Developing high yielding *Brassica rapa* cultivars with resistance to brown girdling root rot, blackleg, white rust and clubroot.

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Research Partner(s):
Alberta Canola Producers Commission, Ward Toma, P.Ag., General Manager
Saskatchewan Canola Development Commission, Catherine Folkerson, Executive Director
BC Peace River Grain Industry Development Council, Barry Willis, P.Ag., Coordinator

Research Centre(s):
AAFC - Northern Agriculture Research Centre, Beaverlodge, AB.
AAFC - Saskatoon Research Centre, Saskatoon, SK.

Research Branch Contact: Kevin C. Falk, Ph.D.

Problem/Opportunity Identified by the Industry:
Summer turnip rape (*B. rapa*) was the dominant species in western Canada in the early 1970's. *B. napus* cultivars were later maturing and could only be grown successfully in the longer season growing areas of Manitoba. In recent years growers have made greater use of new higher yielding *B. napus* cultivars with herbicide tolerance and good disease resistance. Although *B. napus* canola now occupies about 90% of the total acreage sown to canola in western Canada, it is estimated that 25% of this acreage is better suited to *B. rapa*. *B. rapa* has several advantages over *B. napus*. First, it matures 10-14 days earlier and the pods are more shatter resistant than those of *B. napus* allowing the crop to be straight combined. Also, since *B. rapa* matures earlier it is much less likely to produce green seed than *B. napus*. Furthermore, *B. rapa* is typically lower in total saturated fatty acids thereby keeping canola well within the accepted 7% total saturated fatty acid limit. Currently, producers have very few cultivars from which to choose, and only one with resistance to blackleg.

Objectives:
The proposed program has one overall objective: develop high yielding, disease resistant *B. rapa* cultivars. Within this overall objective are four goals: 1) develop a pollination control system, 2) develop brown girdling root rot (BGRR) resistant cultivars, 3) develop blackleg resistant cultivars, and 4) initiate the development of clubroot resistant germplasms/cultivars. The development of a pollination control system will enable ust to develop high yielding hybrid cultivars with resistance to BGRR and blackleg (BL). The program will also continue to produce traditional open-pollinated cultivars and synthetics. Synthetics are intermediate to open-pollinated cultivars and hybrids in that they contain 50% hybrid plants (hybrids contain, in theory, 100%) and 50% (25% from each parent in a two-parent synthetic) parent plants. The development of early maturing canola is crucial to the northern canola growing areas of western Canada. This project will ensure that producers are provided with a high yielding, disease resistant, early maturing alternative to Argentine canola.

1) Develop a pollination control system (goal or objective *italicized*).
The program is currently working on two pollination control systems. Both are based on cytoplasmic male sterility (CMS). Part a) and b) report on the *ogura* system while c) reports on the *yukina* system.

a) Further develop the sterile hybrid system in Polish canola. Develop parental lines and field test new hybrid combinations.
***Ogura* pollination control development.** The program continues to develop new parental lines using the *ogura* cytoplasmic male sterility (CMS) system. As discussed in detail in the project proposal, the *ogura* system in not a complete CMS system in *B. rapa* since no restorers are available. Hence, all hybrids constructed with the system are male sterile and must be augmented with pollinators.

Ogura (1968) reported cytoplasmic male sterility (CMS) in Japanese radish. The production of F_1 hybrids, necessary to exploit heterosis, using CMS requires three breeding lines: A- (male sterile line), B- (maintainer line) and R-lines (restorer). In conjunction with developing an effective Ogura restorer, a number of A- lines are in various stages of development.

Reference: Ogura, H. 1968. Studies on the new male-sterility in Japanese radish, with special reference to the utilization of this sterility towards the practical raising of hybrid seeds. Mem. Fac. Agric. Kagoshima Univ. 6:39-78.

ii) Initiate combining ability studies to ensure that only highly heterotic hybrid parental combinations are produced.
Similarity coefficients (molecular marker work). Nothing new to report.

iv) Continue to develop strategies to best utilize the sterile hybrid concept. Develop experiments to test for multiple pollen donors (versus single) to ensure excellent pollen penetration and therefore seed set on sterile hybrid plants.
Pollinator rate and mix. Previously reported: An experiment testing the effect of number of pollinators (in a male sterile hybrid mixture) and proportion of pollinator in the mix was sown at Beaverlodge (07R510BE) on the 31 May. Various rates of pollinators and combinations of pollinators were used.

b) Initiate large scale testing of the male sterile hybrid concept in Polish canola.
Nothing further to report.

c) Develop A/B and R-lines using the yukina CMS system. Continue to make crosses between putative maintainers or restorers and A-lines (female and R-line development). Assess sterility or fertility under greenhouse and/or field conditions. Increase seed of best A-line plants (needed for maintainer test crosses). Initiate microspore culture of maintainers and restorers. Assess sterility/fertility under greenhouse and/or field conditions.
Development of A/B yukina pairs. Hinata and Konno (1979) reported cytoplasmic male sterility (CMS) in crosses of *Diplotaxis muralis* (L.) DC and *Brassica rapa* L. ssp.

oleifera Metzg. cv. Yukina. Using Schuler's (1989) elite lines as a foundation, Yukina cytoplasm has been introgressed into A- and B-lines at the Saskatoon Research Station and Beaverlodge Research Farm. Current efforts are to develop four isogenic *B. rapa* A- and B- lines. The four recurrent parents, selected based on superior agronomics and genetic divergence (divergent germplasm coefficients), vary in their ability to maintain sterility: 93-6757 (fair); 94-6792 (very good); 95-6855 (good); 00-7874 (good). The 02-8138 line, poor in its ability to maintain sterility, was discontinued.

At time of writing, four putative B-lines (F_6BC_4) and four A-lines (F_1BC_4) have been developed in the last completed greenhouse trial (PR18b_GH08-03) harvested in October, 2008. Appropriate test crosses have been made to check whether the B-lines are indeed maintainers. If four homozygous B-lines have indeed been identified, both near isogenic B-lines and A-lines are ready for ramping of seed production in anticipation of future hybrid trials in 2009.

References:

Hinata, K. and Konno, N. 1979. Studies on a male-sterile strain having *Brassica campestris* nucleus and *Diplotaxis muralis* cytoplasm. I. Breeding processes and some characteristics of this strain. Jap. J. Breed., 29: 305 - 311.

Schuler, Thomas. 1989. *Diplotaxis muralis* induced cytoplasmic male sterility and the development, performance and production of intervarietal hybrids in *Brassica campestris* L. MSc. Thesis. 140 p. U of Saskatchewan.

d) *Obtain accessions from Tibet, screen accessions for adaption to western Canada and quality.*
Completed. Nothing new to report.

2) Develop BGRR resistant cultivars.
a) Complete the mapping of genes controlling BGRR resistance in proven resistant germplasm of Polish canola and produce molecular markers that tag each gene.
b) Determine whether other sources of resistance carry unique and/or complementary genes using molecular markers and field disease nurseries.
Previously reported: At a meeting held in early January 2007, Roger Rimmer and Christine Hammond (technician) presented their most recent work on this project. Contrary to work previously reported where BGRR was thought to be associated with one major and three minor genes (PR25a) it appears that four QTLs have been associated with resistance. For example, our 7664 source of BGRR resistance may be determined by QTLs at both R4 and R8. Furthermore, the 7865 source may have QTLs at R7 and R9. The third source (7864) showed no association. It is interesting to note that this source and populations derived from it were dropped from the program last year due to poor resistance to BGRR. Clearly the molecular work confirmed our suspicions. Beginning in May of 2007, Christine will continue to mine existing nursery material based on the above findings to confirm QTLs on more advanced material assessed in the field since the initial screens were done. Currently, the most resistant and susceptible selections from the advanced material based on phenotypic results from two field seasons are being

screened. Each year will be screened separately, using markers throughout the A genome with a focus on three QTL regions. Furthermore, DNA has been extracted and bulked for the R101 and R102 selections from 2005, and mapping panels set up. Also, samples from 2006 were re-screened with QTL markers and other A genome markers.
Update: DNA from 1104 R101 and R102 plants were extracted individually, then pooled according to seed package, then arranged in R-S-R-S order in a 96-well plate. R102 Dec06 DNA was bulked and arranged into R-S-R-S order in a 96-well plate. Mapping panels were created using 5 markers per linkage group, including markers near QTL regions. The same mapping panels were used for both plates, PCR was performed, followed by Megabace analysis.

c) Identify new BGRR resistant germplasms using field disease nurseries. Introgress resistance genes into germplasms that are adapted to the short season growing areas of western Canada.
BGRR. Population development for lines in the marker assisted selection study for BGRR with two populations continues with a crossing block to be grown in the greenhouse in Fall 2008 and to be evaluated for root rot in Summer 2009. A root nursery trial for another stream was to be evaluated in 2008, but due to poor seed set in greenhouse, a crossing block was established and single plants harvested instead. This material will be assessed in 2009. In 2009 the root rot nursery will be comprised of 322 plots + appropriate checks.

d) Initiate full plot replicated yield trials of adapted BGRR resistant Polish canola cultivars developed by the program.
Replicated full plot testing of advanced BGRR populations (also described below). Two advanced lines selected for resistance to brown girdling root rot and one line selected for blackleg resistance were tested in a prelim trial for good agronomics and at Beaverlodge, Fort Vermilion, Dawson Creek, Fort St John and Ellersie. Data are being processed. Also, within the *Brassica rapa* preliminary population trial a number of lines representing improved germplasms. Two sets of trials were sown at each location: 13 entry and 17 entry, each with four replications. In addition, a private Coop with eight entries, four replicates was sown at these locations plus Fairview and Falher, AB. Trials have been harvested. Results are pending.

3) Develop blackleg resistant cultivars.
a) Further improve resistance to blackleg in breeding populations using recurrent selection and/or back-cross breeding procedures. Two unique sources of blackleg resistance will be utilized.

Since CB9937 and CB9939 were found to be segregating for resistance, other germplasms or advanced material 9319 (02KF004BE-071), 01-7939 and 03-8336 was used for selfing and crossing to CV2. Crosses were made and seed currently being harvested.

Performed a second round of selection on 9272 and 9273, where all lines with at least a 4S:1R ratio were advanced; 24 seeds from each were planted and screened with WA51

and Leroy. Double-resistant plants were transplanted to the greenhouse for seed increase and crossing to CV2. F_1 seed has been harvested.

In addition, triploids from interspecific crosses (LepR1 and LepR2) were backcrossed to CV2: seed of each line from wt5 (D5-8 X D4-8) and wt8 (D5-5 X D4-5) were tested with WA51 and 87-41. Selected double, single, and reverse single resistance plants were transplanted, selfed and also crossed to CV2. Seed has been harvested.

b) Screen germplasms with improved blackleg resistance in field disease nurseries and in the greenhouse (Richard Gugel).
Field screening for resistance to blackleg. Two populations were sown for BL screening this year – one consisting of 303 progeny + 45 checks and the other 266 progeny + 38 checks. On a scale of 0 to 5 (zero being no infection or resistant), the checks rated 1.0, on average. On that basis the test was abandoned. The blackleg nursery, which will be sown in SK in 2009 will contain 672 entries + checks.

c) Test microspore culture protocol on putative blackleg resistant populations. Modify microspore culture protocol (if necessary). Produce doubled haploids and increase seed of each for field testing.
This is an ongoing process. DH production was relatively successful over the winter months but the number which could be increased for field evaluation was small and therefore field evaluation was deferred until 2009.

d) Initiate the pyramiding of different resistance genes to produce B. rapa populations with increased levels of blackleg resistance.
Once resistant forms are developed in each of three sources this part of the project will be initiated.

4) Initiate the development of clubroot resistant germplasms.
a) Work in collaboration with Alberta pathologists to develop strategies to manage the spread of clubroot in crucifers.
b) Assist in screening B. rapa cultivars and germplasms for resistance/susceptibility to Plasmodiophora brassicae.
c) Initiate work to determine the correlation of disease expression by single races and their mixtures on both B. rapa and B. napus.
One source of resistance has been secured and we are continuing discussions with the Alberta group led by Murray Hartman.

Population development. Overview of nursery work (population development program). Approximately 1100 progeny rows were sown in 2008 in Beaverlodge. Progeny are selected on the basis of agronomics and quality. The nursery is comprised of three distinct canola quality populations ranging in size from just under 200 to 600. Tradtional recurrent selection procedures are being used to select superior canola quality progeny. At this time, progeny have not been harvested.
In addition, seven composite crossing blocks were sown to provide seed (individual plant harvest) for the next recurrent selection cycle. In 2009 the nursery will be comprised of

approximately 1800 plots + appropriate checks.

Replicated full plot testing (preliminary and advanced yield trials). Replicated full plot multi-location trials were sown at Beaverlodge, AB., Fort Vermilion (two sites), AB., Fort St. John, B.C. and Dawson Creek, B.C.. Trials were arranged as randomized complete blocks (RCB) with four replicates. Data for each location including days to first flower, days to mature, seed yield, seed oil and meal protein content, total NIR glucosinolate content (coarse measurement) and NIR chlorophyll content follow. Seed oil and meal protein contents are listed for each entry in the accompanying tables. All data is based on NIR. In most instances entries exceed or are not significantly different than the check AC Sunbeam. Glucosinolate data is also based on NIR. Since glucsinolate determinations by NIR are not as reliable as those by gas chromatograph (GC), a final decision for advancement (to the next stage of testing or development) will not be made until GC data is available. At this time most plots have not been harvested owing to late seeding.

In addition, a Private Data Coop with nine entries, two checks and four replicates was sown at Beaverlodge, Dawson Creek, Fort St. John and Fort Vermilion. From these trials four entries will move forward for COOP testing. Three are first year entries and one a second year entry.

List of collaborators (and contribution):
Mr. Clair Langlois, Agronomist, B.C. Grain Producers Association (trial site manager).
North Peace Applied Research Association (trial managers).
Mr. Joe Unrah, Agronomist, AAFC - Fort Vermilion (trial manager).

List of AAFC staff (and contribution):
Dr. Henry Klein-Gebbinck, Pathologist/Statistician (BGRR and statistics).
Mr. Lance Lewis, Biologist (Beaverlodge field and laboratory coordinator).
Mr. Richard Gugel, Pathologist (blackleg).
Dr. Roger Rimmer, Molecular Pathologist (BGRR and blackleg work).
Ms. Annette Zatylny (Saskatoon field and laboratory lead breeding technician)
Ms. Holly Spence (breeding technician).
Ms. Lori Bobowski (breeding technician).
Ms. Christine Hammond (molecular/pathology technician).
Dr. Rong Zhou (chemist)
Ms. Dawnne Campbell (quality technician)
Mr. Gerald Serblowski (quality technician)