Due Date

05/31/2022

Report

Project Overview

Project number:	2018F159R
Project title:	Rahman - Agronomic and seed quality improvement of the clubroot resistant canola germplasm of canola × rutabaga cross and fine mapping of the resistance gene
Project start date:	
Project completion date:	
This is an interim report for the reporting period to	

Research Team

Principal Investigator:	
Name:	**Institution:**
Habibur Rahman	University of Alberta
Research team members:	

Non-technical summary

Provide a summary of the project results which could be used by the funder(s) 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 livestock and meat industry, how they will impact industry stakeholders and/or consumers, and the economic benefits for the industry.

Among the different threats to canola (Brassica napus) production in Canada, the clubroot disease caused by Plasmodiophora brassicae is one of the most important ones. This disease not only result in a significant yield loss but also adversely affect seed oil content and increase chlorophyll content in the oil. In the last years, efforts have been made to introduce clubroot disease resistance from different

sources, such as the European winter canola, rutabaga and its allied species Brassica rapa, into canola. While introducing a trait from allied species and exotic germplasm, several undesirable traits including the traits adversely affecting crop productivity and seed quality are also introduced; therefore, repeated cycles of breeding are needed to improve canola carrying the clubroot resistance to develop a resistant cultivar. The development of a clubroot resistant canola cultivar can be greatly accelerated by the use of DNA-based molecular markers linked with the resistance gene. Thus, the key objectives of this research project were (i) to improve the performance of the canola lines carrying clubroot resistance of rutabaga (Brassica napus var. napobrassica) for the development of clubroot resistant canola cultivars, and (ii) to fine map the clubroot resistance gene by use of next-generation sequencing techniques including SNP markers for the development of markers that are tightly linked to the resistance gene.

To achieve the first objective, we carried out pedigree and doubled haploid (DH) breeding for the development of elite clubroot resistant lines and cultivars, and also converted the selected lines to cytoplasmic male sterile (CMS) lines for the development of hybrid cultivars in collaboration with the industry. Over the last four years, we grew 13,064 plants in the greenhouse and tested 11,347 early and advanced generation lines in 15,737 plots for agronomic and seed quality traits; 3,921 lines in 7,361 clubroot disease evaluation plots; seeds harvested from field plots were analyzed for oil, protein, glucosinolates, and fatty acid contents. Based on this, 33 lines were selected and used to develop CMS lines. In addition to this, in 2022, one clubroot resistant non-GMO cultivar has been entered into Public Coop trials and two RoundUp herbicide tolerant clubroot resistant cultivars have been entered into extensive Private Coop trials in collaboration with seed companies for possible registration in February 2023.

For the second objective, we followed two approaches. The first approach included the use of whole-genome resequencing (WGRS), single nucleotide polymorphism (SNP) genotyping, and construction of a linkage map for all 19 B. napus chromosomes for mapping of the clubroot resistance (CR) genes and the development of markers for this resistance, and the second approach employed transcriptome sequencing (RNA-Seq) for the identification of putative candidate genes involved in clubroot resistance and development of molecular markers based on differentially expressed genes (DEGs). Following the first approach, we delineated the location of the major CR gene of the A8 chromosome of rutabaga cv. Brookfield at about 3 cM genomic region, and thus the flanking markers of this locus can be used in breeding. This resistance has been used extensively in breeding. In addition to this major CR locus of A8, we identified an additional locus on A3 conferring resistance to P. brassicae pathotype 3 (3H) demonstrating that the rutabaga cv. Brookfield, indeed, carries two CR genes. Based on this research, we also demonstrated that the CR genes of the rutabaga cv. Brookfield does not exert any negative effect on days-to-flowering and seed quality traits.
In the second approach, RNA-Seq of the canola lines carrying clubroot resistance of the rutabaga cv. Brookfield was carried out to understand the molecular basis of clubroot resistance and develop

molecular markers based on the DEGs. RNA-seq analysis has been carried out by several researchers; however, mostly by using a single CR gene or resistance source to study host-pathogen interaction and the molecular basis of resistance. In this study, we also analyzed canola lines carrying clubroot resistance of the European winter canola cv. Mendel to carry out a comparative analysis of the DEGs upregulated in these two types of resistant lines to identify the candidate genes which might play a fundamental role in resistance and, thus, to further extend our knowledge of the molecular-genetic basis of resistance. To our knowledge, such study has so far not been conducted. Based on this study, we not only identified increased expression of the genes involved in primary and secondary metabolic pathways, but also demonstrated the importance of primary metabolism in clubroot resistance through studying the effect of trehalose, a nonreducing disaccharide which may serve as a source of energy and carbon in plants, eliciting resistance in early plant development stage. Comparative analysis of the transcriptomes based on these two types of resistances showed that the gene CRF4 might play a fundamental role in clubroot resistance. We developed SSR markers from the DEGs involved in primary and secondary metabolic pathways, and demonstrated their genetic linkage with clubroot resistance for use in breeding. We further extended our effort to understand the role of the differentially expressed (DE) long non-coding RNAs (lncRNAs) in clubroot resistance by using transcriptome sequence data from the canola lines carrying rutabaga cv. Brookfield- and Mendel-resistance, and showed that the genes involved in primary and secondary metabolic pathways, plant-pathogen interaction, and plant hormone signaling can be regulated by lncRNAs. Comparative analysis of the DE lncRNAs of the rutabaga- and Mendel-resistance identified a few DE lncRNAs that might play a fundamental role in resistance to this disease. We also demonstrated the prospect of developing molecular markers from the DE lncRNAs for use in breeding.

Thus, the elite clubroot resistant lines and cultivars developed in this project will increase the pool of clubroot resistant germplasm for sustainable production of this crop on the Canadian prairies. The knowledge of host-pathogen interaction gained and molecular markers developed in this study will increase the efficiency of canola breeding by reducing the turn-around time for cultivar development.

Project details

Project team

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

Dr. Habibur Rahman was the principal investigator of this project. Several personnel and graduate students were involved in this project in research activities carried out in the laboratory, greenhouse and growth chamber, and fields. The following personnel were involved in this project:
• Jose Salvador Lopez, Senior Field Trial Technician: Carried out field trials and seed increase in greenhouse.
• Jory Lane Underwood, Technician: Assistance in field trials, seed increase in greenhouse, and chemical analysis.
• Riley Coppicus, Technician (Jory Underwood's replacement): Assistance in field trials, seed increase in greenhouse, and chemical analysis.
• Hysent Nikang, Technician (Riley Coppicus's replacement): Assistance in field trials, seed increase in greenhouse, and chemical analysis.
• Aarohi Summanwar, PhD student: Transcriptome sequencing (RNA-Seq) and development of molecular markers.
• Jakir Hasan, PhD student: Whole-genome resequencing (WGRS), QTL mapping, and development of molecular markers.
• Aleya Ferdausi, PDF: Preparation of the final report.
• Berisso Kebede, Research Associate: Assistance in molecular marker works; doubled haploid production, and compiling data for interim and final reports.
• Karanjot Singh Gill, Research Assistant: Help in laboratory and greenhouse works.
• Junye Jiang, Summer help: Assistance in field trials and greenhouse works.
• Will Feindel, Summer student: Assistance in field trials and greenhouse works.
• Kevin Neil Lockwood, Summer help: Assistance in field trials and greenhouse works.
• Ivan Duenas, Summer help: Assistance in field trials and greenhouse works.
• Prinjiya Dhungel, Casual helper: Help in different field and greenhouse operations.
• Russell Johnson, Casual helper: Help in different field and greenhouse operations.
• An Vo, Senior Chemical Analysis Technician: Assisted in chemical analysis of the materials for oil, protein, glucosinolates and fatty acids. Also assisted graduate students and PDFs in different operations including the maintenance of the pedigree database.
• Agnes Pieracci, Finance Administrator: Project account management.
• Sandra L. Doerr, Finance Administrator: Project account management.
In addition to this, Dr. Nat Kav, Professor, Department of Agricultural, Food and Nutritional Science, University of Alberta has also been involved in this research, especially in transcriptome sequencing.

Abbreviations

Define ALL abbreviations used.

AAFC = Agriculture and Agri-Food Canada
CMS = Cytoplasmic male sterile
CON = Conventional type canola, non-GMO
CR = Clubroot resistance
DH = Doubled haploid
DEG = Differentially expressed gene
DSI = Disease severity index
ET = Ethylene
ETI = Effector-triggered immunity
FPKM = Fragments Per Kilobase pair of exon model per Million fragments mapped
GH = Greenhouse
GM = Genetically modified (by using gene from another organism)
GO = Gene ontology
IMI = Imidazoline herbicide tolerant canola
JA = Jasmonic acid
KASP = Kompetitive allele-specific PCR
lncRNA = long non-coding RNA
MAS = Marker-assisted selection
Md = Population carrying clubroot resistance gene of the winter canola cv. Mendel
MR = Moderately resistant
MS = Moderately susceptible
ncRNA = non-coding RNA
qRT-PCR = Quantitative real time polymerase chain reaction
QTL = Quantitative trait loci
R = Resistant
RP = Resistant parent
RPA = Resistant parent assembly
RR = RoundUp herbicide tolerant canola
Rt = Population carrying clubroot resistance gene of the rutabaga cv. Brookfield
S = Susceptible
SP = Susceptible parent
SDW = Sterile distilled water
SNP = Single nucleotide polymorphism
SSR = Simple sequence repeat
UofA = University of Alberta
WGRS = Whole-genome resequencing

Background

a. Review the project background and update as needed.
b. State the related scientific and development work that has been completed to date by your team and/or others.

Since the identification of clubroot disease in canola fields in Canada, extensive genetics and breeding research has been carried out by the Canola Program of the University of Alberta (UofA) and Agriculture and Agri-Food Canada (AAFC), Saskatoon. Both programs identified several clubroot resistant germplasm for use in canola breeding (Hasan et al. 2012; Peng et al. 2014). The AAFC's research mostly focused on understanding the genetic and molecular basis of clubroot resistance in B. rapa, B. oleracea and B. nigra (e.g. Huang et al. 2017, 2019; Yu et al. 2017; Dakouri et al. 2018, 2021; Chang et al. 2019; Karim et al. 2020) as well as introgression of clubroot resistance from B. rapa into Canadian canola (for review, see Rahman et al. 2014; Chu et al. 2013, 2014; Yu et al. 2022). The UofA program has been involved in breeding-research for the development of clubroot resistant B. napus canola germplasm. This program introgressed clubroot resistance from the winter canola cv. Mendel and rutabaga cv. Brookfield (Rahman et al. 2011, 2014), and recently from different B. rapa, B. oleracea and rutabaga (Hasan et al. 2021b; Wang et al. 2022; The Plant Genome, under review) into Canadian canola, and made some of these resistances available to industry to develop clubroot resistant cultivars. The program has also identified the genomic regions of different resistances including the most widely used Mendel- and Brookfield-resistance (Fredua-Agyeman and Rahman 2016; Hasan and Rahman 2016; Rahman and Franke 2019; Hasan et al. 2021c), and has developed, in collaboration with industry, the first clubroot resistant canola cultivars (PV580 GC and PV858 GC, http://provenseed.ca/) carrying stacked resistance genes for durable resistance to this disease. Among the different clubroot resistances introgressed into canola, the clubroot resistance of rutabaga cv. Brookfield (Rutabaga-BF) confers resistance to multiple pathotypes (Hasan and Rahman 2016) and, therefore, it is considered as one of the best resistances introgressed into canola. However, the flanking markers of this clubroot resistance (Hasan and Rahman 2016) often showed high recombination with resistance which is an impediment to the use of the markers in marker-assisted selection (MAS). Therefore, the development of tightly linked markers, and preferentially, from the resistance gene is needed for efficient use of this resistance in MAS.

Rutabagas produce turnip-shaped roots and require vernalization for flowering. Seeds of rutabaga contain a high level of erucic acid in the oil and glucosinolates in the meal. The use of this type of exotic germplasm in breeding often introduces undesired traits in breeding populations such as lateness of flowering and maturity, high erucic and saturated fatty acid contents in seed oil, high glucosinolate content in seed meal, etc. This is a major hindrance of using the clubroot resistant lines developed from canola × rutabaga cross in practical applications, such as the development of commercial cultivars. The clubroot resistant canola lines derived from the first cycle of breeding were late flowering and maturing, and had lower oil content than the spring canola check cultivars. Therefore, an additional cycle of breeding with this germplasm was needed for commercial utilization of the results from this research.

Objectives

a. Review the original objective(s).
b. Indicate any modifications to the objective(s) that occurred over the course of the project.

The objectives of this research project were:
1. Improvement of the agronomic and seed quality traits of the canola lines carrying clubroot resistance of the rutabaga cv. Brookfield and to develop elite canola lines for commercial use.
2. Fine mapping of the clubroot resistance of rutabaga cv. Brookfield by using next-generation sequencing techniques including whole-genome resequencing and transcriptome analysis to develop markers linked to the resistance gene.

Research design and methodology

In summary, describe the project design, methodology, laboratory and statistical analysis 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.

1. Improvement of the agronomic and seed quality traits of the canola lines carrying clubroot resistance of the rutabaga cv. Brookfield and develop elite canola lines for commercial use.

Prior to the start of this project in 2018, germplasm development in the UofA Canola Program was mostly focused on the development of clubroot resistant canola in RoundUp (RR) herbicide tolerance (gene construct RT73) background. Around this time, the TruFlex™ Roundup herbicide tolerance trait was released by Monsanto, and all seed companies started to use this trait/gene instead of RT73. Monsanto was not allowing the UofA Canola Program to use this gene. Under this situation by following advice from the seed companies, the UofA Canola Program focused on the development of clubroot resistant canola germplasm in non-GM background (conventional type, CON) so that they can be used by the companies as parents for the development of TruFlex™ Roundup herbicide tolerant hybrids. With the announcement of the patent expiry of RT73 in April 2022, the program again started to develop clubroot resistant germplasm in RT73 background as well in imidazoline (IMI) herbicide tolerance background. During this project period, the program identified another clubroot resistant rutabaga, viz. cv. Polycross (Wang et al. 2022); this resistance found to exhibit strong resistance to multiple P. brassicae pathotypes including the recently evolved ones; therefore, Polycross-resistance has also been used extensively in the development clubroot resistant germplasm.

Population development and field tests: For this, crosses were made in a greenhouse (GH), and the F1 plants were self-pollinated to produce F2 seeds. In some cases, the F1's were backcrossed to the elite

parent to develop BC1 and their self-pollinated generation populations. Some of the F1's were also used for the production of doubled haploid (DH) lines. The F2 and BC1-derived populations were grown in GH and the next generation families were grown in field nursery plots.
Field tests were carried out in three types of plots: (i) Nursery plots, where early generation (F3, DH, BC1F2) populations were tested in 1.5 × 1.3 m plots, or in 1.5 or 3.0 m single rows plots without replication. Nursery plots were mostly grown at Edmonton Research Station (South Campus) of the UofA. (ii) Yield trial plots, where F4, DH and advanced generation families/lines were grown in 5.0 × 1.8 m plots in 2 to 3 replications. Trials were grown at Edmonton Research Station (South Campus) and St. Albert Research Station of the UofA. The advanced generation (mostly F6 and advanced) lines were also tested in Saskatchewan and Manitoba in collaboration with Agriculture and Agri-Food Canada, Cybus, and Farmers Business Network. (iii) Seed increase plots, where the advanced generation lines were grown in 1.5 × 1.3 m plots at Edmonton Research Station. The advancement of generation of the selected families were carried out through self-pollination of single plants under bag isolation.
Field plots were evaluated for different agronomic traits, such as vigour at seedling and rosette stages (1-9 scale where 9 = most vigorous), days to flowering, days to maturity, plant height (cm), lodging resistance (1-9 scale where 9 = most resistant) and seed yield (kg/plot or kg/ha). Seeds harvested from the field plots were analyzed for seed oil, protein and glucosinolate contents using a Foss NIRsystem (FOSS, Hillerød, Demark) and following near-infrared spectroscopy method. The fatty acid profile of the seed oil was estimated by using a gas chromatograph (Agilent, CA, USA). All these analyses were carried out in the Analytical Laboratory of the Canola Program of the UofA; this laboratory is certified by the Canadian Grain Commission for these analyses.

Evaluation for clubroot resistance in the field: The advanced generation lines, which were included in replicated field trials or grown for seed increase in field, were tested in two P. brassicae-infested fields in Spruce Grove and Leduc, Alberta for clubroot resistance. The field in Spruce Groove carries multiple P. brassicae pathotypes including 5x (Alberta Agriculture and Forestry, personnel communication), while the field in Leduc carries mostly pathotype 3H. For this, 0.5 g seed was seeded in 3 m long single-row plot in one replication with 70 cm space between the rows; the susceptible cv. Hi-Q was seeded at every 10th row as a check. Clubroot resistance was assessed at the end of flowering by uprooting 25 plants from each row and scoring the plants on a 0-3 scale, where 0 = no gall, 1 = small galls on less than 1/3rd of the roots, 2 = small to medium-sized galls on 1/3rd to 2/3rd of the roots, and 3 = severe galling with medium to large-sized galls on more than 2/3rd of the roots. The disease severity index (DSI) of different generation families was calculated using the following formula: DSI (%) = $\sum(n \times 0+n\times 1+n\times 2+n\times 3))/(N \times 3)\times 100$, where, n is the number of plants in each disease severity class and 0, 1, 2 and 3 are the disease symptom severity classes, and N is the total number of plants. A DSI of less than 20% was considered resistant (R) and more than 70% was considered susceptible (S); while DSI of >20 to 40% and >40 to 70% were considered as moderately resistant (MR) and moderately susceptible (MS), respectively.

Greenhouse assay for clubroot resistance: Single spore isolates of P. brassicae used in this study were obtained from Dr. Stephen Strelkov, University of Alberta. These isolates were preserved in galls of the susceptible B. napus cv. Hi-Q at -18 °C until use. To prepare the inoculum of the resting spores, approximately 37 g galls were soaked in 1000 ml distilled water for 2 h and homogenized in a blender (Ninja Professional Blender 1100 W) at medium speed for 2 min. The homogenate was filtered through cheesecloth and the concentration of the spore suspension was adjusted to 1 × 107 spores/ml, and was stored at 4 °C overnight before inoculation. Inoculation was carried out at 7-10 days after germination with 1 ml inoculum following pipette method. In this method, the inoculum was pipetted at the base of the seedling, and the inoculation was repeated on the following day to ensure successful infection. Scoring of the plants for clubroot resistance and data analysis was carried out as mentioned above. The cv. Hi-Q was used as a susceptible check

Blackleg test: Tests for blackleg resistance were carried out at Edmonton Research Station (South Campus) of the UofA on the most advanced generation lines; the cv. Westar was used as a susceptible check. For this, the lines were grown in 3.0 m single rows plot with one to three replications. Leptosphaeria maculans spore suspension, at 5x107 spores/ml concentration, was purchased from InnoTech Alberta, Vegreville, and this was diluted to 1.5 liters of inoculum in 15 liters of water. The plots were inoculated either very early in the morning or very late in the evening by spraying at 4 leaf-stage, and the second inoculation was carried out 7 days after the first inoculation. Scoring for blackleg resistance was carried out at the mature stage when 20% silique were ripe by pulling 25 plants from each plot and cutting the plants at the crown of the plant. A 0 to 5 scale was used for scoring, where 0 = no disease, 1 = diseased tissue occupied up to 25% of cross-section, 2 = diseased tissue occupied 26-50% of the cross-section, 3 = diseased tissue occupied 51-75% of the cross-section, 4 = diseased tissue occupied more than 75% of the cross-section, and 5 = diseased tissue occupied 100% of the cross-section. The ratings were converted to a percent severity index for each line, and the following scale is used to describe the level of resistance in comparison to the cv. Westar: R (resistant) <30%; MR (moderately resistant) 30 – 49%; MS (moderately susceptible) 50 – 69%; and S (susceptible) 70 – 100%.

Fusarium wilt test: Test for fusarium wilt disease was carried out in a growth chamber following the Screening for Fusarium Wilt Disease in Canola – Indoor Screening (Method 1) protocol listed in Appendix B of the Procedures of the Western Canada Canola/Rapeseed Recommending Committee (WCC/RRC) incorporated for the Evaluation and Recommendation for Registration of Canola/Rapeseed Candidate Cultivars in Western Canada. For this, 10 seedlings of each line were grown in fine-grained industrial quartz sand (Granusil® 4020, Unimim Canada Ltd, Quebec) contained in a metal steamer tray which was placed in a 24°C water bath inside a growth chamber under controlled conditions (16 h photoperiod with temperatures of 22°C day/20°C night). At 10–12 days after seeding, seedlings were gently removed from the sand, roots rinsed with sterile water, and inoculated by submerging roots in a 5 × 106 conidia mL−1 spore suspension of F. oxysporum f.sp. conglutinans (kindly provided by Dr Ralph

Lange of InnoTech Alberta, Vegreville, AB) for one hour prior to re-planting in the sand. The cultivars 'Lolinda' and 'SP Banner' were used as susceptible and resistant checks, respectively. The inoculated seedlings were then returned to the growth chamber. Disease severity scoring was carried out two weeks after inoculation on a 0–9 scale, where 0 = no stunting and no disease symptoms on the seedlings; 1 = slight stunting and slight chlorosis of leaves; 3 = moderate stunting and most tissue chlorotic; 5 = moderate stunting, severe chlorosis and some necrosis; 7 = severe stunting and most tissue necrotic; 9 = dead plants. The average disease severity score for each line was calculated, and lines with a disease score of less than 3.0 were considered as resistant.

2. Fine mapping of the clubroot resistance of rutabaga cv. Brookfield by using next-generation sequencing techniques including whole-genome resequencing (including linkage map) and transcriptome analysis to develop markers linked to the resistance gene.

2.1 Whole-genome resequencing (WGRS) and linkage map-based approach

The detailed methodology of this research can be found in Hasan and Rahman (2016) and Hasan et al. (2021b), and briefly described below.

Plant materials: For this research, a DH population derived from rutabaga cv. Brookfield (Rutabaga-BF) × UA AlfaGold (Hasan and Rahman 2016) and advanced generation inbred lines derived from Rutabaga-BF × Hi-Q and Rutabaga-BF × A07-26NR crosses were used.

Greenhouse assay for clubroot resistance: A similar approach, as described above, was followed.

Whole-genome resequencing (WGRS): WGRS was carried out on three samples: Resistant parent (RP) rutabaga cv. Brookfield (Rutabaga-BF), and one resistant bulk (R-pool; bulk of 10 DH lines) and one susceptible bulk (S-pool; bulk of 10 DH lines) carrying clubroot resistance of the cv. Brookfield (Hasan and Rahman 2016). Samples were sequenced on Illumina HiSeq 2500 (Illumina Inc., San Diego, CA, USA), and data analysis, including single nucleotide polymorphism (SNP) calling and alignment to the reference genome, was done by Novogene Inc. (8801 Folsom Blvd, Sacramento, CA, USA). Based on this sequence data, a consensus whole-genome sequence for the resistant parent (RP) Rutabaga-BF was generated using B. rapa cv. Chiifu-401 whole-genome assembly v1.5 as reference. The consensus sequence assembly of the resistant parent (hereafter referred to as RP assembly, RPA) was developed by substituting B. rapa cv. Chiifu-401 v1.5 nucleotides with variants from RP. The sequence read with read-depth >7 and base quality ≥100 from R- and S-pool were aligned onto the reference-guided RPA and used to calculate their SNP index. The SNP index of the R- and S-pool was calculated using the formula: SNP index = (Number of alternate (alternate to RPA) nucleotide reads in bulk) / (Total number of nucleotides reads in bulk). Based on this SNP-index, delta (Δ) SNP-index was calculated by subtracting

the R-pool SNP-index from the S-pool SNP-index. Only those SNP positions with ΔSNP-index = −1 were considered the causal SNPs for the trait of interest. ΔSNP-index = −1 indicates that the resistant bulk allele is the same as that of the resistant parent, while the alternate base is present in the susceptible bulk, indicating the association of the SNPs with the trait.

Marker development based on WGRS data: Based on WGRS data, a total of 106 SNP-allele-specific PCR (AS-PCR) primers were designed from the chromosome region associated with resistance to P. brassicae pathotype 3 (3H). The DH population was genotyped using these AS-PCR markers. Electrophoretic separation of the PCR amplicons was conducted on 2.0% agarose gel, stained with SYBR Green 1, and then imaged with a Typhoon FLA 7000 laser scanner (GE_Healthcare 2010).

Genotyping by Brassica 60K SNP array: In addition to the above-mentioned DH lines, a total of 47 + 38 = 85 inbred lines of Rutabaga-BF × Hi-Q and Rutabaga-BF × A07-26NR crosses were genotyped by Brassica 60K Illumina Infinium SNP genotyping array (San Diego, CA, USA). Genotyping, SNP data analysis including alignment to the B. napus reference genome was done by the Delta Genomics, Edmonton, AB (http://www.deltagenomics.com/), and a total of 1,437 A-genome SNP markers polymorphic between the resistant (Rutabaga-BF) and susceptible parents (Hi-Q and A07-26NR) were identified and used for single-marker analysis.

Marker development based on 60K SNP data: The Brassica 60K SNP genotyping data was also used to design kompetitive allele-specific PCR (KASP) primers for genotyping the DH mapping population (Hasan and Rahman 2016). For this, 72 KASP markers were designed based on 72 SNPs from the A8 QTL region (Hasan and Rahman 2016). The KASP analysis, including designing the primers and genotyping the DH population, was carried out by LGC Genomics LLC (100 Cummings Centre, Beverly, MA, USA; current name LGC Biosearch Technologies; https://www.lgcgroup.com/).

Genotyping the DH population: Genotyping of the DH population (Hasan and Ragman 2916) was carried out using simple sequence repeat (SSR), AS-PCR, KASP and SNP markers. The details of the genotyping by SSR markers have been reported elsewhere by Hasan and Rahman (2016). This population was also genotyped by 24 polymorphic 24 AS-PCR markers designed based on the WGRS approach. The DH lines and their parents were also genotyped using a sub-set of 1,378 SNP from the Brassica 60K Illumina Infinium SNP genotyping array (San Diego, CA, USA) at Cargill Seed Innovation Centre (Fort Collins, CO).

Construction of a genetic linkage map: A genetic map of the DH population was constructed using the SSR, AS-PCR, KASP and SNP genotype data in the software program JoinMap v.4 (Van Ooijen 2006). A threshold recombination frequency of 0.25 and logarithm of the odds (LOD) value of 3.0 was used for grouping the loci to construct the framework map; however, a higher threshold for recombination frequency (0.35) but the same LOD (3.0) was used to integrate distorted markers in the framework map.

The genetic distance between the pairs of markers was calculated by using the Kosambi mapping function, and the linkage groups were named following the recommendation by the Multinational Brassica Genome Project (MBGP) (http://www.brassica.info/resource/maps/lg-assignments.php). The chromosome maps were illustrated with MapChart v. 2.32 (Voorrips 2002) using linkage distribution calculated by JoinMap v.4 (Van Ooijen 2006).

Quantitative trait loci (QTL) analysis: QTL analysis was conducted using the software program QTL IciMapping version 4.2 (Meng et al. 2015). To declare a QTL, the empirical threshold of LOD (p-value < 0.05) was obtained from 1,000 permutations (Churchill and Doerge 1994). The likelihood of a QTL and its effects at every 0.1 cM interval was estimated in a window of 10 cM. The QTL detected in different environments was considered the same if their C.I. (Confidence Interval) overlapped with 2-LOD (equal to 95% C.I.). On the other hand, if the C.I. of one QTL from one environment did not overlap the C.I. of a QTL in a different environment, the two QTL were considered independent.

Single marker analysis: Single marker analysis was carried out using 1,437 SNP data from the Brassica 60K array and DSI for resistance to pathotype 3 (3H) of the pedigree lines. The analysis was performed using the software program QTL IciMapping version 4.2 (Meng et al. 2015). The likelihood of association between the marker and the trait was performed following a stepwise regression probability of 0.001. To declare a marker-trait association, the empirical threshold of LOD (p-value < 0.05) was obtained from 1,000 permutations (Churchill and Doerge 1994).

Physical mapping of the genetic markers: The physical position of the marker was determined by aligning the forward and reverse primer or the marker sequences to the B. napus cv. Darmor-bzh whole-genome assembly v4.1 (Chalhoub et al. 2014; www.genoscope.cns.fr/brassicanapus) or to the B. rapa cv. Chiifu-401 whole-genome assembly v1.5 (Cheng et al. 2011; www.brassicadb.cn) by using BLASTn search tool in Brassica Database server (BRAD, http://brassicadb.cn). When the forward and reverse primer sequences aligned with the DNA sequence of a chromosome and the deduced length of the fragment between the forward and reverse primer sequences or the markers matched with the known or predicted amplicon size, the position of the marker was considered to be the physical position. The genome sequences of B. napus and B. rapa, flanked by the QTL markers, were scanned for putative candidate genes using Generic Genome Browser v2.56 in Brassica Database (BRAD, http://brassicadb.cn). The putative candidate genes identified in the Brassica genome were aligned with the Arabidopsis genome (The Arabidopsis Information Resource TAIR, http://www.arabidopsis.org) to identify the homoeologous genes with known functions.

Study of the effect of Rutabaga-BP resistance on other traits: In addition to mapping of the resistance, we also investigated the effect of the Rutabaga-BF resistance on other traits. For this, the DH population (Hasan and Rahman 2016) and their parents were evaluated in four field trials over three years at

Edmonton Research Station of the UofA. The trials were grown as two types of experiments: Direct seeding in the field (Exp. 1) or seeding in the greenhouse and transplanting the plants into the field after vernalization (Exp 2). In the case of Exp. 1, seeding was done in eight-foot-long single-row plots in three replications, and these two trials were designated as 2012NV and 2013NV. In the case of the Exp. 2, the seedlings were grown in 2-inch × 2-inch cells in a greenhouse at 21±2 ºC temperature and 16/8 h (day/night) photoperiod for about four weeks and were vernalized for eight weeks at 4 °C with an 8/16 h (day/night) photoperiod. After vernalization, the seedlings, acclimatized at 10 °C for one week and 15 °C for another week, were transplanted into the field plots. The two vernalized trials were designated as 2012V and 2016V, respectively. In the case of Exp 2, the number of plants grown in a plot was eight, and the number of replications was two to three.
Days to flowering (DTF) data were recorded as the number of days from seeding or transplanting to the date when 50% of the plants in a plot had at least one open flower. Seed oil, protein and glucosinolate contents were estimated on open-pollinated bulk seeds harvested from Exp 2 using the near-infrared spectroscopy (NIRS) method on a FOSS NirSystems 6500 (FOSS, Denmark), and data was adjusted at 8.5% seed moisture basis. Erucic acid (EA) content in seed oil was estimated following the methodology to measure methyl ester of fatty acids of the oil on an Agilent 7890a gas chromatograph (Agilent, USA).

2.2 RNA-Seq approach

To understand the genetic and molecular basis of the clubroot resistance as well as to develop molecular markers based on the differentially expressed genes (DEGs), transcriptome analysis was carried out with resistant and susceptible lines. The detailed methodology of this research can be found in Summonwar et al. (2019, 2020 and 2021); however, it is briefly described below.

Plant materials: Two doubled-haploid (DH) populations were used in this study. The first DH population (Rt) was derived from F1 of Rutabaga-BF × UA AlfaGold (Hasan and Rahman 2016). The second DH population (Md), consisting of the populations, #1333 and #1330, which carried clubroot resistance of the cv. Mendel (Fredua-Agyeman and Rahman 2016). RNA-Seq on the Mendel-resistance (Md) population, in addition to the Rt population, was carried out to identify the genes to be commonly involved in clubroot resistance. Identification of the candidate genes involved in general regulation of clubroot resistance would be a significant contribution to our knowledge; this knowledge can be used for genetic improvement of clubroot resistance through other avenues, such as gene editing. For this, a total of 12 resistant (RtR) and 12 susceptible (RtS) DH lines of the Rt population carrying Rutabaga-BF resistance, and 12 resistant (MdR) and 12 susceptible (MdS) DH lines of the Md population carrying Mendel-resistance were grown in a greenhouse and were inoculated, following the procedure described above, with P. brassicae pathotype 3 (or 3H) to obtain root tissue for RNA extraction. The two experiments were repeated three times representing three biological replicates.

Greenhouse assay for clubroot resistance: A similar approach, as described above, was followed for inoculation of the above-mentioned populations used for RNA-Seq.

RNA isolation, library preparation, sequencing, mapping to the reference genome and transcriptome assembly: Based on the histopathological study, we observed that the initial infection and disease symptom development occur 10 days after inoculation; therefore, root tissues of the susceptible and resistant lines collected at this stage were used for RNA-seq analysis. In order to accomplish this, root tissues of three seedlings from each of the 12 RtS and 12 RtR lines as well as 12 MdS and 12 MdR lines were used for RNA isolation, and the samples were sent to Novogene (USA) for library preparation and sequencing using Illumina HiSeq platform. Novogene's in-house Perl transcripts were used to remove the low-quality reads, adapter sequences and unrecognized bases. The clean reads were aligned to the B. napus reference genome v5.0 (Chalhoub et al. 2014). Expression analysis of the mRNAs for each sample was calculated based on Fragments Per Kilobase pair of exon model per Million fragments mapped (FPKM) by taking the length of the fragments and count of the mapped reads into account and using Cuffdiff (v2.1.1). The genes were considered differentially expressed if the adjusted p-value was < 0.05. To acquire the Gene Ontology (GO) annotations, enrichment analysis was carried out using GOSeq R package. GO terms with corrected p-value less than 0.05 were considered significantly enriched by the differentially expressed genes. For further annotation of the DEGs from the significantly enriched pathways, KEGG Orthology-Based Annotation System KOBAS v2.0 software (http://www.genome.jp/kegg/) was used.

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis: Expression patterns of 12 candidate genes known to be involved in plant defense responses and identified through RNA-Seq in both Rt and Md DH populations were validated using qRT-PCR. Prior to cDNA synthesis using iScript™ cDNA Synthesis Kit (Bio-Rad, USA), RNA from root tissues of the inoculated susceptible and resistant plants of both DH populations were treated with DNase. All qRT-PCR reactions were performed using PowerUP SYBR Green Master Mix from Applied Biosystem (Thermo Fisher Scientific, USA) on QuantStudio 6 Flex Real-Time PCR System (Thermo Fisher Scientific, USA). Two technical replicates of each biological replicate were analyzed. Ubiquitin conjugating enzyme 9 (UBC9), a housekeeping gene, was used as an endogenous control. Fold change of the candidate genes was calculated using the formula: $2^{-\Delta\Delta Ct}$= [(Ct gene of interest – Ct endogenous control) R – (Ct gene of interest – Ct endogenous control) S].

Comparative analysis of the DEGs of Rutabaga-BF and Mendel-resistance: Transcriptome analysis has been conducted by different researchers by using only a single resistance gene. To our knowledge, no transcriptome study has so far been conducted by using multiple resistance genes to understand the molecular basis of clubroot resistance. We carried out comparative analysis of data from the Rt and Md populations to identify the genes which could play a central role in clubroot resistance.

Investigation of the putative CR genes involved primary metabolism on clubroot resistance: It is well established that the carbon from the primary metabolic pathway generally flows to the secondary metabolic pathway for the synthesis of different secondary metabolites such as phenols, lignins, flavones, alkaloids, and stilbenes. To understand the role of sugars in resistance to P. brassicae, we examined the effect of priming the susceptible and resistant DH lines with trehalose for resistance to clubroot disease. For this, seeds of each of seven RtS and RtR DH lines (Hasan et al. 2016) were surface sterilized using 10% bleach for 15 min and 70 % ethanol for 1 min, followed by washing three times with sterile distilled water (SDW). The sterilized seeds were placed on Whatman filter paper Grade 2 (GE Healthcare; USA) in Petri dishes containing SDW (T0 sample) and trehalose sugar solution of 30 mM, 60 mM, 90 mM, and 120 mM concentration for seed priming. In total, eight seeds from each of the susceptible and resistant DH lines were grown for each trehalose treatment, and the experiment was carried out in three replications. The Petri dishes were sealed using Parafilm (Fisher Scientific, USA) and placed in an incubator at 22/15ºC temperature (day/night) with a 16 h photoperiod for five days or until germination. The germinated seedlings were inoculated with the pathogen by dipping the roots in a resting spore suspension (1 × 107 spores/ml) of P. brassicae pathotype 3 (3H) for 20 sec and were transferred to soil (Sunshine Professional Growing Mix). The plants were grown for 45 days in the greenhouse and phenotyped for resistance to clubroot disease, and the disease severity index was calculated as described above.
To evaluate the expression of the genes following trehalose treatment and pathogen challenge, six seedlings of each of the three RtS and RtR lines were used in each biological replicate, and the experiment was repeated three times. The roots of the treated seedlings were harvested at 10 dpi and flash-frozen until qRT-PCR analysis was done. Vacuolar ATP synthase subunit G1 (VHA-G1), a housekeeping gene, was used as an endogenous control for this experiment.

Development of SSRs based on RNA-Seq data: The DEGs from the RNA-seq carried out between the RtS and RtR were analyzed, and those from chromosome A8 showing upregulation in the resistant plants (<-1fold change) were selected for the development of molecular markers. For this, the candidate genes sequences were downloaded from the Brassica napus genome resource Genoscope (http://www.genoscope.cns.fr/brassicanapus/) and scanned for the presence of simple sequence repeat (SSR) motifs by using the web-based microsatellite finder tool MISA-web (https://webblast.ipk-gatersleben.de/misa/). Twenty-four primer pairs were generated for 17 SSRs from 15 DEGs located on A8 and were used to investigate their genetic linkage with resistance to P. brassicae pathotype 3 (3H) using the Rt DH population (Hasan and Rahman 2016). For this, genomic DNA of five resistant and five susceptible DH lines and their parents were used for identification of the polymorphic markers. PCR was carried out as described by Fredua-Agyeman and Rahman (2016). The polymorphic markers from A8 were used to genotype the whole DH Rt population by gel electrophoresis.
The objective of this research project was to develop markers tightly linked with CR of the rutabaga cv. Brookfield located on the A8 chromosome. The Mendel resistance located on A3 is also commonly used

in breeding, and mapping of this resistance has been reported previously (Fredua-Agyeman and Rahman 2016). To get the full benefit of the RNA-Seq, an effort was also made to develop markers based on DEGs of the Mendel-resistance population. For this, the DEGs obtained from the RNA-seq of MdS and MdR lines were analyzed and those from A3 showing upregulation in the resistant plants (<-1 fold change) were selected for the development of SSR markers. In this case, 47 primer pairs were designed from the flanking sequences of 34 DEGs and nine primer pairs were designed based on sequences of nine DEGs. All these 56 SSR markers were tested for polymorphism, and the polymorphic markers were tested on the DH populations (Fredua-Agyeman and Rahman 2016) carrying Mendel-resistance using ABI sequencer 3730 (Applied Biosystems, USA) for linkage association with the CR.

Results, discussion and conclusions

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.

1. Improvement of the agronomic and seed quality traits of the canola lines carrying clubroot resistance of the rutabaga cv. Brookfield and develop elite canola lines for commercial use.

During this project period, a total of 907 crosses were made involving canola lines carrying clubroot resistance of the rutabaga cvs. Brookfield and Polycross, and winter canola cv. Mendel to develop improved clubroot resistant canola in conventional (CON; non-GMO), or in RoundUp (RR) or Imidazoline (IMI) herbicide tolerance background. For this, pedigree breeding was mostly applied and a total of 13,064 plants, mostly of early generations (F1, F2 and F3), were grown in greenhouse (Table 1.1). Seed harvested from greenhouse were evaluated in field plots for agronomic and seed quality traits.
During the project period, a total of 11,347 different generation pedigree (F3 to advanced) and doubled haploid (DH) lines were tested in one to two replications tests in a total of 15,757 plots. There were three categories of plots: Nursery plots, where the earliest generation families (mostly F3 and DH) were grown; seed increase plots, where more advanced generation families/lines (>F4 and DH) were grown; and yield trial plots, where advanced generation families/lines (>F4 and DH) were grown. The nursery and increase plots were grown in one location with one replication, while the yield trial plots were grown in two to three replication trials. In total, 7,109 (63% of the total families/lines) lines/families were grown in nursery plots and 2,768 (24% of the total families/lines) families/lines were grown in increase plot (Table 1.2). Self-pollination of single plants by bag isolation was carried out in these plots, and a total of 15,693 plants were self-pollinated in field over the four years. A total of 1,470 (13% of the total families/lines) lines/families were tested in 5,880 yield trial plots over the four years. The early generation yield plots were grown in one to two locations (University of Alberta South Campus and St. Albert), while the advanced generation plots were grown in two to six locations. Of the total number of families/lines

tested in field trials, about 10 % were IMI herbicide tolerant type, 18% were RR herbicide tolerant type, and the remaining were conventional (CON) type (Table 1.2). All plots were evaluated for agronomic and seed quality traits (Table 1.4 to 1.15), and selection performed for these traits as well as for disease resistance.

Clubroot resistance: Over the years 2018 to 2021, a total of 3,921 lines, which also tested in yield trials and seed increase plots, were evaluated for clubroot resistance in a total of 5,322 field plots in Spruce Groove and Leduc, Alberta. The field in Spruce Groove known to carry pathotype 5X while pathotype 3H was predominant in the field in Leduc. Of the total number of lines tested, about 65% of the lines had DSI less than 30%, i.e. they were considered to be resistant, and about 30% of the lines did not show any disease symptom (DSI = 0), i.e. they were highly resistant under field conditions (Fig. 1.1). In each year, selection was performed for the resistant lines, and this reduced the occurrence of the susceptible lines to almost zero in the population tested in 2021 (Fig. 1.1).

Blackleg resistance: Field test for resistance to blackleg disease was carried out in 2019, 2020 and 2021. Disease infection in 2019 was low; therefore, most of the lines showed resistance phenotype. Of the total lines tested in 2020 and 2021, majority showed moderately resistant or moderately susceptible phenotypes; however, about 14-24% lines with disease severity less than 30%, i.e. resistant, could be selected in both 2020 and 2021 (Fig. 1.2).

Fusarium wilt resistance: Test for resistance to this disease was carried out on the candidates before entering into Private and Public Coop trials. All three lines entered into these trials were completely resistant to this disease (Table 1.19).

Cultivar and elite lines developed from this project

Based on evaluation of the breeding populations in field and greenhouse, a clubroot resistant non-GMO line UA21-15 NA has been entered into Public Coop trials (≈2nd year official trial) for registration in 2023. There is a demand for non-GMO canola in Canada and this cultivar will meet this demand. Seed yield of UA21-15 NA in Private Coop trials (≈1st year official trial) was 11% less than the hybrid cultivar 45H33; however, UA21-15 NA had 0.4% higher oil and 0.2% higher protein, and 0.3% less saturated fatty acids than 45H33 (Table 1.16). Other agronomic trait of UA21-15 NA is comparable to 45H33; UA21-15NA is also resistant to blackleg, fusarium wilt and clubroot disease.
Two clubroot resistant RoundUp herbicide tolerant lines UA21-26NR and UA21-27NR has been transferred to Farmers Business Network (FBN) through a material transfer agreement for extensive field trials (at least 12 locations) in 2022 for possible registration in 2023. These two lines were tested in Private Coop trials in 2021 where UA21-26NR gave only 5% less yield than the hybrid cultivar 45H33 (Table 1.17). Seed oil and protein contents of both lines was higher and saturated fatty acids was lower

than 45H33. Both lines were resistant to clubroot, blackleg and fusarium wilt disease.
In addition to the above-mentioned lines, a total of 33 elite lines were selected for conversion of CMS lines for the development of hybrid cultivars in collaboration with industry. This included 11 conventional, 9 Imidazoline herbicide tolerant and 13 RoundUp herbicide tolerant lines. They are in BC1 (first backcross generation) to BC3 (third backcross generation) stage of conversion to CMS line. The BC3 lines will be transferred to the seed companies in Fall 2022 through material transfer agreement for joint hybrid production in Chile in 2022-23 winter.

2. Fine mapping of the clubroot resistance of rutabaga cv. Brookfield by using of next-generation sequencing techniques including whole-genome resequencing (including linkage map) and transcriptome analysis to develop markers linked to the resistance gene.

2.1 Whole-genome resequencing (WGRS) and linkage map-based approach

Identification of the genomic region for clubroot resistance based on WGRS data: Calculation of ΔSNP-index identified 2,159 SNPs from 10 A-genome chromosomes. Among these, chromosome A8 carried 93.8% (2,025/2,159) of the SNPs indicating that the gene for resistance to pathotype 3 is present on this chromosome (Fig. 2.1.1a). Of the total number of SNPs of A8, the great majority are located at about 10.0 to 15.0 Mb region (Fig. 2.1.1b), suggesting that this genomic region carries resistance to this pathotype. Based on the highly confident SNPs from A8, 118 SNP-based AS-PCR primers (118 × 3 = 354 primers) were designed.

Linkage map construction: Of the 118 SNP AS-PCR and 72 KASP markers, 24 (20.3%) AS-PCR and 42 KASP (58.3%) markers were polymorphic between the two parents Rutabaga-BF and UA AlfaGold. The DH population was genotyped using the polymorphic 24 AS-PCR and 42 KASP markers. In the case of the SNP markers from the Brassica 60 K Illumina (San Diego, CA, USA) Infinium SNP genotyping array (genotyped at Cargill Seed Innovation Centre), 1,378 SNP probes were tested on the two parents, of which 40.28% (555/1,378) were polymorphic. Thus, the genotypic data of the 227 SSR (Hasan and Rahman 2016), 24 AS-PCR, 42 KASP and 555 SNP markers were used to construct the linkage map. Among these, a total of 585 markers, which included 108 SSR, 13 AS-PCR, 29 KASP and 435 SNP markers, could be assigned to 19 linkage groups (Fig. 2.1.2). This linkage map had a total length of 1,202.1 cM, where 648.3 cM for the A-genome and 553.8 cM for the C-genome chromosomes. The smaller size map, as compared to many of the published maps, apparently resulted from limited genome coverage for some of the chromosomes, such as A5, C6, C7 and C8. The linkage map's average marker density was 2.05 cM between the markers, where chromosome A8 had the highest density (0.83 cM). The number of marker loci in the linkage groups varied from 10 (A2) to 108 (A8), and the length varied from 24.9 cM (A5) to 104.1 cM (C3). As compared to the genetic map of A8 reported by Hasan and

Rahman (2016), the A8 of this map contained 73 additional markers (5 SSR, 13 SNP AS-PCR, 29 KASP and 26 SNP) and allowed fine mapping of the Rutabaga-BF resistance. The inclusion of a greater number of markers from this chromosome also reduced the length of this chromosome from 107.60 cM to 90.1 cM.

QTL for clubroot disease resistance: The disease reaction data of the DH lines to P. brassicae pathotypes (Hasan and Rahman 2016), estimated qualitatively (disease score '0' as resistant and scores 1, 2, and 3 as susceptible) and quantitatively (DSI ranging from 0 to 100), were used to map the QTL associated with resistance. QTL analysis using the quantitative data (DSI) identified one QTL, qCR_A8, on chromosome A8 and one QTL, qCR_A3, on chromosome A3 (Table 2.1.1). qCR_A8, located within a 3 cM region (20.4 to 22.8 cM) (Fig. 2.1.2), and explained 84.4%, 74.6%, 80.1%, 89.1% and 89.1% of the total phenotypic variance for resistance to pathotype 2, 3, 5, 6 and 8, respectively. BLAST alignment of the flanking marker sequences, UACSSR3667 and UACAS4263(T), positioned the qCR_A8 in an interval of about 3 Mb (9,303,840 to 12,229,696 bp) of the A8 of B. rapa cv. Chiifu-401 genome-assembly v1.5 (Cheng et al. 2011). The QTL qCR_A3, located within a 0.6 cM region (15.5 to16.1 cM) (Fig. 2) of chromosome A3 and explained about 12.4% of the total phenotypic variance for resistance to P. brassicae pathotype 3 (3H) (Table 2.1.1). QTL analysis using the qualitative data (resistant or susceptible) also broadly confirmed the two QTL mentioned above on A3 and A8. In all cases, the resistance alleles were derived from the parent Rutabaga-BF.

Single marker analysis of the pedigree lines, derived from Rutabaga-BF × Hi-Q and Rutabaga-BF × A07-26NR crosses, detected 17 SNP markers with LOD>10 associated with resistance to pathotype 3 (3H) (Fig. 2.1.3). These markers are positioned at 9,644,955 to 12,930,447 bp of A8, i.e. within a 3 Mb region, where the greatest number of markers is located at about 10 Mb position. This further confirms the location of the qCR_A8 QTL.

Previously, following the approach of marker analysis followed by the construction of a genetic linkage map of chromosome A8, we (Hasan and Rahman 2016) identified a genomic region of this chromosome carrying resistance to P. brassicae pathotypes 2, 3, 5, 6, and 8. In this research project, by using a genetic map of 19 B. napus chromosomes, we further confirmed the A8 QTL and reduced the flanking marker intervals to about a 3 cM region (Fig. 2.1.2), and also detected an additional QTL at about 0.6 cM region of A3 contributing resistance to pathotype 3 (3H). Pathotype 3 (3H) has been one of the most prevalent and virulent pathotypes in Canada. A recent study showed that the rutabaga cv. Brookfield also carries resistance to the newly evolved pathotypes, such as 3A and 5X (Shaikh et al. 2021). Our additional study from a different research project and using a population carrying clubroot resistance of the rutabaga cultivar Polycross (Wang et al. 2022) demonstrated that the A3 QTL together with A8 QTL plays an important role in resistance to the newly evolved pathotypes including 3A.

Previous reports have identified at least five race-specific CR loci, such as Crr1, CRs, Rcr3, Rcr9wa and qBrCR38-2, on chromosome A8 (for review, see Hasan et al. 2021a). Among these, Crr1, CRs, Rcr3 and Rcr9wa are located at about 9.6 - 11.8 Mb and qBrCR38-2 at about 18.7 - 20.2 Mb position of A8 of B.

rapa cv. Chiifu-401 whole-genome assembly v1.5 (Cheng et al. 2011) (for review, see Hasan et al. 2021a). These CR loci of chromosome A8 have been reported independently by different researchers by using different pathotypes. Therefore, the possibility of naming the same locus with different names cannot be ruled out, as has been reported in the case of CRa and CRb of chromosome A3 (Hatakeyama et al. 2017). Based on the physical position of the CR loci reported on A8, the qCR_A8 that we detected in this study would correspond to the Crr1, CRs, Rcr3 or Rcr9¬wa. Thus, the results from this study including comparison of the A8 CR locus of Rutabaga-BF with the previously reported CR loci would help canola breeders to avoid confusion with the A8 CR loci for their rational use in breeding. The chromosome A3 carries two genomic regions (about 14.4 - 15.4 Mb and 23.5 - 25.0 Mb), harbouring multiple CR loci (for review, see Hasan et al. 2021a). The 14.4 - 15.4 Mb region carries CRd, CRk and Crr3, while the 23.8 - 25.0 Mb region carries CRa/CRb, CRq, Rcr1, Rcr2, Rcr4 and Rcr5 (for review, see Hasan et al. 2021a). However, based on the flanking marker sequences of qCR_A3, which we detected in this study, this locus could be positioned at 15.1 – 15.8 Mb region of A3 of B. rapa cv. Chiifu-401 whole-genome assembly v1.5 (Cheng et al. 2011). Therefore, it is likely that the locus qCR_A3 corresponds to CRd, CRk or Crr3; thus, we demonstrated that an additional CR locus can be found in the rutabaga cv. Brookfield.

Effect of clubroot resistance on days-to-flowering and seed quality traits: The disease reaction data of the DH lines to P. brassicae pathotypes 2, 3, 5, 6 and 8, reported by Hasan and Rahman (2016), were analyzed with growth habits, days-to-flowering, oil, protein, erucic acid and glucosinolate data. Resistance to these pathotypes did not show significant correlation with the growth habit, flowering, and seed quality traits (Table 2.1.2). To further confirm this, the DH population was partitioned into resistant and susceptible types for each pathotype; and these two groups were compared for flowering and seed quality traits. Two-sample t-tests showed no significant difference between the resistant and susceptible groups for growth habit (winter or spring), days-to-flowering, seed oil, protein, glucosinolate, and erucic acid contents (Table 2.1.3). QTL mapping of clubroot resistance and agronomic and seed quality traits as well as analysis of the DH population for all these traits (Fig. 2.1.2) also demonstrated that clubroot resistance of the rutabaga cv. Brookfield does not exert any effect on vernalization requirement and days to flowering, as well as on seed oil, protein, glucosinolate and erucic acid contents.

In conclusion, by employing the WGRS approach and developing a high-density linkage map of 19 B. napus chromosomes, we located the major CR gene of the rutabaga cv. Brookfield introgressed into canola on chromosome A8 at an interval of about 3 cM region; the flanking markers (Fig. 2.1.2) (Hasan et al. 2021c) of this locus can be used in breeding. In addition to this A8 QTL, we identified an additional QTL on A3 conferring resistance to P. brassicae pathotype 3. The results from this research also demonstrated that the clubroot resistance of rutabaga cv. Brookfield does not exert any negative effect on days-to-flowering and seed quality traits.

2.2 RNA-Seq approach

Histopathological study: The DH population segregating for clubroot resistance of the rutabaga cv. Brookfield was used for the histopathological study of the infection by P. brassicae. Examination of the resistant (R) and susceptible (S) DH lines showed that infection by P. brassicae can occur in both S and R plants at 10 days post inoculation (dpi); however, the infection did not progress further in the R plants (Fig. 2.2.1). Based on this result, we focused on the root samples collected at 10 dpi for RNA-Seq.

Transcriptomic analysis (RNA-Seq): Transcriptomic analysis (RNA-Seq) was carried out on roots of the clubroot resistant and susceptible DH lines (Hasan and Rahman 2016) at 10 days post-inoculation (dpi). In case of the CR of Rutabaga-BF, a total of 7,379 genes were found to be differentially expressed in the resistant and susceptible DH lines. Among these, 3,184 genes were upregulated in the resistant DH lines of which 64 genes were uniquely expressed in the resistant lines and 38 genes in the susceptible lines (Fig. 2.2.2). Based on functional enrichment analysis, the differentially expressed genes (DEGs) associated with biosynthesis of secondary metabolites, phenylpropanoid biosynthesis and pathways of primary metabolism, especially carbon metabolism and amino acid biosynthesis, were found to be involved in mediating resistance to this disease (Fig. 2.2.3). RNA-Seq data showed that the DEGs involved in plant-pathogen interaction included the transcription factors, pathogenesis-related proteins, calcium-ion influx and respiratory burst oxidase homolog (RBOH) protein. In addition, expression of the genes encoding pathogenesis-related PR1 and PR5 proteins, disease resistance proteins, cytochrome 450 family, dehydration-responsive proteins and ethylene (ET) pathway signaling genes and many cell-wall proteins were found to be upregulated in the resistant lines. Thus, the results suggest that the defense response during primary infection (at 10 dpi) seems to be a coordinated effort between some of the proteins encoded by the DEGs of the Effector-triggered immunity (ETI) and primary metabolic pathways.

Quantitative real time-PCR (qRT-PCR) validation of gene expression: To confirm the results of the RNA-seq, 24 DEGs identified in this study that may play a role in plant defense in the RtR and MdR lines were selected for qRT-PCR assay. The selected genes belong to plant hormone signaling and phenylpropanoid pathways, encode defensins, PR genes, and the genes involved in plant-pathogen interaction. In all these cases, qRT-PCR results were consistent with gene expression data from RNAseq analysis (Fig. 2.2.4).

Comparative analysis of the DEGs of Rutabaga-BF and Mendel-resistance: Through comparative transcriptome analysis, we identified a total of ~7,500 genes differentially expressed (DE) between the RtS and RtR lines as well as between the MdS and MdR lines. Among them, ~3,500 were upregulated and ~4,000 were downregulated in both the RtR and MdR lines (Fig. 2.2.5), where 64 genes were uniquely expressed in the RtR plants, whereas 38 were expressed only in the RtS plants. Similarly, 40

genes were expressed only in the MdR plants and 86 were uniquely expressed in the MdS plants. In order to further investigate which DEGs may be responsible for clubroot resistance in both types of resistant lines, we focused on the genes that were observed to be upregulated in the RtR and MdR plants and their roles in plant defense related pathways.

Previously, we reported that the major clubroot resistance gene of the rutabaga cv. Brookfield is located on the chromosome A8 (Hasan and Rahman 2016) and the Mendel-resistance is located on A3 (Fredua-Agyeman and Rahman 2016). We annotated a total of 44 genes from A8 of the RtR lines which were upregulated and found to be involved in host-pathogen interaction (Fig. 2.2.6A); one of them is the PR disease resistance RPP5-like gene (BnaA08g20170D). This gene was upregulated only in RtR but not in MdR lines (Fig. 2.2.6A). Similarly, a total of 92 genes, which were upregulated and found to be involved in host-pathogen interaction, were annotated from A3 of the MdR lines (Fig. 2.2.6B). Among these, three genes viz. BnaA03g46200D, BnaA03g48510D and BnaA03g03830D were upregulated in the MdR lines but not in RtR lines (Fig. 2.2.6B). Furthermore, among these 92 genes, the most upregulated gene (fold change: -5.78042) was found to be an aspartic proteinase-like protein 1 (BnaA03g39150D) from A3 of the MdR lines. However, the aforementioned genes which expressed in the MdR lines, but not in the RtR lines, can be considered to be specific to MdR. Some of the genes from jasmonic acid (JA), brassinosteroid and auxin signalling pathway were upregulated in the MdR lines (Fig. 2.2.6B), whereas not a single gene from these pathways was expressed in the RtR lines (Fig. 2.2.6A). Overall, a greater number of genes involved in plant response to biotic stress were found to be expressed in the MdR lines as compared to the RtR lines (Fig. 2.2.6A and 2.2.6B).
We also identified several orthologous genes, which were upregulated in either A8 of RtR or A3 of MdR lines and were involved in biotic stress response. Six orthologous genes, viz. probable WRKY transcription factor 19, serine carboxypeptidase-like 29, serine carboxypeptidase-like 42, probable xyloglucan endotransglucosylase/hydrolase protein 18, ethylene-responsive transcription factor called “cytokinin response factor 4 (CRF4)”, and ethylene-responsive transcription factor ERF060, were identified in both A8 and A3 (Fig. 2.2.7). Among these, only the CRF4, which is an ethylene-responsive transcription factor, was upregulated in both types of resistant lines (Fig. 2.2.7). The CRF4 (BnaA08g13940D) of A8 is located at 11,929,168 – 11,930,155 bp, while the CRF4 (BnaA03g48910D) of A3 is located at 25,137,421 – 25,138,399 bp position of the B. napus genome. Thus, the upregulation of CRF4 in both resistant lines indicated that this gene might play a fundamental role in resistance to P. brassicae.

Evaluation of the canola lines treated with trehalose and expression of the defense responsive genes: Of the four concentrations of trehalose we used in this study, pooled data of the seven susceptible DH lines of the Rt (Rutabaga-BF resistance) population treated with 30 mM and 60 mM trehalose showed a partial resistance to this disease with DSI of about 45%, while the treatment with 120 mM trehalose and sterile distilled water (SDW) did not change the phenotype of the DH lines, i.e. they were completely

susceptible with DSI of 100% (Fig. 2.2.8A). The differences between the DSI values for the trehalose treatments were statistically significant ($P < 0.05$). Priming of the resistant lines with trehalose did not exert any negative effect on the growth and development of the plants, as well as on resistance to clubroot disease.
Expression analysis of six plant defense genes, viz. chitinase (CHI), pathogenesis-related 1(PR1), pathogenesis-related 2 (PR2), phenylalanine ammonia lyase (PAL), lipoxygenase (LOX) and peroxidase (PER), in response to priming of the three clubroot susceptible DH lines of the Rt population with trehalose was carried out. Among the six genes, a consistent expression of the genes in the three DH lines was found for PR2, LOX and PAL. The PR2 gene was significantly upregulated in T30 treatment as compared to the T0 treatment ($P < 0.05$). The LOX was significantly upregulated in both T30 and T60 treatments ($P < 0.05$) (Fig. 2.2.8B), while the gene PAL was upregulated in T30 treatment ($P < 0.05$). Thus, the treatment with trehalose resulted an increased expression of some of the defense related genes in T30 and T60 treatments of the susceptible lines (Fig. 2.2.8A, B). On the contrary, it was evident from the results observed in T90 and T120 treatments that the expression of these defense-related genes is statistically similar to the expression observed in the T0 treatment; however, expression of all six plant defense genes, on average, was lower in the T120 treatment as compared to the T30 and T60 treatments. Treatment of the susceptible plants with trehalose suggested that the modulation of primary metabolism can increase resistance to clubroot.

Development of SSRs based on RNA-Seq data: Of the total 7,379 DEGs identified through RNA-seq approach (Fig. 2.2.2), 465 were from chromosome A8 and 115 DEGs were found to be involved in plant defense. In total, 84 of the 465 DEGs were upregulated in the resistant DH lines of which 44 were involved in plant defense. To develop gene based SSR primers, we screened sequences of all 84 upregulated DEGs where 15 were found to carry 17 SSRs. Twenty-four gene-based SSR primer pairs were designed from these 15 upregulated DEGs for genotyping the mapping population. These 15 DEGs are located at the following positions: five at 157,029–6,790,400 bp, two at 10,299,331–10,318,442 bp, seven at 12,256,423–14,314,535 bp and one located at A08_random 1,822,640–1,824,044 bp. Among the SSR markers tested on the Rt population, the marker designed from the sequence of the DEG BnaA08g03250D (forward primer: CACGAACTATACTGGTGATCGG; reverse primer: TGGGAGACTACAACATCCACAG) based on (AGC)5 repeat exhibited polymorphism. Genotyping of 81 DH lines of this mapping population with this marker showed genetic linkage with clubroot resistance (Fig. 2.2.9A); however, 2.4% recombination occurred between the marker and resistance.
In case of Mendel-resistance, 119 DEGs from A3 were upregulated in the MdR lines; however, nine DEGs were found to contain SSRs. A total of nine primer pairs were designed from the nine DEGs; these DEGs were located at the following positions: three at 12,392,309–15,561,056 bp, four at 18,474,534–22,509,061 bp and two were located at 28,491,805–28,617,965 bp. In addition to this, 47 primer pairs were designed from the flanking sequences of 34 DEGs. All 56 (47+9) markers were tested for polymorphism between the resistant and susceptible parents of the DH mapping populations

(Fredua-Agyeman and Rahman 2016). Among these, two SSR markers designed from the sequences of the DEG BnaA03g41300D (forward primer: TCCTCCACCACCAGAGATAGA; reverse primer: GAAAGGCCAAAAGAGAGAGGA) and the DEG BnaA03g44400D (forward primer: GTTCCGTGGCTGTCTTCTTT; reverse primer: GAGGAAGAGGAGGAGGAGGA) based on sequence repeats (TCC)6 and (CAT)5, respectively, exhibited polymorphism (Fig. 2.2.9 B, C). Genotyping of the 96 DH lines of the two mapping populations showed genetic linkage of these markers with resistance. However, the marker from BnaA03g41300D showed about 3.12% recombination, while the marker from BnaA03g44400D showed about 1.04% recombination

RNA-seq has been implemented by several researchers mostly by using single clubroot resistance gene to study the transcriptomic changes that occur during the interaction between Brassica host and P. brassicae and to identify the putative candidate genes (reviewed in Summanwar et al. 2021). In this study, we conducted a comparative analysis of the DEGs upregulated in the lines carrying a major clubroot resistance gene of rutabaga located on chromosome A8 and the DEGs upregulated in the lines carrying the Mendel-resistance located on A3 to identify the candidate genes to be involved in clubroot resistance, as well as to identify the genes which might play a fundamental role in resistance and, thus, to extend our knowledge of the molecular-genetic basis of clubroot resistance. To our knowledge, such study has so far not been conducted in canola. Transcriptome analysis identified increased expression of the genes involved in primary and secondary metabolic pathways. The importance of primary metabolism in clubroot resistance was also evident from the effect of trehalose eliciting resistance in the early plant development stage. Comparative analysis of the transcriptomes from the plants carrying A8 or A3 resistances suggested that the gene CRF4 might play a fundamental role in clubroot resistance. This gene belongs to a subfamily of ERF transcription factors and is a component of the cytokinin signaling pathway; however, further study will be needed to delineate its role. The development of SSR markers from the DEGs involved in primary and secondary metabolic pathways and their genetic linkage with clubroot resistance demonstrated the potential of developing markers from gene expression studies. Thus, the results from this study provided not only information on the putative candidate genes to be involved in clubroot resistance, but also extended our knowledge of the molecular-genetic basis of resistance. Finally, the molecular markers developed from this study could be used in Brassica breeding for clubroot resistance.

3. Additional study based on transcriptome analysis: lncRNA approach

Intergenic regions in the genome have been shown to be involved in transcriptional and post-transcription regulation of gene expression. These regions are ubiquitously transcribed into RNAs and, these transcripts are classified based on their sizes and their regulatory functions. These types of functional RNAs are classified as non-coding RNAs (ncRNAs). The long ncRNA (lncRNAs) are defined to

be greater than 200 bp and have been implicated in various gene regulatory processes including the reprogramming of gene expression in plants in response to a variety of biotic and abiotic stresses (for review, see Summonwar et al. 2020a).
To get the maximum benefit of the RNA-Seq study that we carried out in this research project, we extended our investigation on the differentially expressed (DE) long non-coding transcripts. The objective of this extended research was to identify the lncRNAs involved in the regulation of clubroot resistance and to develop molecular markers based on these. The details of this study can be found in Summanwar et al. (2019 and 2020a). In this report, we present, in brief, a comparative analysis of the DE lncRNAs of the lines carrying Rutabaga-BF- and Mendel-resistance, and the prospect of the development of molecular markers based on the DE lncRNAs.

Comparative analysis of DE lncRNAs: We identified 530 DE lncRNAs in response to infection by P. brassicae, in the roots of the canola plants carrying resistance introgressed from Rutabaga-BF (A8), and 464 DE lncRNAs in the canola plants carrying Mendel-resistance (A3). Based on a comparative analysis of these two types of materials, we identified 123 (23-26%) common DE lncRNAs in these two types of resistant lines. Of the 123 DE lncRNAs, 12 (LNC_001220, LNC_000496, LNC_000801, LNC_000980, LNC_002687, LNC_002381, LNC_003929, LNC_004310, LNC_003624, LNC_003305, LNC_004780 and LNC_001968) showed a similar trend of upregulation with fold change >1 in both types of lines, i.e. the lines carrying Mendel-resistance or rutabaga-resistance; therefore, these lncRNAs might play an important role in resistance to clubroot disease. Among them, 10 lncRNAs (excluding LNC_002687 and LNC_004310) were predicted to regulate the genes involved in different defense-related pathways (Fig. 3.1a), while the two, LNC_002687 and LNC_004310, are predicted to regulate only the neighbouring genes. qRT-PCR analysis further confirmed the upregulation of these lncRNAs and their target genes in the lines carrying Mendel-resistance or rutabaga-resistance in response to infection by P. brassicae at 10 dpi (Fig. 3.1b).

Identification of simple sequence repeats (SSRs) within the DE lncRNAs and development of molecular markers: To demonstrate the prospects of developing SSR markers from the DE lncRNAs associated with clubroot resistance, we used one of the two populations used in RNA-Seq study. For this, we choose the Mendel-resistance population as resistance in this population controlled by a single locus located on A3 (rutabaga resistance controlled by a major locus on A8 and a minor locus on A3). For this, we analysed all the 464 DE lncRNA sequences identified by using Mendel-resistance for the presence of SSRs. Among these, 196 sequences, which were distributed throughout the 19 B. napus chromosomes (Fig. 3.2), contained a total of 269 SSR motifs, where 57 sequences contained more than one SSR motif. The proportion of mononucleotide SSRs (70.6%) was greater than the di-, tri-, and tetranucleotide SSRs (Fig. 3.3a,b), while no penta- or hexanucleotide SSRs could be detected; 22 were identified as compound SSRs (Fig. 3.3b). Among the dinucleotide repeats, the AT repeats were the most frequent (Fig. 3.3c). Thus, the results from this study demonstrated that the DE lncRNAs, in response to infection by P.

brassicae, contain SSRs that can be used in molecular breeding.
To study the linkage association between the SSRs from the DE lncRNAs with Mendel-resistance, we focused on the SSRs from the DE lncRNAs of the chromosome A3. A total of 17 SSRs could be identified within 13 lncRNAs located on this chromosome; this included 12 mononucleotides, four dinucleotides, and one compound SSR (Fig. 3.3d). Of the 13 lncRNAs, LNC_000318 and LNC_000424 contained two and four SSRs, respectively, while all others contained only one SSR. A total of 18 primer pairs were designed based on seven mononucleotides, four dinucleotides, and one compound SSR, and they were tested for polymorphism; among these, the marker designed based on the compound SSR [(TA)12(T)12] identified within the LNC_000424 was found to be polymorphic. Genotyping of the 153 DH lines of the mapping populations (Popl#1330 and Popl#1333) carrying Mendel-resistance with this marker showed an association with clubroot resistance (Fig. 3.4); however, this association could not be established in two lines of Popl#1330 and one line of Popl#1333. Thus, this SSR marker can potentially be used in marker-assisted breeding for identification of the resistant plants with an accuracy of greater than 98%. This marker can also be for the pyramiding of multiple genes including A8-resistance to develop clubroot-resistant canola cultivars; however, recombination between the marker and the resistance needs to be taken into account.

Thus, the development of an SSR marker from the lncRNA LNC_000424 and the establishment of its association with clubroot resistance has paved the way for exploring the Brassica lncRNAs for use in breeding. Expression analysis showed that LNC_000424 was slightly upregulated in the resistant plants. This lncRNA is located at 22,295,257-22,297,928 bp position of A3 of the B. napus genome (Genoscope B. napus Genome Browser, http://www.genoscope.cns.fr/ brassicanapus/), and the SSR occurred at 22,295,388-22,295,423 bp position; the primer pairs of the SSR marker from this lncRNA exhibiting association with clubroot resistance are located at 22,295,341 and 22,295,531 bp position of A3. The clubroot resistance locus CRa (GenBank accession No. AB751516; Ueno et al. 2012), which is probably present in the cv. Mendel (Fredua-Agyeman and Rahman 2016), is located at 22,862,712-22,873,697 bp on the A3 of the B. napus genome (Genoscope B. napus Genome Browser, http://www.genoscope.cns.fr/brassicanapus/). The physical distance between the lncRNA-based SSR marker that we developed in this study and the CRa locus is about 0.56 Mb. Through bioinformatics analysis, we predicted that the lncRNA LNC_000424 transregulates 45 target genes and cis-regulates 27 target genes (Fig. 3.4); some of the target genes trans-regulated by this lncRNA are involved in plant defense (Fig. 3.4). However, the failure of the marker designed from this lncRNA to detect the resistance phenotype in about 2% of cases indicated that this lncRNA might not be involved in the regulation of clubroot resistance in Brassica. It is possible that this lncRNA is located about 2 cM away from the clubroot resistance gene, and this might have resulted in the observed recombination between the marker and the resistance phenotype. Further investigation would be needed to confirm the possible role of this lncRNA in mediating resistance to clubroot disease in B. napus.

The following research papers published from this research project:

Hasan J, Shaikh R, Megha S, Harrmann DT, Kebede B, Rahman H, 2021. Mapping of flowering time, seed quality and clubroot resistance in rutabaga × spring canola populations and their association. Euphytica 217:160.

Hasan J, Megha S, Rahman H, 2021. Clubroot in Brassica: Recent advances in genomics, breeding and disease management. Genome 64:735-760.

Shaikh R, Farid M, Rahman H. 2021. Inheritance of resistance to the newly evolved Plasmodiophora brassicae pathotypes in Brassica napus L. Can J Plant Path 43:256–266.

Summanwar A, Farid M, Basu U, Kav N, Rahman H, 2021. Comparative transcriptome analysis of canola carrying clubroot resistance from 'Mendel' or Rutabaga and the development of molecular markers. Physiological and Molecular Plant Pathology 114:101640.

Summanwar A, Basu U, Kav N, Rahman H, 2021. Identification of lncRNAs in response to infection by Plasmodiophora brassicae in Brassica napus and development of lncRNA-based SSR markers. Genome 64:547-566.

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The following PhD thesis evolved from this research project:

Hasan MJ, 2021. Genetics of clubroot (Plasmodiophora brassicae) disease resistance in Brassica. A PhD thesis, submitted to the University of Alberta. No. pages = 256.

Summanwar A, 2020. Transcriptomic responses in spring canola carrying clubroot resistance introgressed from rutabaga or “Mendel”. A PhD thesis, submitted to the University of Alberta. No. pages = 186.

The following cultivars and lines developed from this research project:
• One conventional (non-GMP) and two RoundUp tolerant clubroot resistant cultivars expected to be registered in February 2023.

• Thirty-three CMS lines for the development of hybrid cultivars in collaboration with industry.
• A few thousand early and advanced generation clubroot resistant canola lines.

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

Literature cited

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

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Benefits to the industry

a. Describe the impact of the project results on Alberta's agriculture and food industry (results achieved and potential short-term, medium-term and long-term outcomes).
b. Quantify the potential economic impact of the project results (e.g., cost-benefit analysis, potential size of market, improvement in efficiency, etc.).

The crop canola (B. napus) contributes about $29.9 billion to the economic activity in Canada. Among the different threats to canola production in Canada, the clubroot disease is one of the most important ones. This disease can cause a yield loss of about 30% (Tewari et al. 2005). Aside from yield loss, clubroot can result in 2-6% decrease in seed oil content (Engqvist 1994; Pageau et al. 2006) and about 50% increase in chlorophyll content in oil (Engqvist 1994). Therefore, the development of clubroot resistant canola cultivars will secure the production of this crop and also maintain the quality standard of the Canadian canola.

The clubroot resistance of the European winter canola cv. Mendel has been introgressed into Canadian spring canola by different seed companies as well as by the UofA Canola Program (Rahman et al. 2011, 2014), and this resistance has been used extensively for the development of clubroot resistant canola cultivars for Canada. However, a breakdown of this resistance has been reported after growing a cultivar carrying a single resistance gene for a few years in an infested field (Strelkov et al. 2016). Clubroot resistance has also been introgressed from other sources into Canadian canola and used in breeding. Among the different sources of clubroot resistance used in Canada, the resistance of the rutabaga cv. Brookfield, introgressed by the UofA Canola Program, is considered as one of the best resistances conferring resistance to multiple pathotypes (Hasan and Rahman 2016; Shaikh et al. 2021). This resistance has been made available to the industry to develop clubroot resistant canola cultivars, such as PV585GC. Recently, the canola program has also introgressed another resistance from a rutabaga cv. Polycross; this resistance has been found to be even better than the Brookfield-resistance (Wang et al. 2022). In this regard, the development of elite canola lines carrying clubroot resistance of the rutabaga cvs. Brookfield and Polycross and the development of canola cultivars and hybrid parent lines carrying these resistances will increase the diversity of the clubroot resistance genes in Canadian canola, ensuring sustainable production of this important crop on the prairies.

The knowledge of the molecular basis of clubroot resistance and putative candidate genes that we identified in this research project would enable researchers to develop other strategies, such as genome editing, to combat this disease. The knowledge of the genetic basis of clubroot resistance and the molecular markers that we developed would enable canola breeders to employ marker-assisted selection, and thus, accelerate breeding to develop elite canola lines and cultivars resistant to this disease. Molecular markers developed from this project will also help breeders to pyramid the clubroot resistance genes of the rutabaga cv. Brookfield with other clubroot resistance genes for the development of hybrid canola cultivars carrying multiple clubroot resistance genes for durable resistance to this disease in this crop. Pyramiding of multiple clubroot resistance genes in Chinese cabbage has been shown to confer robust resistance to multiple isolates of P. brassicae (Matsumoto et al. 2012).

Performance Measures

Collaboration and partnerships

# of Industry partners: 5

# of Public partners: 2

# of international partners: 1

Training of Highly Qualified Personnel (HQP)

# of Undergraduate students trained: 1

# of graduate students trained: 2

# of postdoctoral fellows trained: 1

# of research associates trained: 1

# of technicians trained: 5

Others (Such as Visiting scientist): 0

Technology Transfer & Commercialization

# of Peer reviewed scientific publications: 7

# of scientific presentations, posters and abstracts:	11
# of industry communications:	4
# of patents and licences:	0
# of new innovations / products / practices:	3

Project resources

Statement of revenues and expenditures

In a separate document certified by the organisation's accountant or other senior executive officer, provide a detailed listing of all cash revenues to the project and expenditures of project cash funds. 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).

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RES0040694_(M)_SAE_Mar2018_-Feb2022.pdf
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Total Files: 2

Provide a justification of project expenditures and discuss any major variance (i.e., ± 10%) from the budget approved by the funder(s).

For this project, a total of $522,000 was used over four years, and this was funded by RDAR with $241,000 and ACPC/AI with $281,000. The budget under different categories was:
Personnel = $408,000;
Supplies = $105,000;
CDL & Travel = $9,000.
We considered the total amount from RDAR, ACPC and AI as one pot of money to be used for this research. The total expenditure was:
Personnel = $323,882.52;
Supplies = $194,962.48;
CDL & Travel = $3,155.00.
Thus, $84,117.48 was less spent for salary, while $89,962.48 was overspent for supplies. The reasons for the difference in salary line was primarily due to the use of less time of the Research Associate (RA) and more time of the technicians which was needed to carry out field trials with a large number of materials and growing the materials in greenhouse; and sharing the PhD student's salary with Dr. Nat Kav. Thus, saving from RA and grad student's salary resulted this less spending in the salary line. On the other hand, almost a similar amount of money was overspent in supply line, which was primarily due to expenditures associated with field trials and greenhouse propagation in 4th year of the project (no cost extension) as well as increased user fees for the use of land, lab and greenhouse. Of the total budget for publication and travel, a total of $3,155 was used. This was partly due to COVID-19 travel restrictions, and sharing of publication fee with Dr. Kav, as well as for publishing in journals which does not require a page charge.

Resources

Provide a list of all external cash and in-kind resources which were contributed to the project.

External sources of funding for the entire project.

Clearly indicate any changes to confirmed sources of funding as well as any new sources of funding.

Government Sources

Industry Sources

Attachments

Attachments

Please attach any supplemental documents

2018F159R_-_Rutabaga_CR_-_Tables_and_Figures.docx
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Genome_2021_-_Clubroot_resistance_Review.pdf
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Genome_2021_-_lnc-RNA_Mendel_vs_Rutabaga___Mol_marker.pdf
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Mol_Plant_Microbe_Interactions_2019_-_LncRNA_Rutabaga_CR.pdf
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Physiol_Mol_Plant_Path_2021_-_RNA-Seq_Mendel___Rutabaga_CR.pdf
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Plant_Sci_2020_-_Lnc-RNA_Review_-_Summanwar_et_al.pdf
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Total Files: 8

Does your agreement with RDAR include funds from the Canadian Agricultural Partnership (CAP) Program?

No

RPO Comments

Revisions Required