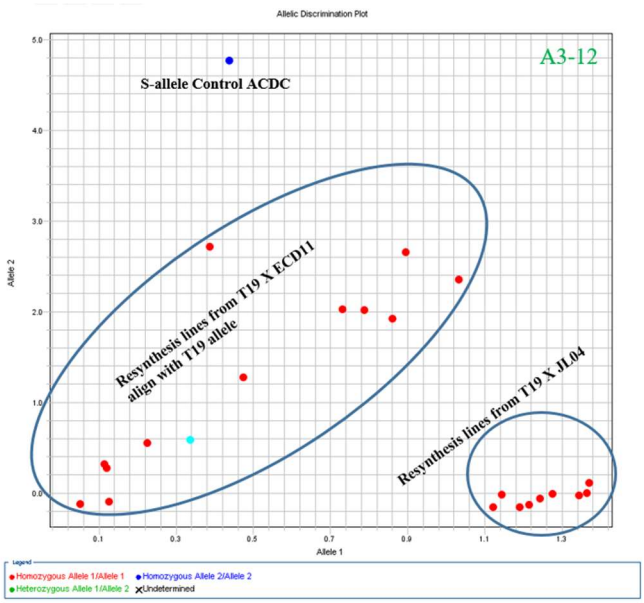
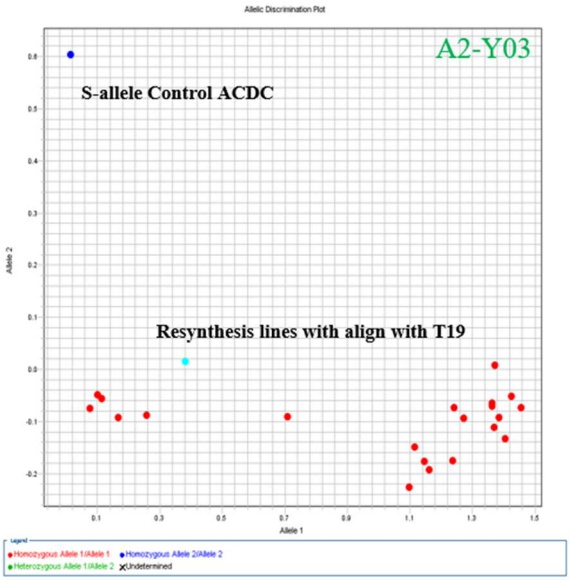


Figure 8. Allelic discrimination plot of re-synthesized *Brassica napus* (AACC) lines from the crosses T19 × ECD11 and T19 × JL04 with ; *Rcr8* linked SNP marker A2-Y03 and A2-Y06 were used for KASP analysis, ACDC was used as susceptible check.



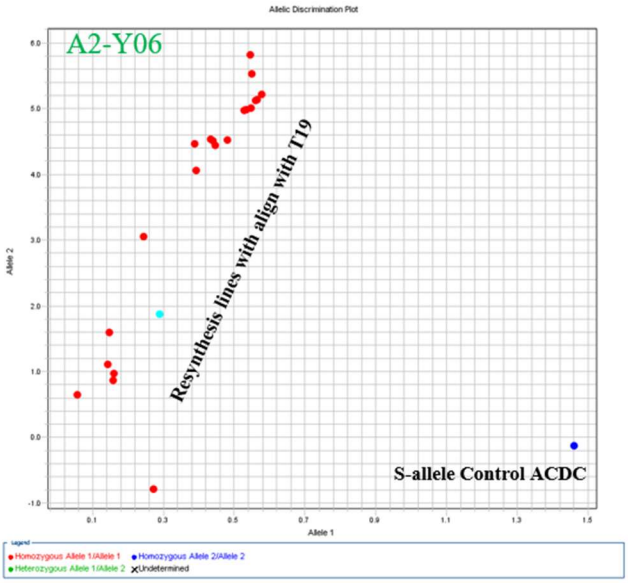


Figure 9. Allelic discrimination plot of re-synthesized *Brassica napus* (AACC) lines from the crosses T19 × ECD11 and T19 × JL04, *Rcr8* linked SNP markers DTS12 and DTS25 were used for KASP analysis, ACDC was used as susceptible check.

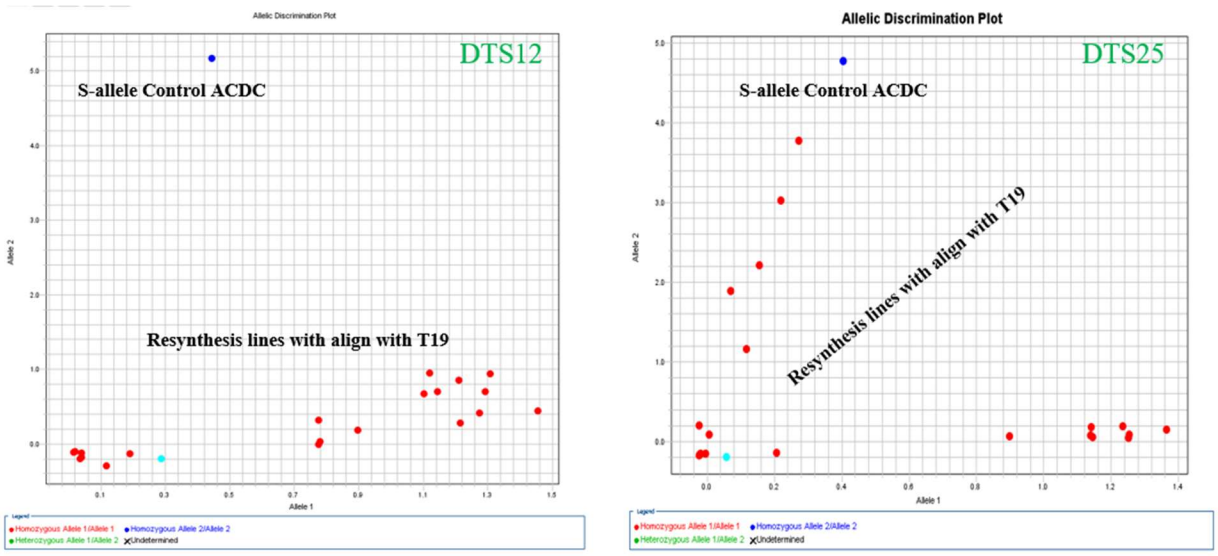


Figure 10. Allelic discrimination plot of re-synthesized *Brassica napus* (AACC) lines from the crosses T19 × ECD11 and T19 × JL04 ; *Rcr9* linked SNP marker A8-Y07 was used for KASP analysis, ACDC was used as susceptible check.

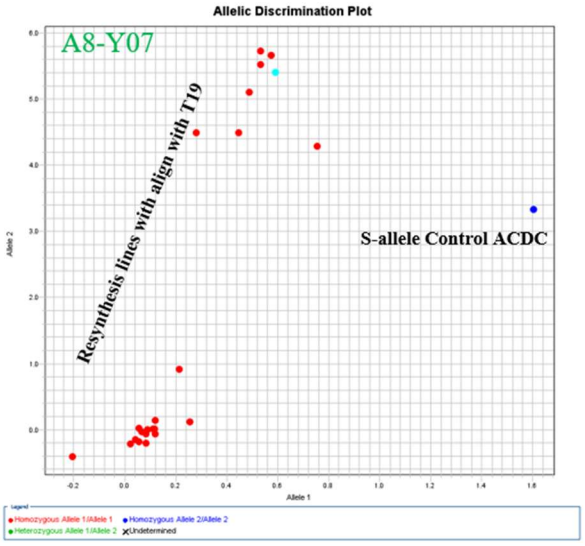


Figure 11. Allelic discrimination plot of re-synthesized *Brassica napus* (AACC) lines from the crosses T19 × ECD11 and T19 × JL04; *Rcr9* linked SNP marker M22 and M23 were used for KASP analysis, ACDC was used as susceptible check.

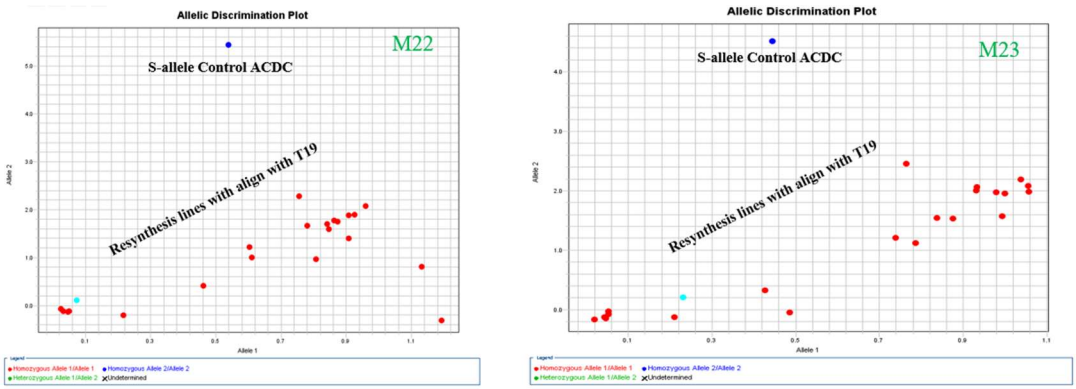


Figure 12. Allelic discrimination plot of re-synthesized *Brassica napus* (AACC) lines from the crosses T19 × ECD11 and T19 × JL04; *Rcr_ECD11_C3* linked SNP markers DC3-19, DC3-21 and DC3-22 were used for KASP analysis, DH3 was used as C-genome susceptible check

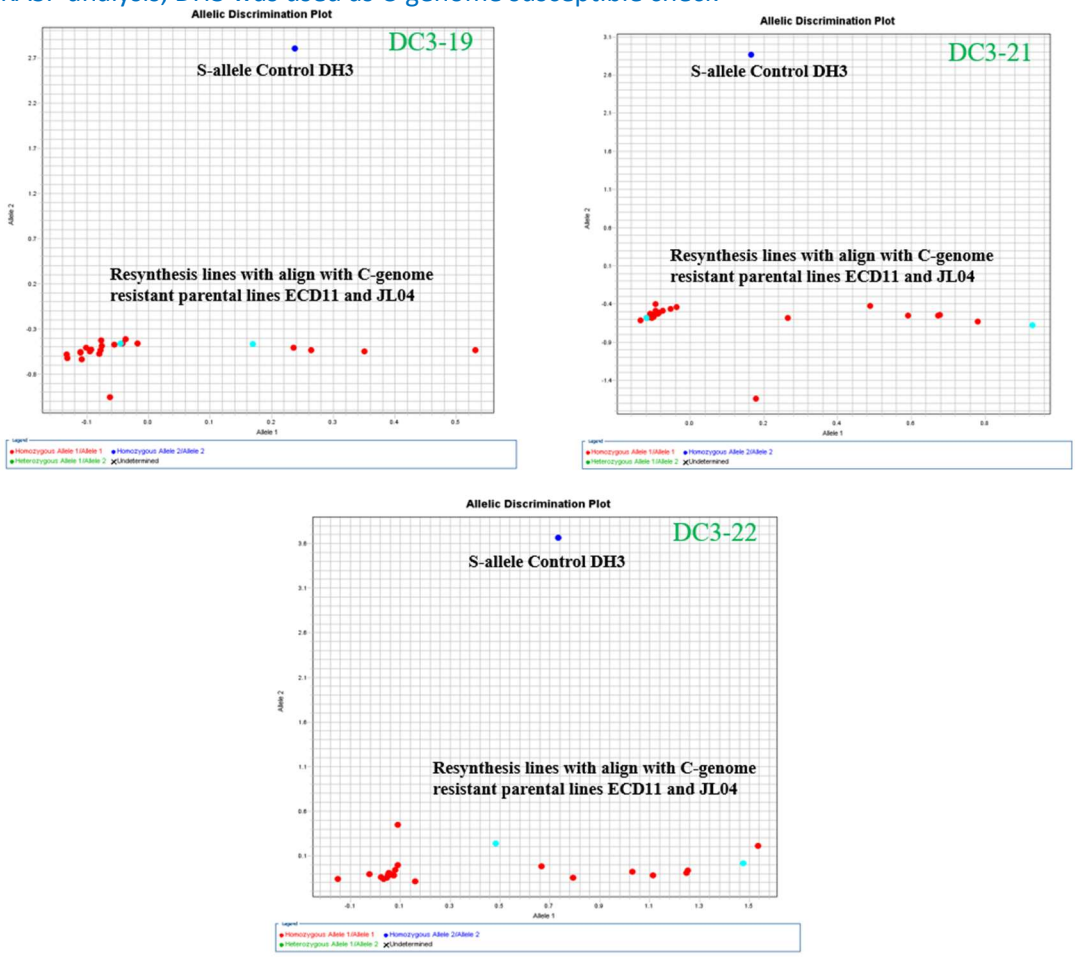
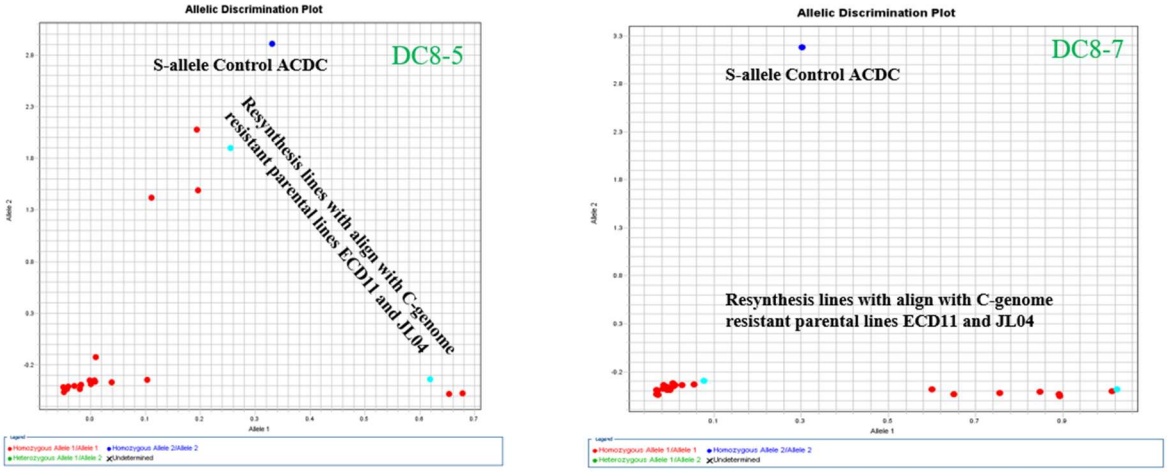


Figure 13. Allelic discrimination plot of re-synthesized *Brassica napus* (AACC) lines from the crosses T19 × ECD11 and T19 × JL04; *Rcr_ECD11_C8* linked SNP markers DC8-5 and DC8-7 were used for KASP analysis, DH3 was used as C-genome susceptible check.



Tables

Table 1. Cross ability of interspecific hybridization between *Brassica rapa* (AA) parental line T19 and *Brassica oleracea* (CC) parental lines ECD11 and JL04.

Cross combination (♀ × ♂)	No. of parental line used	Flower Pollinated (a)	Silique set (rate, %)	F1(AC) germinated	F1 (AC) survived in soil	F1-hybrid (AACC) produced seed (b)	Cross ability (b/a, %)
T19 × ECD11	T19: 6plants ECD11: 3 plants	221	137 (61.9)	13	10	04	1.8
ECD11 × T19	T19: 6 plants ECD11: 3 plants	57	26 (45.6)	3	1	0	0.0
T19 × JL04	T19: 10 plants JL04: 6 plants	269	230 (85.5)	35	34	04	1.5
Total		547	393 (78.1)	50	47	8	1.5

Table 2. Amount of seed produced from resynthesized *Brassica napus* (AACC) and test cross lines.

Plant ID	Cross combination (♀ × ♂)	Amount of seed (g)					
		F1	F2	F3	F4	F5	F6
Re-4	T19 × ECD11	Ovary culture	0.07	10 seed	0.06	No seed	No seed
Re-10	T19 × ECD11	Ovary culture	0.49	1.03	5 seed	0.41	0.07
Re-11A	T19 × ECD11	Ovary culture	7 seed	2.09	2.68	1.71	0.18
Re-11B	T19 × ECD11	Ovary culture	11 seed	1.92	0.89	4.00	1.33
TJ1-B	T19 × JL04	Ovary culture	0.09	0.51	1.00	-	-
TJ1-E	T19 × JL04	Ovary culture	0.09	0.29	0.68	-	-
TJ1-G	T19 × JL04	Ovary culture	0.09	1.05	9.08	-	-
TJ4	T19 × JL04	Ovary culture	0.01	-	-	-	-
Test cross	DHT × Re-4(F2)	3.9 g	-	-	-	-	-
	DHT × Re-10(F2)	5.0 g	-	-	-	-	-
	DHT × Re-11A(F2)	2.5 g	-	-	-	-	-
	DHT × Re-11B(F2)	4.9 g	-	-	-	-	-

Table 3. List of resynthesized *Brassica napus* (AACC) lines used for KASP analysis to confirm the presence of A and C-genome CR gene/ QTL.

Resyn. lines from T19 × ECD11	Resyn. lines from T19 × JL04	Parental lines	S allele control
Re4-1-3-1 (F4)	TJ1-B1 (F3)	T19 (A- gen. parent)	ACDC (A- gen. control)
Re4-1-3-2 (F4)	TJ1-B2 (F3)	ECD11 (C- gen. parent)	DH3 (C-gen. control)
Re4-1-3-3 (F4)	TJ1-B3 (F3)	JL04 (C- gen. parent)	
Re10-4-1 (F3)	TJ1-E1 (F3)		
Re10-4-2 (F3)	TJ1-E9 (F3)		
Re10-4-3 (F3)	TJ1-E10 (F3)		
Re11A-5-1 (F3)	TJ1-G1 (F3)		
Re11A-5-2 (F3)	TJ1-G2 (F3)		
Re11A-5-3 (F3)	TJ1-G8 (F3)		
Re11B-4-1 (F3)			
Re11B-4-2 (F3)			
Re11B-4-3 (F3)			

Table 4. List of SNP markers linked with 7 CR QTL/gene of A and C-genome used for marker confirmation in resynthesized *Brassica napus* (AACC) lines along with A and C-genome control.

Chr.	QTL/ gene name	Marker name	Position	KASP results	
				Resyn. lines from T19 × ECD11	Resyn. lines from T19 × JL04
A3	***Rcr4***	A3-8	24371044	NA	NA
		A3-12	24375572	Segregated with T19	Segregated with T19
A2	***Rcr8***	A2-Y3	18080035	Segregated with T19	Segregated with T19
		A2-Y6	18504402	Segregated with T19	Segregated with T19
		DTS-12	DM_19953009	Segregated with T19	Segregated with T19
		DTS-14	DM_20067428	NA	NA
		DTS-24	DM_21158647	NA	NA
		DTS-25	DM_22090678	Segregated with T19	Segregated with T19
A8	***Rcr3***	A8-Y07	9015777	Segregated with T19	Segregated with T19
		M12	V1.5_9997211	NA	NA
		M16	V1.5_10228875	NA	NA
A8	***Rcr9***	M22	V1.5_10659607	Segregated with T19	Segregated with T19
		M23	V1.5_10705386	Segregated with T19	Segregated with T19
		M28	V1.5_10850444	Segregated with T19	Segregated with T19
C3	***Rcr_ECD11_C3***	DC3-19	D134_11174237	Segregated with ECD11	Segregated with JL04
		DC3-20	D134_11339341	Segregated with ECD11	Segregated with JL04
		DC3-22	D134_12650068	Segregated with ECD11	Segregated with JL04
C8	***Rcr_ECD11_C8***	DC8-5	D134_23698705	Segregated with ECD11	Segregated with JL04
		DC8-6	D134_23698933	NA	NA
		DC8-7	D134_24249890	Segregated with ECD11	Segregated with JL04

5. Literature cited

Provide complete reference information for all literature cited throughout the report.

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6. Project team (max ½ page)

Describe the contribution of each member of the R&D team to the functioning of the project. Also describe any changes to the team which occurred over the course of the project.

Dr. Fengqun Yu collected materials including the parental lines, strains of *P. brassicae*, prepared the research proposal, designed the KASP primers and managed the overall project. Dr. Md Masud Karim conducted all the experiments for the project. He performed crosses, embryo rescue, colchicine treatment, seed increasing, KASP analysis and evaluating plants for resistance. Ms. Melissa Kehler assisted Dr. Md Masud Karim in conducting some of the experiments.

The team would like to thank Drs. Stephen Strelkov, Alireza Akhavan, Barbara Ziesman, Lee Anne Murphy and Xiaowei Guo for providing clubroot strains, Dr. Kevin Falk (AAFC, Saskatoon)

for *B. rapa* T19, Dr. Dr. G. R. Dixon (The University of Warwick, Wellesbourne, Warwick, UK) for *B. oleracea* ECD11 and Dr. Zhen Huang (Northwest A&F University, Yangling, Shaanxi, China) for *B. oleracea* JL04), Alberta Innovates, ACPC & Alberta Government for funding the project.

7. Benefits to the industry (max 1 page; respond to sections *a)* and *b)* separately)

a) Describe the impact of the project results on the Alberta or western Canadian agriculture and food industry (results achieved and potential short-term, medium-term and long-term outcomes).

Clubroot is a very serious problem in western Canada especially in Alberta. Using new sources of resistance is the cornerstone to control the disease. C-genome resistance had not been used in canola breeding programs when we initiated the project. We developed *B. napus* germplasm containing resistance from C-genome that can be directly used for canola breeding programs for resistance to clubroot. In addition, by pyramiding resistance genes from three race specific resistance genes *Rcr4*, *Rcr8* and *Rcr9* in *B. rapa* and two race non-specific QTLs *Rcr_ECD11_C3-1, Rcr_ECD11_C8-1* in *B. oleracea*, the re-synthesized *B. napus* lines provide new sources of *B. napus* germplasm with more durable clubroot resistance. One of the re-synthesized *B. napus* lines has been disseminated to five breeding companies (BASF, Bayer, Cargill, Corteva, Nutrien). An AAFC Plant variety/germplasm disclosure form for the two sources of the re-synthesized *B. napus* lines have been submitted.

b) Quantify the potential economic impact of the project results (*e.g.,* cost-benefit analysis, potential size of market, improvement in efficiency, etc.).

Hard numbers are lacking with respect to the total dollar value for losses associated with clubroot in Alberta, but working on the assumption that approximately one-quarter of the traditional canola growing area in this province is at risk for the disease, and using a moderate estimate of 25% yield losses, then 25% of one-quarter of the provincial canola cash receipts could be lost, totaling about $44 million per year (Strelkov et al 2015. https://www.google.ca/?gws_rd=ssl#q=2010C014R). The financial support received for this project ($41,000 per year) would represent approximately a 1,100-fold return on investment.

8. Contribution to training of highly qualified personnel (max ½ page)

Specify the number of highly qualified personnel (*e.g.*, students, post-doctoral fellows, technicians, research associates, etc.) who were trained over the course of the project.

Research funding at $41K per year for three years can support Dr. Md Masud Karim (biologist) at 0.3 FTE to work on the project at the Saskatoon Research and Development Centre, AAFC. Ms. Melissa Kehler (technician, supported by AAFC A-base fund) assisted Dr. Md Masud Karim at 0.3 FTE to conduct the experiments. They both received training in the areas of genetics, plant tissue culture and plant pathology through the project.

9. Knowledge transfer/technology transfer/commercialisation (max 1 page)

Describe how the project results were communicated to the scientific community, to industry stakeholders, and to the general public. Please ensure that you include descriptive

information, such as the date, location, etc. Organise according to the following categories as applicable:

a) Scientific publications (*e.g.*, scientific journals); attach copies of any publications as an appendix to this final report
   A manuscript on developing the re-synthesized *B. napus* line is in preparation.
b) Industry-oriented publications (*e.g.*, agribusiness trade press, popular press, etc.); attach copies of any publications as an appendix to this final report
   NA
c) Scientific presentations (*e.g.*, posters, talks, seminars, workshops, etc.); attach copies of any presentations as an appendix to this final report
   NA
d) Industry-oriented presentations (*e.g.*, posters, talks, seminars, workshops, etc.); attach copies of any presentations as an appendix to this final report
   NA
e) Media activities (*e.g.*, radio, television, internet, etc.)
   NA
f) Any commercialisation activities or patents
   1) The re-synthesized *B. napus* line Re-11B from T19 X ECD11 was disseminated to five breeding companies (BASF, Bayer, Cargill, Corteva, Nutrien) in April 2022 through the AAFC Clubroot Consortium II.
   2) A Plant variety/germplasm disclosure form for two re-synthesized *B. napus* lines Re-11A from T19 X ECD11 and TJ1-G from T19 x JL04 was submitted in March 2022.

N.B.: Any publications and/or presentations should acknowledge the contribution of each of the funders of the project, as per the investment agreement.

Section D: Project resources

1. **Provide a detailed listing of all cash revenues to the project and expenditures of project cash funds in a separate document certified by the organisation's accountant or other senior executive officer, as per the investment agreement.** Revenues should be identified by funder, if applicable. Expenditures should be classified into the following categories: personnel; travel; capital assets; supplies; communication, dissemination and linkage (CDL); and overhead (if applicable).
   Please see the financial report.
2. **Provide a justification of project expenditures and discuss any major variance (*i.e.*, ± 10%) from the budget approved by the funder(s).**
   NA
3. **Resources:**
   Provide a list of all external cash and in-kind resources which were contributed to the project.

Total resources contributed to the project		
Source	**Amount**	**Percentage of total project cost**
Agriculture Funding Consortium	$124,000	41.2%
Other government sources: Cash		%
Other government sources: In-kind (AAFC: Fengqun at 0.10 FTE, Melissa at 0.33 FTE)	$177,000	58.8%
Industry: Cash	0	%
Industry: In-kind	0	%
Total Project Cost	**$301,000**	**100%**

External resources (additional rows may be added if necessary)		
Government sources		
Name (no abbreviations unless stated in Section A3)	Amount cash	Amount in-kind
Industry sources		
Name (no abbreviations unless stated in Section A3)	Amount cash	Amount in-kind

Section E: Research Team Signatures and Authorised Representative's Approval

The Principal Investigator and an authorised representative from the Principal Investigator's organisation of employment MUST sign this form.

Research team members and an authorised representative from their organisation(s) of employment MUST also sign this form.

By signing as an authorised representative of the Principal Investigator's employing organisation and/or the research team member's(s') employing organisation(s), the undersigned hereby acknowledge submission of the information contained in this final report to the funder(s).

Principal Investigator

Principal Investigator	
Name: **Fengqun Yu**	**Title/Organisation:** **Biology Study Leader/AAFC**
Signature: Fengqun Yu Digitally signed by Fengqun Yu Date: 2022.05.18 08:38:01 -06'00'	**Date:** **May 18, 2022**
Principal Investigator's Authorised Representative's Approval	
Name: **Felicitas Katepa-Mupondwa**	**Title/Organisation:** **Director, Research and Development /AAFC**
Signature: Felicitas Katepa-Mupondwa Digitally signed by Felicitas Katepa-Mupondwa Date: 2022.05.18 16:23:41 -06'00'	**Date:** May 18, 2022

Research Team Members (add more tables as needed)

1. Team Member	
Name:	**Title/Organisation:**
Signature:	**Date:**
Team Member's Authorised Representative's Approval	
Name:	**Title/Organisation:**
Signature:	**Date:**

2. Team Member	
Name:	**Title/Organisation:**
Signature:	**Date:**
Team Member's Authorised Representative's Approval	
Name:	**Title/Organisation:**
Signature:	**Date:**

Section F: Suggested reviewers for the final report

Provide the names and contact information of four potential reviewers for this final report. The suggested reviewers should not be current collaborators. The Agriculture Funding Consortium reserves the right to choose other reviewers. Under *Section 34* of the *Freedom of Information*

and Protection Act (FOIP) reviewers must be aware that their information is being collected and used for the purpose of the external review.

Reviewer #1
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Reviewer #3
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Reviewer #4
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