PROJECT DETAILS

- **Title**: Assessment of seed germination and seedling performance of spring canola at low temperatures
- **Funders:** Alberta Crop Industry Development Fund, Alberta Canola, and Manitoba Canola Growers Association
- **Research program:** Canola Agronomic Research Project (CARP)
- **Principal investigator:** Ludovic J.A. Capo-chichi, Alberta Innovates – Technology Futures
- **Collaborators/additional investigators:** Isobel Parkin, Jan Slashki, Ralph Lange and Limin Wu
- **Year completed:** 2014

Final report

Project Background

The Canadian Prairies is the major growing area for canola in North America with an average annual production of 9 million tonnes on which the revenues of more than 52,000 Canadian farmers depend (Canola Council of Canada, 2008). The export of canola contributed $14 billion to the Canadian economy in 2008 (Canola Council of Canada, 2008). A recent study of the economic impact of canola by province ranked Alberta the second highest contributor to the Canadian economy at $4.5 billion (Canola Council of Canada, 2008). Although Canada has increased canola acreage in recent years to boost production, average yield did not show much improvement and is less than half that realized in Western Europe (USDA Statistics, 2012).

A major factor affecting spring canola production in Canada is frost during seedling development in the late spring and seed maturation in the early fall, resulting in significant yield reduction and low seed quality (Canola Council of Canada, 2008). Losses result from the need to reseed or delay seeding to be sure of avoiding catastrophic losses to frost. In Alberta, scientists showed that delaying spring canola seeding for few weeks resulted in a 20% decline in grain yield (Degenhardt and Kandra, 1981). This yield loss translated to a loss of 1.3 million tones and $2.6 billion in revenue.

Frost seeding has been shown to give a higher yield at lower cost, but it is risky. With the high cost of canola seed and limited availability to re-seed frost killed fields, it may not be a worthwhile option (Manitoba Agriculture Food and Rural Initiatives, 2000). Early seeding, on the other hand, has outperformed normal seeding. However, improving frost tolerance of spring canola by few degrees could allow frost seeding which would greatly improve yield and seed quality. Tools to screen canola lines for frost tolerance and early seedling vigor would enable producers to select canola varieties that would perform better under low soil temperature and frost conditions.

As part of the proposed project, association mapping was conducted to identify candidate genes for cold tolerance related traits. Similar to QTL mapping, association mapping seeks to identify a statistically significant genetic association between genetic markers and a quantitative trait of interest. However, in association mapping, the genetic markers usually lay within candidate genes suspected to contribute to the variation in that trait, and the goal is to identify the actual genes affecting that trait, rather than just chromosomal segments (Panzea, http://www.panzea.org/). While QTL mapping requires development of a mapping population, which takes time and resources, association mapping can be conducted on existing varieties or germplasm and also taking advantage of historic phenotypic data.

Objectives:

The proposed project addressed one of the research priorities identified by the provincial grower groups. Specifically, the overall objective was to determine the effects of cold on the performance of spring canola. Specifically:

- Determined the effects of low temperature on seed germination of *Brassica napus*, *Brassica rapa*, *Brassica oleracea* genotypes;
- Determined the effects of frost on seedling performances in *Brassica napus*, *B. rapa*, *B. oleracea* genotypes;
- Performed association mapping to identify markers linked to alleles for improved cold tolerance;
- Validated new techniques to screen large canola populations for cold tolerance.

Deliverables:

- Knowledge of the genetic variation for cold tolerance in Canadian spring canola germplasm pool;
- Ranking of canola genotypes for cold tolerance;
- Novel tools to screen canola for frost tolerance;
- Annual and final reports, scientific papers describing results. Active engagement of the growers in the evaluation of the research progress in a form of field days, workshops and site meetings.

Chapter I: Emergence responses to low temperatures and seed size in *Brassica napus, Brassica rapa* and *Brassica juncea*

Objective 1: Determine the effects of low temperature on seed germination of *Brassica napus*, *Brassica rapa*, *Brassica oleracea* genotypes.

Seedling emergence and uniform crop establishment of spring canola (*Brassica napus*, *B. rapa*, or *B. juncea*) is often delayed by low soil temperatures on the Canadian Prairies, often causing significant productivity losses.

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

The aim of this study was to determine the extent of inter- and intra-specific genetic variation in spring canola emergence responses to low temperature, and to evaluate effects of seed size on emergence. The study was conducted on 169 genotypes of four Brassica species (*B. rapa, B. napus, B. oleracea and B. juncea*) originating from different climatic regions. We measured seedling emergence at temperatures ranging from 5°C to 15°C for 169 genotypes from four oilseed *Brassica* species. As expected, the time to onset of emergence, 50% (T_{50}) emergence, and maximum emergence was positively correlated with temperature. The effects of decreasing temperature varied among species and genotypes. Onset of emergence began within 2 to 4 days of seeding for all genotypes at 15°C, most of which reached 99% emergence. At 5°C, however, there was significant variability in seedling emergence among genotypes, ranging from 10 to 20 days after seeding. Time required to reach $T_{50}$and maximum emergence was much longer compared to that recorded at higher temperatures, suggesting 5°C may be considered a desirable temperature for selecting spring canola tolerant to low temperature. In addition, some genotypes did not achieve T_{50}, revealing a greater sensitivity to low temperature. On the other hand, several genotypes exhibited excellent germination at the lowest temperature. Seed size had a slight effect on seedling emergence. As temperature decreased, smaller-seeded genotypes tended to emerge slightly faster than larger-seeded genotypes. However, a few larger-seeded genotypes emerged slightly faster than smaller-seeded genotypes, suggesting an interaction between genotypes and seed size. Interspecific variation in the emergence response suggested that *Brassica juncea* exhibited greater lower temperature germination potential than *Brassica napus, Brassica rapa,* and *Brassica oleracea*.

Chapter II: Quantifying responses of seedling performance to freezing temperature: Variation among genotypes of Brassica species

Objective 2: Determine the effects of frost on seedling performances in *Brassica napus*, *B. rapa*, *B. juncea*, *B. oleracea* genotypes.

Frost during seedling development in the late spring and seed maturation in the early fall is one of the major factors affecting spring canola production in Canada, resulting in significant yield reduction and low seed quality. In order to investigate the potential of chlorophyll fluorescence to reveal plant tolerance to low temperature, we used a collection of Brassica species and compared their fluorescence response. Untreated seeds of 169 genotypes including *Brassica napus*, *Brassica rapa*, *Brassica juncea*, and *Brassica oleracea* were sown in pots containing field soil and placed in a germination chamber at 15°C. Light conditions were set at 16/8 h light/dark. Germination was determined by counting the number of seed with 2 mm or more of radicle growth. Seedlings at the cotyledon stage were freeze-shocked at -5°C for 60 minutes after dark adaptation for 60 min. Frost injury was visually assessed as well as being inferred from the measurement of the chlorophyll fluorescence parameter ($F_V/F_M = (F_M - F_0)/F_M$). Genotypes were significantly different ($P < 0.05$) for seed germination and emergence, depending on the tested temperature. Chlorophyll fluorescence of leaf strips was

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

measured before and at different time intervals (2 and 24 hours) after freezing. The response of the lines to freezing-shock was different as assayed by measuring the chlorophyll parameters F_V/F_M, F_V/F_0, F_M, F_0, and F_V. Prior to freezing shock treatment, the values of the F_V/F_M varied from 0.830 to 0.751, averaging 0.784. Two hours after freezing-shock, the F_V/F_M values recorded varied from 0.805 to 0.198, averaging 0.501 across the *Brassica* species. A measure carried out 24 hours after freezing shock varied from 0.781 to 0.014. The values recorded for F_V/F_0 before freezing shock ranged from 4.447 to 3.028, averaging 3.738. Two hours after freezing shock, the F_V/F_0 varied from 4.138 to 0.245. A measure carried out 24 hours after freezing shock ranged from 3.564 to 0.013. A freezing-shock treatment (-5°C for 60 min) caused a dramatic F_V/F_M decrease in non-hardy genotypes. This effect was irreversible during recovery in the growth chambers. In hardy genotypes, the F_V/F_M did not significantly change and remained at a level between 0.805 and 0.781 while the F_V/ F_0 varied from 4.447 to 3.564. Changes in F_V/F_M and F_V/F_O after freezing shock of the seedlings before and after freezing shock are shown in Figure 1. The F_V/F_M showed higher correlation to plant survival than any other parameters, such as F_V/ F_0, F_M, and F_V. A general association between F_V/F_M 24 hours after freezing shock and the percent survival was evident. The regression values (R^2) calculated 24 hours after freezing shock between F_V/F_M and percent survival was 0.74 while it was 0.69 between F_V F_0 and percent survival. Upon freeze-shock at -5°C, the distribution of the ratio of variable to maximum fluorescence (F_v/F_m) indicated that leaves of tolerant genotypes maintained higher rates of electron transport than leaves of sensitive genotypes.

Chlorophyll fluorescence of leaf strips after freezing treatment varied among genotypes within species. In *B. juncea*, the F_V/F_M values varied from 0795 to 0.784 two hours after freezing shock. A measure carried out 24 hours after freezing shock ranged from 0.776 to 0.755, averaging 0.766. In *B. napus*, the F_V/F_M ranged from 0.804 to 0.197 two hours after freezing treatment. A measure carried out 24 hours after freezing shock varied from 0.780 to 0.014, averaging 0.384. This suggests that *B. juncea* exhibited greater freezing temperature at the cotyledon leaf stage than *B. napus*.

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

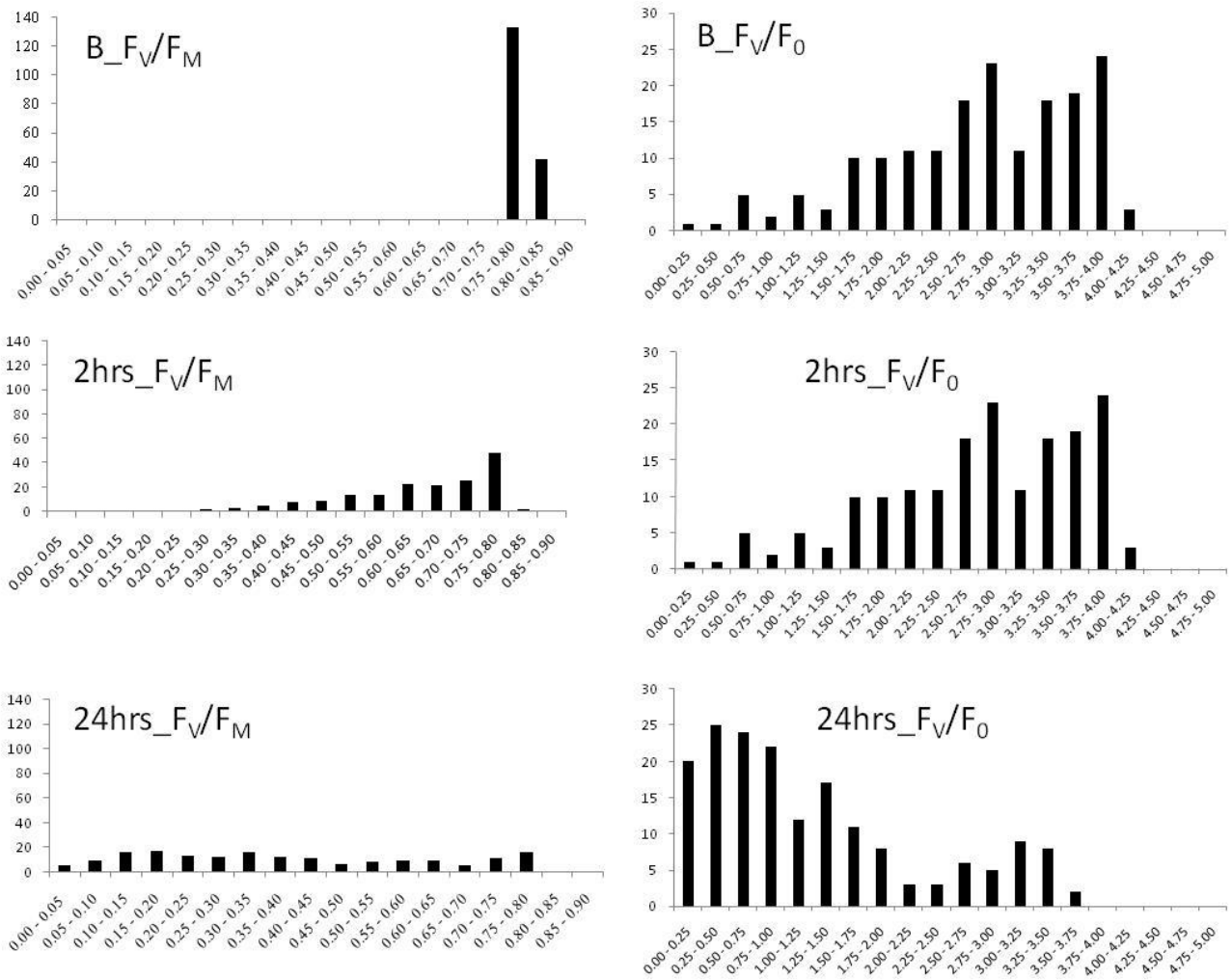

Figure 1. Frequency distribution of F_V/F_M and F_V/F_0 values measured on 169 genotypes of *Brassica* species before and after freezing at -5°C for an hour: B_F_V/F_M = before freezing test, 2hrs_F_V/F_M= 2 hours after freezing test, 24hrs_F_V/F_M= 24 hours after freezing test, B_F_V/F_0= before freezing test, 2hrs_F_V/F_0= 2 hours after freezing test, 24hrs_F_V/F_0= 24 hours after freezing test.

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

Chapter III: Association mapping for low temperature tolerance in spring canola.

Objective 3: Conduct association mapping to identify markers linked to alleles for improved cold tolerance;

The Brassica Illumina Infinium arrays was hybridized and scanned according to the manufacturer's instructions using DNA isolated from 165 *B. napus* lines and 21 *B. rapa* accessions. After filtering, there was initially 47,304 single nucleotide polymorphism (SNP) loci. From the 47,304 SNPs on the array, 2,237 SNPs which are not anchored to the genome that are assigned to unmapped scaffolds were removed from further analysis. In addition, 1,061 SNPs with >20% missing data, and 7,927 SNPs with minor allele frequency (MAF) of <0.05 were also excluded from the downstream analysis. In total 36,079 SNPs were retained for the purposes of association analysis. Of which, 6, 416 high quality SNPs evenly distributed across the genome have been selected for characterizing the population structure and relationship among the lines (Table 1). Initially the software STRUCTURE was used to identify underlying populations among the lines and this was confirmed through PCA analysis. Including the *B. rapa* lines in the analysis biased the analysis such that we could only differentiate between the *B. napus* and *B. rapa* lines (Figure 1 and 2), so the analysis was repeated with only the *B. napus* lines. The analysis of the *B. napus* lines identified two populations, shaded separately in red and green in Figures 2 and 3. Calculation of linkage disequilibrium and association analysis is on-going.

Table 1. Number of SNPs of selected for each chromosome, based on adjacent SNPs being separated by a physical distance of at least 35 Kb.

Chromosome	Number of SNPs
A1	305
A2	287
A3	344
A4	207
A5	268
A6	264
A7	215
A8	245
A9	354
A10	165
C1	430
C2	492
C3	581
C4	480
C5	308
C6	337

C7	422
C8	352
C9	360
Total	6416

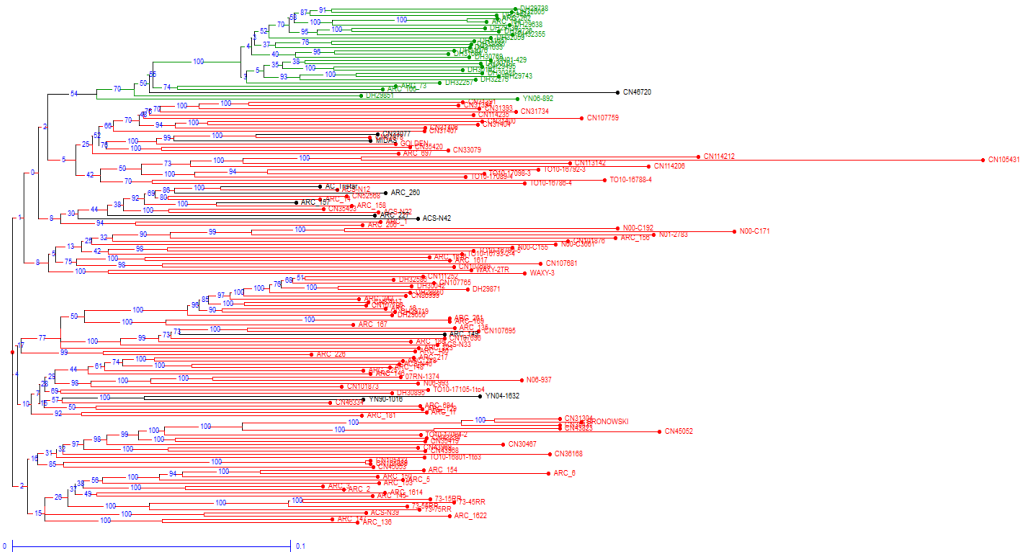

Figure 2. Phylogenic analysis of 161 *B. napus* using SNPs.

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

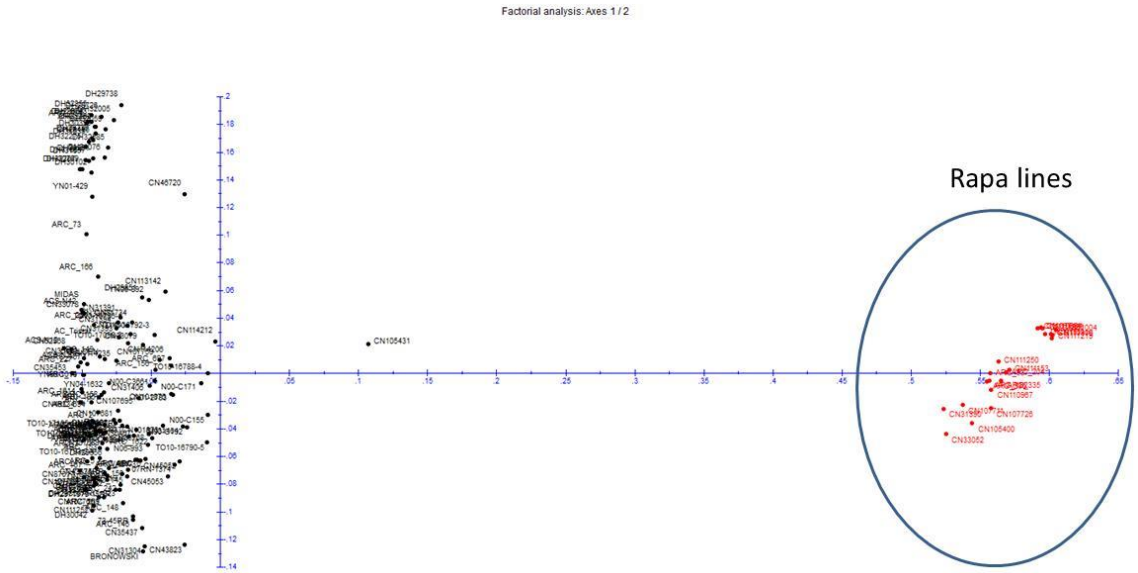

Figure 3. Factorial analysis of 161 *B. napus* and 20 *B rapa.*

Conclusion

Breeding for low temperature tolerance can be very challenging. While some progress has been made in spring seeded canola, the gain was masked by location variation in cold spring soil from year to year.

To improve efficiency and increase the tolerance of low temperature, canola breeder must exploit new screen techniques to complete previously used methods. This project has generated a huge amount of information and tools that will be useful in breeding programs and other projects aimed at understanding the physiology and genomics of low temperature tolerance in spring canola. The study demonstrated that chlorophyll fluorescence parameters can be used in combination with molecular markers to achieve efficient gain.

Suggestions for future research

The following additional studies are recommended:

1. Genomics and proteomics studies needed to identify candidate genes that can be used for potential genetic engineering of new high productive varieties;
2. An introgression of cold hardiness from genotypes expressing high tolerance to low temperature into high yield commercial genotypes must be made. We have already identified these genotypes with high and low tolerance.

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.