Canola Agronomic Research Program (CARP)
FINAL REPORT

The Final Report should fully describe the work completed for the year, noting the personnel involved and any deviations from the original plan and next and/or corrective steps as may be required if deviations are noted. A final financial statement summary of expenses must also be submitted. In the event of major changes within the budget, supporting notes are required. The final report is a summary of activity for the final year and an overview of the entire project.

Project Title: ***Towards better understanding of genetics in Leptosphaeria-Brassica interactions via international collaborations to standardize the nomenclature of blackleg resistance genes***

Research Team Information

Lead Researcher:		
Name	***Institution***	***Project Role***
Hossein Borhan	AAFC Saskatoon	Principal Investigator
Research Team Members (add rows as required)		
Name	***Institution***	***Project Role***
Gary Peng	AAFC Saskatoon	Co-Investigator
Dilantha Fernando	University of Manitoba	Co-Investigator

Project Start Date: April 1, 2019 **Project Completion Date:** March 31, 2024

Reporting Period: April 1, 2023 **to** March 31, 2024

CARP Project Number: **2019.27**

Instructions: This Final Project Report shall be completed and submitted on or about February 15th of the fiscal year that the agreement is in effect (upon completion of the project). The Lead Researcher of the project in question shall complete and submit the report on behalf of his/her complete research team.

This Report is a means by which to provide a detailed account upon completion of the project. Final project financial reporting should be provided at this time.

The following template is provided to assist you in completing this task. Please forward the completed document electronically to the CCC contact listed below.

In addition to the Final Research Report, a *Final Research Abstract/Extension Report* is due upon completion of the project, maximum 2-3 pages, to be used for publication on the Funders' websites and in the *Canola Digest*. Content will be used in extension material, for consumers and/or industry. Include an Executive Summary, brief project description, key findings and conclusions (with a summary graph/table or supporting image for the project), translation of key findings into best management practices and/or relevance to the canola sector and future research, and funding acknowledgment as determined in the grant award letter. The Final Extension Report

is intended to support messaging to all audiences. Information needs to be clear, concise and in "grower-friendly" language.

Please include the funding acknowledgements outlined in your research agreement in all deliverables (publications, presentations, etc.) from this project.

1. Date of completion & status of activity (please check one)

Date of completion: March 31, 2024

☐ **Ahead of Schedule** ☐ **On Schedule** ☐ **Behind Schedule** ☐ **Completed**

Comments: Project completed

2. Summary - Maximum of one page. This must include project objectives, results, and conclusions.

Blackleg caused by the ascomycete fungus *Leptosphaeria maculans* (*Lm*) occurs in most canola (*Brassica napus*) growing countries. To combat the threat of this economically important disease, governments and private industry have supported blackleg research for the past several decades. Researchers in Canada, France, the UK and Australia have conducted multi-faceted research programs covering genetics and genomics of host plant (*B. napus*) resistance and pathogen (*Lm*) virulence as well as study of pathogen epidemiology and evolution (1). Despite significant research achievements of the international blackleg community, there has not been a coordinated effort to standardize research terminologies and protocols or to create well defined common genetic resources for the pathogen and the host. To address this issue, the present project aimed at developing a common nomenclature for referring to plant resistance (R) genes, pathogen Avr genes and creating a common set of genetic material through collaboration and coordination of the international blackleg research community.

Through collaboration with INRA scientist in France we introduced gene naming convention that incorporates locus information alongside phenotypic interactions, departing from previous naming practices solely based on phenotypic characteristics. For example *L. maculans* Avr genes *AvrLmS* and *AvrLep2* that were proved to be allelic have now been renamed as *AvrLmS-Lep2*. We have also expanded the Brassica differentials to total of 12 lines by generating and making publicly available Topas introgression lines *Rlm7, Rlm9, Rlm11, LepR5 and LepR6*. Through collaboration between our labs, we have defined the Avr gene profile of close to 200 *Lm* isolates using both phenotyping and molecular markers. We recently introduced a marker that determines the *AvrLms-Lep2* genotype. Our Australian collaborators have used the sequence of *LepR3, Rlm2, RLm4 , Rlm7* and *Rlm9* (2-5) to develop R gene specific molecular markers (6). Additionally, we have developed specific and nonspecific markers for the *LepR1* gene. These markers have been used to verify the presence of the R genes in Canadian canola varieties. In another project led by Dr. van de Wouw from Australia a collection of 250 Lm isolates were made available to public research institutions (7). We have also established the CRISPR-Case gene editing system first reported for *L. maculans* by our Australian colleagues Alexander Idnurm (8) to engineer *Lm* isolates with desired features for research that so far has not been identified from natural collections. We have generated a genome sequence synteny viewer for 58 *Lm* isolates. Another set of valuable data contributed by us is the profile of R genes in a collection of *B. napus* accessions available through gene-banks (e.g. PGRC) and canola breeding companies.

3. Introduction – Brief project background, rationale, and objectives.

Genetic resistance is the best approach to control the blackleg disease of canola caused by the fungus *Leptosphaeria maculans* (*Lm*) (1). However, other disease management practices such as crop rotation and *Lm* race surveillance are also important in reducing the disease pressure and selecting the best R-rated canola

cultivars. To date twenty one race-specific resistance (R) genes (*Rlm* or *LepR*) have been reported. Similarly, the corresponding effector (virulence) genes from various Lm isolates have been identified. With the advances in genome sequencing, databases of Brassica accessions and *Lm* isolates genome sequences, have been generated by different research labs. Canadian and international blackleg research groups have made their own collection of Brassica and *Leptospaheria* differential sets. While some of these data and resources are publicly accessible, generating a common set of host and pathogen genetic materials and databases could help standardize research resources among international blackleg researchers, canola pathologists, and agronomists. This project aims to help with the better coordination of blackleg research and dissemination of information to ensure future success in blackleg research and to provide canola farmers with the best disease management tools. Below is a summary of achievement(s) for each objective:

1- To expand the current *B. napus* host differential lines

2- To generate a common set of *L. maculans* differential isolates
3- To genotype the R gene(s) of current canola varieties in Canada, as well as *B. napus* collections labeled as resistant to blackleg disease
4- To create a common *B. napus-Leptosphaeria* database of genetics and genomics data as well as protocols.

4. Methods – Include approaches, experimental design, methodology, materials, sites, etc. Major changes from original plan should be cited and the reason(s) for the change should be specified.

Pathology test

Seedlings were germinated in 32-cell trays in a controlled growth chamber (20°C, 16 h days (light intensity c. 450 μmol m−2 s−1 at bench level) and 18°C, 8 h nights). Cotyledons of one week old seedlings were inoculated with a spore suspension of *L. maculans* (2 × 105 pycnidiospores per wound site, eight plants per test) and were rated 14 d postinfection. The resistance phenotype of the seedlings was rated using a continuous 0–9 scale modified after. A score of 0–4 was classified as 'resistant', 5 as 'intermediate' and 6–9 as 'susceptible'.

Genotyping *L. maculans* isolates

To determine the genotype of *L. maculans* isolates, each isolates were inoculated on 5-7 days seedlings of Brassica differential lines with known R genes and plant response to *Lm* infection was scored as described above. Genotyping was also confirmed by PCR markers and genome sequencing.

Develop a new set of international *L. maculans* isolates by including Canadian *L. maculans* isolates collected in Western Canada:

To collect *L. maculans* isolates from Manitoba, Alberta and Saskatchewan, the stubble samples were collected from canola fields where blackleg was prominent. Pure cultures of potential *L. maculans* isolates were prepared and verified through genotyping and phenotyping in the greenhouse. Finally the genotyping and phenotyping data were combined and the full Avr profile was determined for each isolate. Once the Avr profiles were determined a selected *L. maculans* isolates were sent to the Australian group (Dr. Van de Wouw group) for validating the Avr profiling, whole genome sequencing and including Canadian isolates into the newly developed international *L. maculans* set.

Finally, these isolates were phenotyped and genotyped for validation and used for whole genome sequence analysis along with other *L. maculans* isolates originally collected from different regions of the world such as the United States, Europe, and Australia. Furthermore, these isolates were used for population analysis, determination of genetic relationships between isolates and the distribution of Avr genes globally.

Production of Introgression Lines

F_1 seed was produced by first, crossing the resistant donor parent lines to the DH Topas DH16516. F_1 seedlings were assayed for resistance to *L. maculans* isolates. A single resistant F_1 selection was then backcrossed to the susceptible *B. napus* Topas DH16516 line to produce BC_1F_1 seeds. Recurrent backcrossing and selection, both for resistance and spring-type growth habit (where applicable), was performed to produce BC_5F_1 seedlings, which were selfed through three additional generations. The selfed lines were tested for homozygous resistance and a final single-plant selection was made.

CRISPR-Cas gene editing

Guide-RNA (gRNA) flanking the gene of interest were delivered with Cas9 and a Hygromycin (Hyg) cassette to *L. maculans*. Transformants were selected on PDA media with Hyg antibiotic selection and confirmed with PCR using primers flanking the mutated locus.

Screening of R genes in Canadian canola varieties

A total of 52 canola varieties provided by seven Canadian breeding companies were tested for the presence of *LepR3*, *Rlm2*, *RLm4* , *Rlm7*, *Rlm9* and *LepR1* using markers.

5. Results – Present and discuss project results, including data, graphs, models, maps, design, and technology development.

Activity 1- To expand the current *B. napus* host differential lines.

As reported previously we have generated five new host differential lines in the B. napus cultivar Topas genotype. Three of these lines are Topas-*Rlm7*, Topas-*Rlm9*, Topas-*Rlm11*, Topas-*LepR5* and Topas-*LepR6* with the three formers are provided to public and private research labs (Larkan &Borhan; unpublished). The Topas *Rlm-LepR* introgression lines consist all the genetically well defined and available Brassica R genes that were identified by our lab (total of 12 lines) (9).

Activity 2- To generate a common set of *L. maculans* differential isolates.
The collection of 250 *Lm* isolates coordinated by Dr. van de Wouw provides a naturally divers Lm isolates collection. We characterized the Avr profile of seven *Lm* field isolates sent to Borhan's lab by Dr. Franco Rossi at the Biotechnology Research Institute of Brazil at Chascomus. In addition we genotyped six IBCN isolates that exist in our lab isolates collection marked as possible *L. maculans* that were determined to be *L. biglobosa* (Appendix Table 1).

The *L. maculans* isolates that we have sent for the collection of international set had multiple Avrs on their profile and all their Avr profiles and mating type information can be found in Van de Wouw et al. (7).
The frequency of each Avr allele of *L. maculans* isolates over the world including Canada was determined (Figure 1) (7).

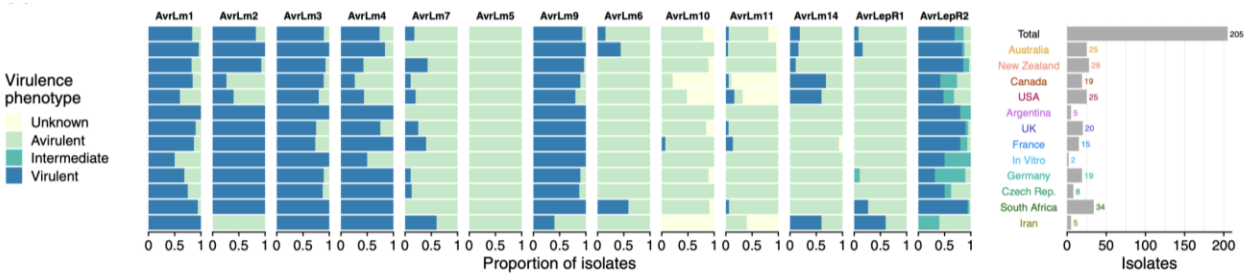


Figure 1:The frequency of virulence phenotypes in L. maculans for each country (7).

According to Figure 1, most of the isolates are virulent mostly on *Rlm1,2,3,4,9* and *LepR2* in many countries however, the frequency of some avirulence genes is varied among the countries. For example, the frequency of *Avrlm2* is higher in Canada, USA, and Iran whereas it is much lower in other countries such as Australia and New Zealand. In Canada, there is a less frequency of *AvrLm3* in *L. maculans* population proving the breakdown of *Rlm3* in Canadian prairies.
The *L. maculans* isolates were used in principal component analysis (PCA) (Figure 2) and found that Canadian isolates are clustered with *L. maculans* isolates from the United States (Figure 2).

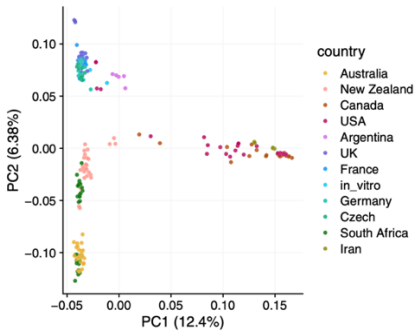


Figure 2: The analysis of population structure through principal component analysis (7).

Furthermore, all the *L. maculans* isolates were subjected to analysis of genomic relationship matrix and found a same pattern as in the principal component analysis (Figure 2). According to the genomic relationship matrix, the Canadian isolates were closely grouped with the United States isolates (Figure 3).

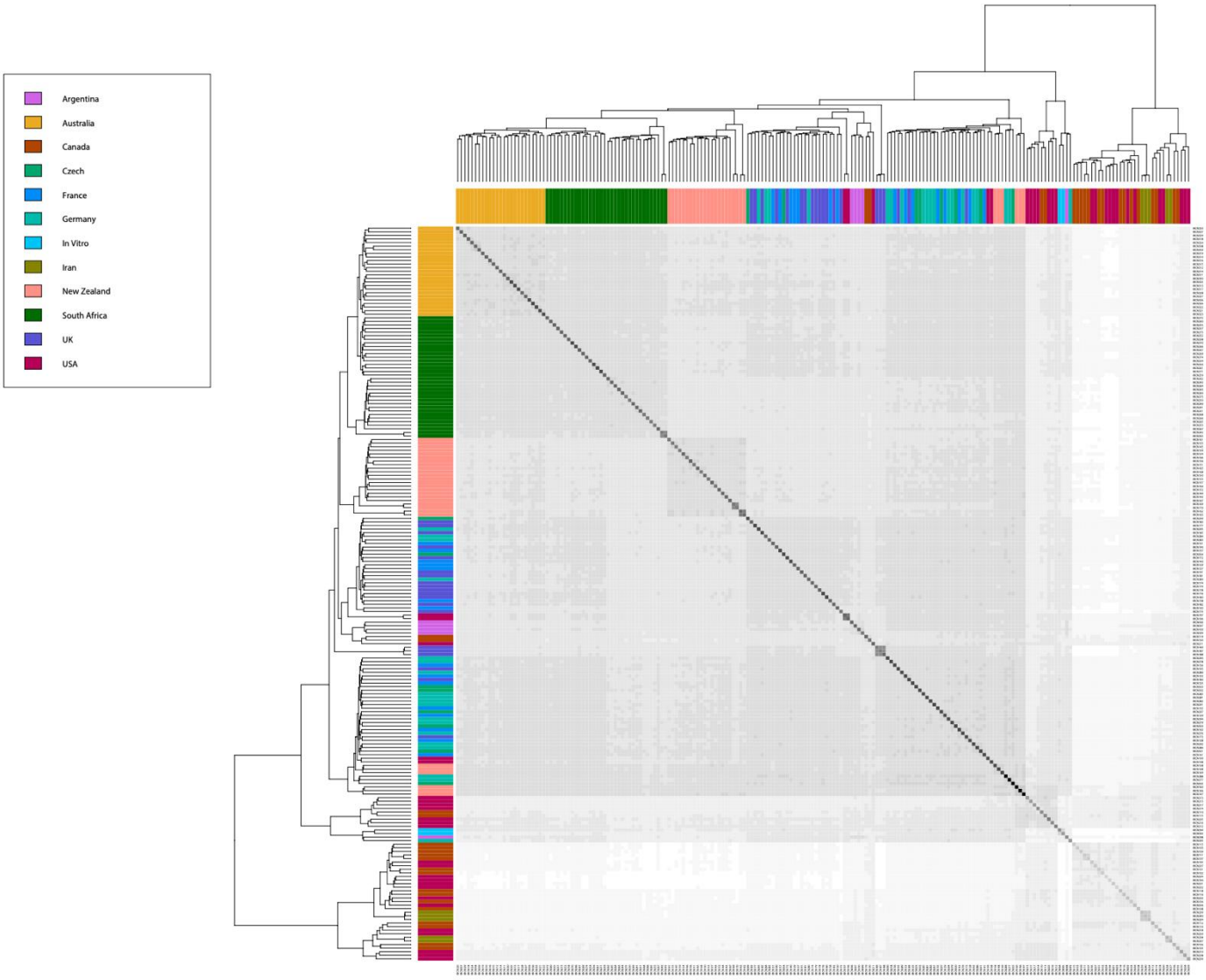


Figure 3: Analysis of the genomic relationship matrix on new international set of L. maculans isolates (7).

Blackleg researchers often require isolates that lack all or most of the Avr genes that are rare or impossible to find within the natural population. CRISPR-Cas technology could be applied to generate such novel isolates or to study the Lm gene function. We applied CRISPR-Cas to knockout Avr genes *AvrRLm5-9* and AvrLm6 in our reference L. maculans isolate 00-100. As shown in figure 4, replacement of AvrLm5-9 and AvrLm6 was confirmed by PCR primers flanking the insertion sites alone and in combination with insert specific primers as additional confirmation.

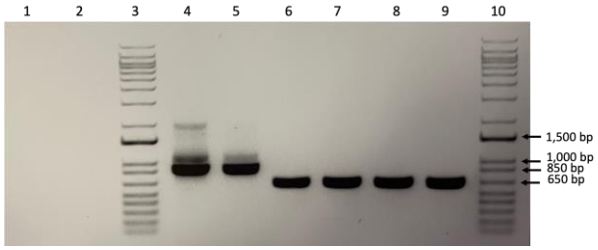


Figure 4a: PCR amplified *AvrLm6* and *AvrLm5-9* with the respective sizes of 909 bp and 671 bp in the L. maculans isolate 00-100. 1) Negative control (water as template and *AvrLm6* primers); 2) Negative control (water as template and *AvrLm5-9* primers); 3) Invitrogen 1 Kb Plus DNA ladder (CAT#10787018); 4 and 5) WT gDNA as template and *AvrLm6* primers; 6-9) WT gDNA as template and *AvrLm5-9* primers; 10) Invitrogen 1 Kb Plus DNA ladder (CAT#10787018).

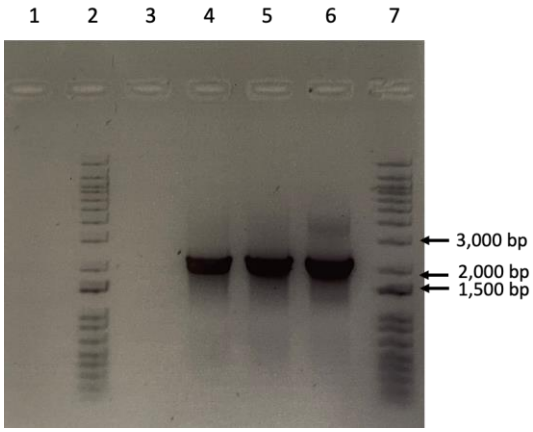


Figure 4b: PCR with gDNA extracted from WT 00100 and *AvrLm5-9* mutants. CRISPR gene knockout was used to replace *AvrLm5-9* with a repair template (PgpdA promoter: hph) conferring resistance to hygromycin to confirm the location of the introduced homologous repair template. The expected size ranges for WT and *AvrLm5-9* mutants are no band and 2,072 bp, respectively. 1) Negative control (water as template); 2) Invitrogen 1 Kb Plus DNA ladder (CAT#10787018); 3) WT gDNA as template; 4-6) *AvrLm5-9 CRISPR* mutant line #4 as template.

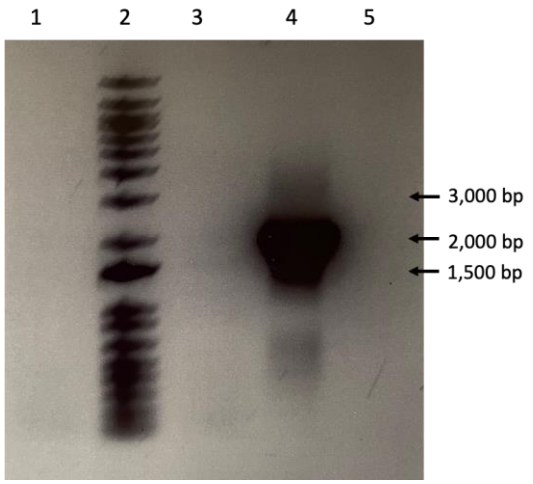


Figure 4c: PCR with gDNA extracted from WT 00100 and *AvrLm6* mutants. CRISPR gene knockout was used to replace *AvrLm6* with a repair template (PgpdA promoter: hph) conferring resistance to hygromycin. The expected size ranges for WT and *AvrLm6* mutants are no band and 2,075 bp, respectively. 1) Negative control

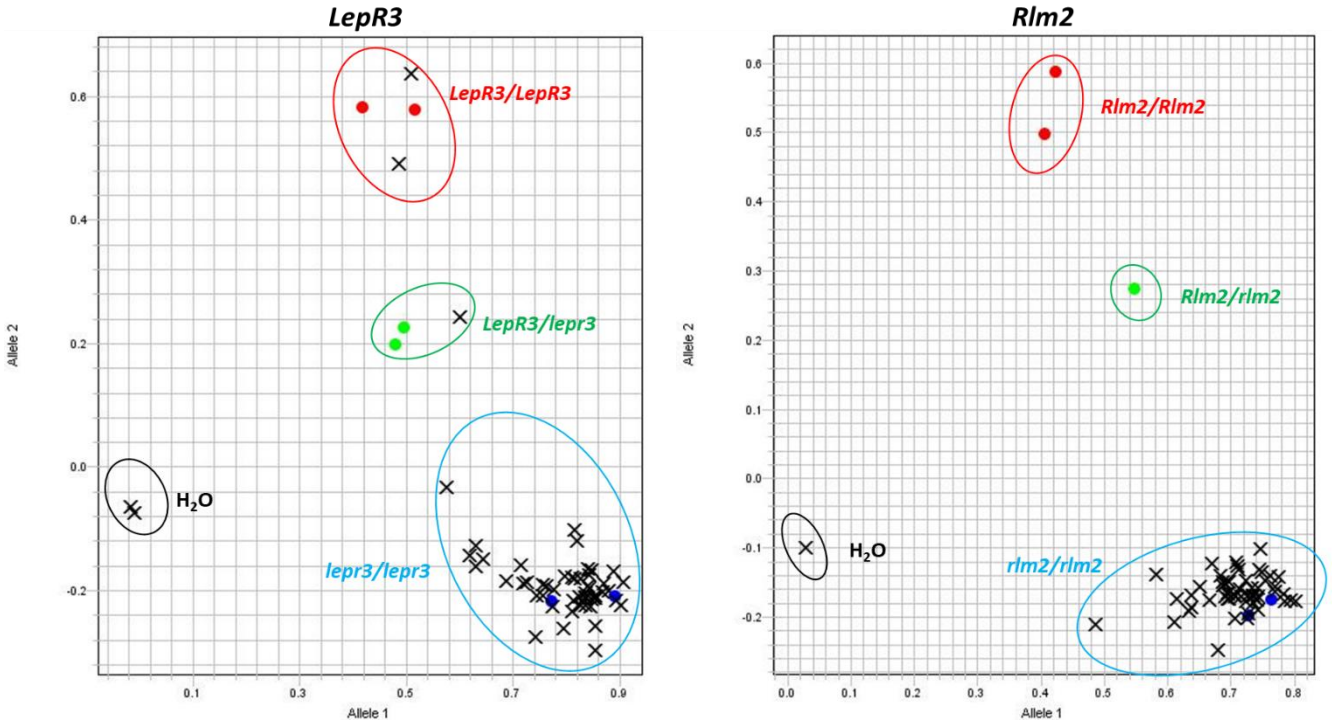
(water as template); 2) Invitrogen 1 Kb Plus DNA ladder (CAT#10787018); 3) WT gDNA as template; 4) *AvrLm6 CRISPR* mutant as template; 6) empty lane.

Activity 3- To genotype the R gene(s) of current commercial canola varieties and *B. napus* collections labeled as resistant to blackleg disease.

We previously reported R gene profile of almost entire *B, napus* accessions that exist in the Plant Genome Resources Canada (PGRC).

Drs. Peng and Fernando have determined the profile of *Rlm-LepR* genes in commercial canola varieties provided by breeding companies.

KASP markers developed by van de Wouw et al (6) were used to detect the presence of *LepR3, Rlm2, RLm4* , *Rlm7* and *Rlm9*. Additionally, specific and nonspecific markers developed in Dr. Peng's lab were used to detect *LepR1*. All these R genes are now present in at least some of the varieties, and there was clear distinction among breeding companies in utilization of genetic resistance; some relied almost completely on quantitative resistance with none of the R genes detected in their varieties (Figures 5 and 6)



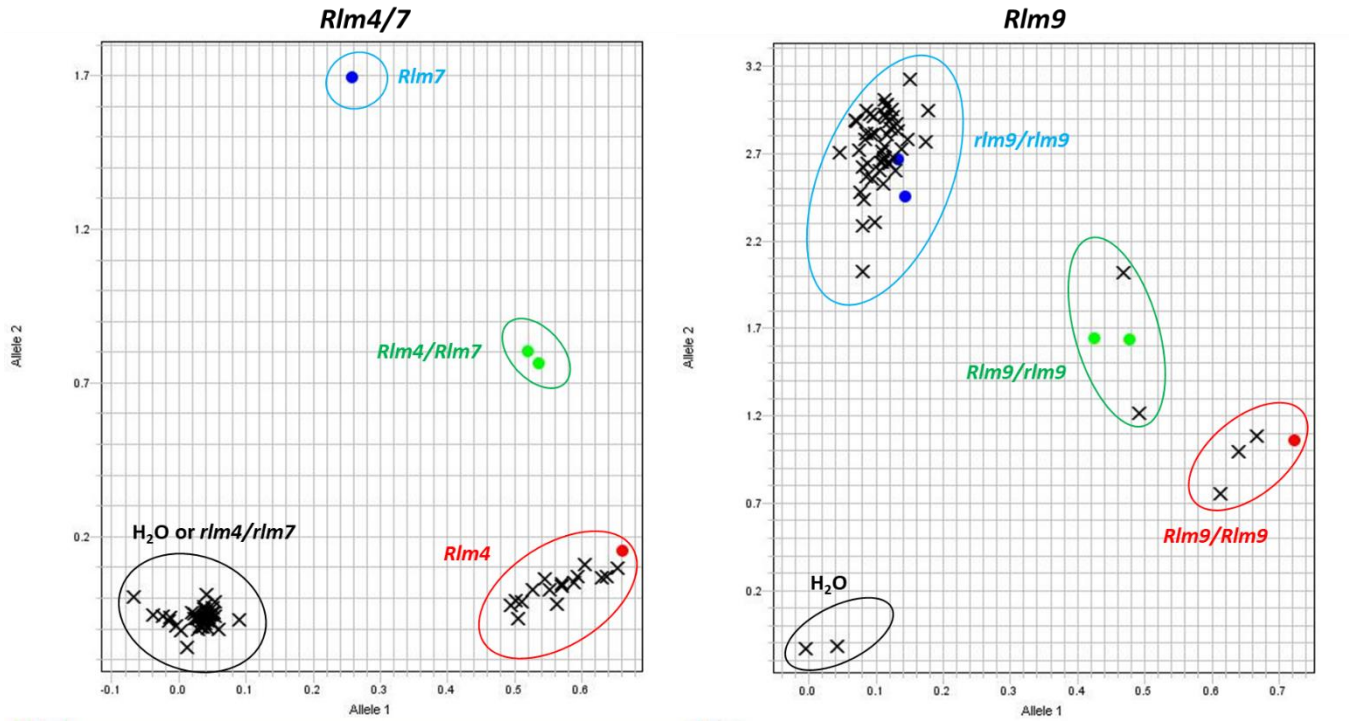


Figure 5. Allelic discrimination plots of KASP assays for *LepR3*, *Rlm2*, *Rlm4*, *Rlm7* and *Rlm9*. Each data point represents the fluorescence signal of a single DNA sample of a variety.

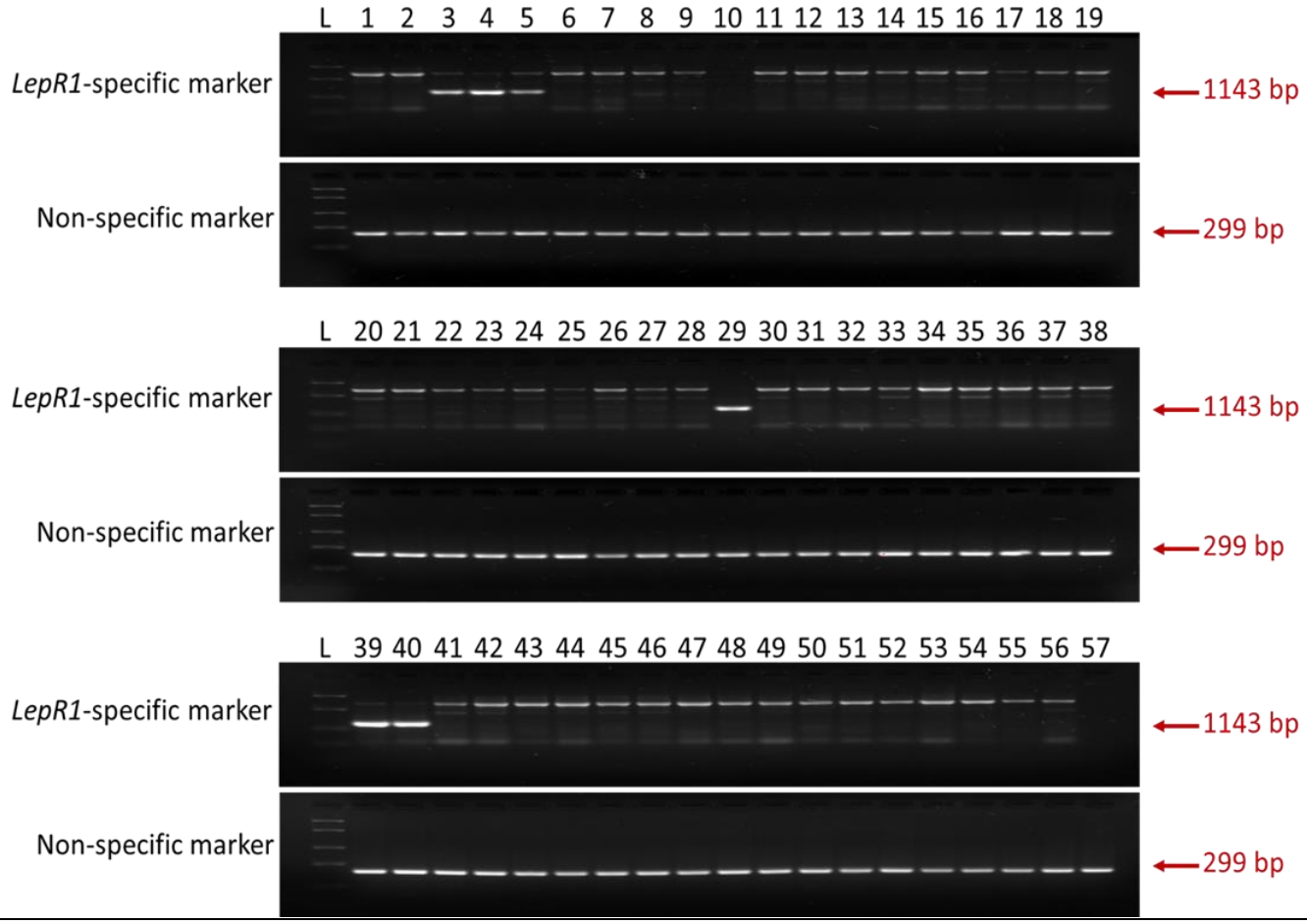


Figure 6. Detection of *LepR1* resistant and susceptible alleles in canola cultivars with *LepR1*-specific and non-specific markers. L: FastRuler middle range DNA ladder; 1-5: Controls, with the lines 3, 4, and 5 carrying LepR1. Lines 6-57 are commercial canola varieties provided by seven breeding companies.

Activity 4- To create a common *B. napus-Leptosphaeria* database of genetics and genomics data as well as protocols.

4-1 Protocols

4.1.1-Seedling pathology assay : Race specific resistance gene evaluation is conducted at the seedling stage via the standard drop inoculation method by placing a 10µl spore suspension on wounded sites of cotyledons and disease evaluation by rating lesion formation on a scale of 0 to 9. While inoculation and disease scoring is standardized among all the blackleg research groups, timing of inoculation and disease evaluation varies based on the growth-chamber setting/ variation in different labs. For example, Canadian researchers inoculate seedling as young as 5 to 7 days post germination and rate the disease progression at 10 to 14 days post inoculation (dpi). Based on published research from French and Australian blackleg researchers, inoculation is performed at 10 to 14 days post seedling germination and rating of disease take place at one to two weeks after inoculation (3 weeks and older seedlings). As mentioned above this variations if time of inoculation and disease scoring between research groups in different countries are likely caused by difference in plant growth facilities and plant genotypes, making it difficult to conduct exactly the same cotyledon assay in different labs. However, since the disease rating scale is the same in every lab there should not be a concern at least for robust resistant and susceptible phenotypes.

4.1.2 Indoor Adult Plant Resistance (APR/QTL) assay: Outdoor (field) based APR screening has been the main method used by public and private blackleg research lab to screen germplasm for APR. But screening for APR under the field is time-consuming, highly variable, and not widely applicable due to difference in climate and race structure of blackleg among and even within canola growing countries. Attempts have been made to develop indoor APR assay by injecting inoculum to the base of stem or placing inoculum drop on petiole at the site of cotyledon excision. Australian researchers have developed an indoor APR assay called spore shower test by placing ascospore producing stubbles over a mesh screen placed above the young seedlings. However this method is not readily applicable to Canadian blackleg research since under the Canadian climate, pycnidiospore (asexual spore) is the main source of inoculum instead of ascospore (10). We have optimized an indoor APR assay that mimics the natural infection by inoculating cotyledon with pycnidiospores and allowing the infection to progress into the stem (Borhan et al., unpublished). Lang et al (personal communication) have successfully applied asexual spore spray on wounded cotyledons and we have further validated this method on *B. napus* accessions with and without APR.

4.1.3 Phenotype and marker based *Lm* isolate genotyping.
The Topas-introgression lines distributed among the public and private blackleg research groups have standardized isolate genotyping based on interaction phenotype. In addition KASP markers were developed to distinguish avirulent (Avr) vs virulent (avr) genes based on the causative SNPs. These markers have been widely used by the blackleg researchers in Canada and internationally and pathogen diagnostic labs.

4.2 databases.

As we reported preciously, despite the initial success of a pilot web based map of blackleg isolates distribution in Canada, the cost of setting up an interactive and updatable website that host maps, genome sequences, protocols and markers was prohibitive. In June 2021, Dr. Borhan submitted a proposal to the AAFC Information System Branch to setup a pathogen genomic database to host *L. maculans* research data and resources, protocols, and a map of pathogen race distribution. Despite initial approval of the project, due to the initial cost

of approximately $100,000 for the project we could not proceed with creating a comprehensive interactive and updatable database. Our lab (Haddadi and Borhan, unpublished) have set up a local website that visualize genome alignment of 58 re-sequenced genome against our reference isolate 00-100. SNPs are colored (blue and read) and homologous sequences are shown in white. Appendix figure 1 is a screenshot of a subset of isolates aligned to isolate 00-100.

6. Conclusions and Recommendations – Highlight significant conclusions based on the discussion and analysis provided in the previous section with emphasis on the project objectives specified above; also provide recommendations for the application and adoption of the project results and identify any further research, development, and communication needs, if applicable.

Blackleg research in Canada has a long history as the crop itself and goes back to the first reports of blackleg as a disease of concern on canola in the mid 70s. Despite research progress in understanding the pathogen and breeding for resistance in Canada Europe and Australia, coordination of research and sharing of research experience and data was limited to separate initiatives taken by individual research labs. This research proposal was in a response to an urgent need to standardize research protocols, nomenclatures and to share resources and massive amount of information and data generated in the past two decades, brought about by the rapid advances in genomics technologies.

During the course of this project we were able to catalogue and make available genetically defined pathogen (Lm) and plant (Brassica Sp.) resources. Similar research initative by our Australian collaborators led to a huge

collection (250) Lm isolates. In addition, we have provided laboratory reference isolates of blackleg to private and public researchers to be used as standard in genotyping Lm field isolates. Prior to this project L. maculans Avirulence (Avr) genes were named based on the interaction phenotype of a given isolate inoculated on a panel of B. napus differential lines such as AvrLm1, matching the R gene Rlm1, AvrLep3 vs R gene LepR3, AvrlmS and AvrLep2 against RlmS and LepR2 respectively. Recent cloning of several Avr and their corresponding R gene led to naming system that represent the corresponding locus/gene. For example AvrLepR2 and AvrLmS being the same locus was re-named as AvrLmS-Lep2. Similarly AvrLm1 being recognized by both LepR3 and Rlm1 was re-named as AvrRlm1-Lep3. Similar naming will be applied to R genes if an R gene recognized 2 or more Avr genes.

Five new Topas introgression lines with single R gene were made available under MTAs. Gene editing protocol for Lm was streamlined by the collective efforts of Australian and the applicants of this proposal making it possible to engineer desired Lm genotypes for research purposes. A fast and reliable indoor APR assay is now available for researchers and canola breeders, overcoming the long and high variability associated with field APR nurseries.

An objective of this proposal was to develop an interactive website that host research data (e.g. genome sequences, plant and pathogen differential lines and R and Avr gene markers) and updatable information for canola farmers and agronomists such as map of Lm filed isolates. Through collaboration with the computer department at the University of Saskatchewan we implemented some these ideas such as Lm map (reported previously) and Lm genome synteny viewer (appendix figure 1). Dr. Borhan submitted a proposal to the AAFC, Information System Branch on June, 2021. Despite the approval of proposal, the estimated cost ($100,000) to develop an interactive and updatable database prohibited pursuing the development of a database hosted by AAFC.

7. Extension and communication activities: (e.g. extension meetings, extension publications, peer-reviewed publications, conference presentations, photos, etc).

Peer-reviewed publications :

1- Van de Wouw, A.P., et al. (2024) A new set of international Leptosphaeria maculans isolates as a resource for elucidation of the basis and evolution of blackleg disease on Brassica napus. Plant Pathology, 73, 170–185.

Conference presentations:

1- FUNCTION OF B. NAPUS CELL SURFACE RECEPTORS IN RESISTANCE AGAINST BLACKLEG DISEASE OF CANOLA. Hossein Borhan, From the deciphering of host pathogen interactions to disease management: The Leptosphaeria maculans /rapeseed case study, ICPP 2023; Lyon, France; Aug. 2023.

2- SUCCESS IN R-GENE LABELING, MULTI GENES, KASP MARKERS AND QR: A GAME CHANGER IN THE CANOLA BLACKLEG PLAYBOOK IN CANADA. Dr. Dilantha Fernando, From the deciphering of host pathogen interactions to disease management: The Leptosphaeria maculans /rapeseed case study. ICPP 2023; Lyon, France; Aug. 2023.

3-

8. Acknowledgements – Include actions taken to acknowledge support by the Funders.

9. Literature Cited

1- Borhan et al. (2022) Molecular Interactions Between Leptosphaeria maculans and Brassica Species. Annu. Rev. Phytopathol., 60: 237-257.

2- Larkan, et al., (2013). The Brassica napus blackleg resistance gene LepR3 encodes a receptor-like protein triggered by the Leptosphaeria maculans effector AVRLM1. The New phytologist, 197(2), 595–605.

3- Larkan, et al. (2015), The Brassica napus receptor-like protein RLM2 is encoded by a second allele of the LepR3/Rlm2 blackleg resistance locus. Plant Biotechnol J, 13: 983-992.

4- Larkan, et al. (2020), The Brassica napus wall-associated kinase-like (WAKL) gene Rlm9 provides race-specific blackleg resistance. Plant J., 104: 892-900.

5- Haddadi, et al. (2022). Brassica napus genes Rlm4 and Rlm7, conferring resistance to Leptosphaeria maculans, are alleles of the Rlm9 wall-associated kinase-like resistance locus. Plant biotechnology journal, 20(7), 1229–1231.

6- Van de Wouw, et al., (2022) Molecular Markers for Identifying Resistance Genes in Brassica napus. Agronomy, 12, 985.

7- Van de Wouw et al. (2023) A new set of international Leptosphaeria maculans isolates as a resource for elucidation of the basis and evolution of blackleg disease on Brassica napus. Plant Pathology. DOI: 10.1111/ppa.13801.

8- Idnurm, et al. (2017) Spontaneous and CRISPR/Cas9-induced mutation of the osmosensor histidine kinase of the canola pathogen Leptosphaeria maculans . Fungal Biol Biotechnol 4, 12. https://doi.org/10.1186/s40694-017-0043-0.

9- Larkan, et al. (2016) Single R Gene Introgression Lines for Accurate Dissection of the Brassica - Leptosphaeria Pathosystem. Front. Plant Sci. 7:1771. doi: 10.3389/fpls.2016.01771.

10- Ghanbarnia, et al. (2011) Comparison of disease severity and incidence at different growth stages of naturally infected canola plants under field conditions by pycnidiospores of Phoma lingam as a main source of inoculum, Canadian Journal of Plant Pathology, 33:3, 355-363

10. Other Administrative Aspects: HQP personnel (PhD and/or MSc students) trained and involved; equipment bought; project materials developed

11. Appendices - If necessary, include any materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications.

Table 1: Field isolates of Leptosphaeria obtained from Dr. Franko Rossi at the National University of General San Martín, Argentina were genotyped using molecular markers.

	AvrLm1	AvrLm2	AvrLm3	AvrLm4-7	AvrLm6	AvrLm11	Avrlm5	avrLm9	CPN60A	KMAT1A	"Avr" genes
IBCN60	No Call	No Call	No Call	No Call	No Call	No Call	No Call	No Call	L.biglabosa	n/a	L.biglabosa, no Avr genes
IBCN61	AvrLm1	No Call	No Call	AvrLm4-7	No Call	No Call	Avrlm5	maybe avrLr	L.biglabosa	n/a	L.biglabosa, but Avr1-47-,
IBCN62	No Call	No Call	No Call	No Call	No Call	No Call	No Call	No Call	L.biglabosa	n/a	L.biglabosa, no Avr genes
IBCN63	No Call	No Call	No Call	No Call	No Call	No Call	No Call	No Call	L.biglabosa	n/a	L.biglabosa, no Avr genes
IBCN64	No Call	No Call	No Call	No Call	No Call	No Call	No Call	No Call	L.biglabosa	n/a	L.biglabosa, no Avr genes
IBCN65	No Call	No Call	No Call	No Call	No Call	No Call	No Call	No Call	L.biglabosa	n/a	L.biglabosa, no Avr genes
Rossi 1	No Call	avrLm2	AvrLm3	AvrLm7	AvrLm6	AvrLm11	Avrlm5	probably avr	L.maculans	n/a	Avr Lm 3-7-6-11-J1
Rossi 2	No Call	avrLm2	AvrLm3	AvrLm7	AvrLm6	AvrLm11	Avrlm5	avrLm9	probably L.m	n/a	Avr Lm 3-7-6-11-J1
Rossi 3	No Call	avrLm2	AvrLm3	AvrLm7	AvrLm6	AvrLm11	Avrlm5	avrLm9	L.maculans	n/a	Avr Lm 3-7-6-11-J1
Rossi 4	No Call	avrLm2	No Call	AvrLm7	AvrLm6	AvrLm11	Avrlm5	avrLm9	L.maculans	n/a	Avr Lm 7-6-11-J1
Rossi 5	No Call	avrLm2	No Call	AvrLm7	AvrLm6	AvrLm11	Avrlm5	avrLm9	L.maculans	n/a	Avr Lm 7-6-11-J1
Rossi 6	No Call	avrLm2	No Call	AvrLm7	AvrLm6	AvrLm11	Avrlm5	avrLm9	L.maculans	n/a	Avr Lm 7-6-11-J1
Rossi 7	No Call	avrLm2	No Call	AvrLm7	AvrLm6	AvrLm11	Avrlm5	avrLm9	L.maculans	n/a	Avr Lm 7-6-11-J1
	no call = avrLm1			no call=avrL	no call = avr	no call = avrLm11					

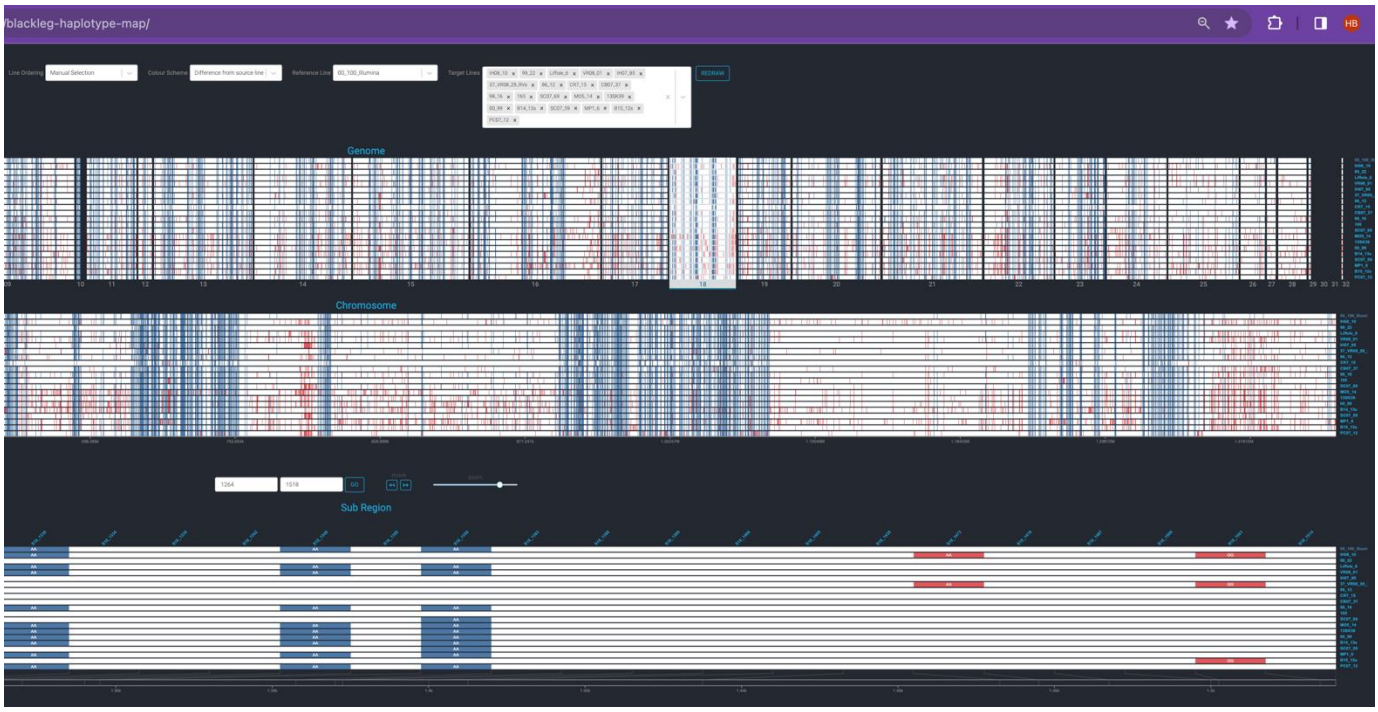


Figure 1: Synteny view of re-sequenced Lm isolates aligned to the high-quality reference genome (Lm isolate 00-100, assembled in 32 contigs). Mutations within the sequenced genomes are shown in color. Lower panel displays SNPs sequences.

12. Financial (to be provided to each Funding Agency (at the addresses indicated in 11.2)

a. Comprehensive Financial Statement that summarizes the total income and expenditures to date attributable to the Funders' Funding.
b. Explanation of variances from budget which are greater than 10%.
c. Invoice

13. Final Report Posting Do you consent to a version of this Final Report (with sensitive information removed) to be posted on the funder's website?	☐ Yes - this version can be posted ☐ Yes - a modified version will be sent ☐ No
14. Research Abstract Posting Do you consent to the 2-3 Research Abstract submitted with this Final Report to be posted on the funders and the Canola Council of Canada's website?	☐ Yes ☐ No

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