

**DEVELOPMENT OF LOW TEMPERATURE TOLERANCE OF SPRING BARLEY FOR  
GENETIC IMPROVEMENT OF YIELD AND MALTING QUALITY**



**DRAFT FINAL REPORT**

**SUBMITTED TO BREWING AND MALTING BARLEY RESEARCH INSTITUTE  
(BMBRI)**

**PRINCIPAL INVESTIGATOR:**

Dr. Ludovic J.A. Capo-chichi

Research Team Members:

Dr. Aaron Beattie, CDC, Saskatoon

Dr. Patricia Juskiw, FCDC, Lacombe

Dr. Joseph Nyachiro, FCDC, Lacombe

Dr. Jan Slaski, AITF, Vegreville

Crop Development and Management  
Ecosystems and Plant Sciences  
Alberta Innovates – Technology Futures,  
P.O. Bag 4000, Vegreville,  
AB, Canada T9C 1T4

## Table of Contents

|  |    |
|--|----|
| Section A: Project overview .....  | 4  |
| Section B: Non-technical summary .....   | 4  |
| Section C: Project details.....  | 6  |
| <b>1.</b> Project team .....   | 6  |
| <b>2.</b> Background .....   | 7  |
| <b>3.</b> Objectives and Deliverables .....  | 9  |
| 3.1.    Objectives .....   | 9  |
| 3.2.    Deliverables .....   | 10 |
| <b>4.</b> Research design and methodology .....  | 10 |
| 4.1.    Plant materials .....  | 10 |
| 4.2.    Methodology.....   | 10 |
| 4.2.1.    Cold treatments .....  | 10 |
| 4.2.2.    Genome wide association study .....  | 12 |
| 4.2.3.    Data analysis.....   | 13 |
| <b>5.</b> Results and discussions .....  | 13 |
| 5.1.    Results .....  | 13 |
| 5.1.1.    Germination.....   | 13 |
| 5.1.2.    Chlorophyll fluorescence under freezing shock .....                                | 13 |
| 5.1.3.    Chlorophyll fluorescence under cold acclimation.....                               | 14 |
| 5.1.4.    Survival rates after freezing shock and cold acclimation.....                      | 16 |
| 5.1.5.    Introgression of cold tolerance from winter barley into spring barley.....         | 18 |
| 5.1.6.    Genome-wide association analysis: Population structure and association analysis. . | 21 |
| 5.2.    Discussions .....  | 24 |
| 5.2.1.    Chlorophyll fluorescence.....  | 24 |
| 5.2.2.    Genome-wide association analysis.....  | 26 |
| <b>6.</b> Conclusions and Recommendations.....   | 27 |
| <b>7.</b> Literature cited.....  | 28 |
| <b>8.</b> Benefits to the industry .....   | 30 |

|  |   |    |
|--|---|----|
| 8.1.   | Impact of the project results on Alberta’s agriculture and food industry..... | 30 |
| 8.2.   | Potential economic impact of the project results                              |    |
|  | 30  |    |
| 9.   | Contribution to training of highly qualified personnel (max ½ page).....      | 31 |
| 10.  | Knowledge transfer/technology transfer/commercialisation (max 1 page).....    | 31 |
| 11.  | Acknowledgements .....  | 32 |
| Section D: Project resources (See separate document) ..... |   | 32 |
| 1.   | Statement of revenues and expenditures: .....                                 | 32 |
| 2.   | Resources:.....   | 33 |
| Section E: The next steps (max 2 pages) .....              |   | 34 |

## **Section A: Project overview**

1. Project number: 2013C001R
2. Project title: Development of low temperature tolerance of spring barley for genetic improvement of yield and malting quality
3. Research team leader: Ludovic Capo-chichi
4. Research team leader's organisation: Alberta Innovates – Technology Futures
5. Project start date: 2013/04/01
6. Project completion date: 2016/03/31
7. Project final report date: 2016/06/30

## **Section B: Non-technical summary**

Frost during seedling development in late spring is one of the major factors affecting spring barley in Canada, resulting in poor crop establishment, mortality, and significant yield reduction. In general, financial losses are the result of reseeded to avoid catastrophic losses due to frost. The current project focused on 1) validating new techniques to screen large spring barley populations for cold tolerance; 2) conducting association tests to identify SNP markers linked to alleles for improved cold tolerance; 3) introgression cold hardiness from winter malting barley into spring malting barley; and 4) screening hybrid lines for germination and seedling cold tolerance.

A set of 96 barley genotypes, sourced from the Western Canadian barley breeding programs, consisting of advanced breeding lines and commercial varieties registered in Canada were used. This included the two-row malting barley association mapping population (91 varieties/breeding lines) used to study limit dextrinase and beta-glucanase (project supported by Brewing and Malting Barley Research Institute), containing elite germplasm that have been in the Western Two-Row Cooperative Registration Tests over the past 15 years and contains a number of

malting barley check cultivars currently registered in Western Canada: ‘AC Metcalfe’, ‘CDC Copeland’, ‘Bentley’, ‘CDC Meredith’, and ‘CDC Reserve’. Four winter malting barley varieties were used as checks and parental lines. These were ‘02Ab431’, ‘02Ab671’, ‘02Ab669’, and ‘2Ab08 X 05W061-208’ (Aberdeen, ID) developed by the US Department of Agriculture. The lines were selected based on their high percentage of winter survival, malt extraction, and seed yield (Data provided by the USDA and the University of Minnesota, Minnesota, USA).

We have successfully developed protocols to screen barley varieties for cold tolerance at the seedling growth stage and ranked lines/genotypes for their ability to tolerate low temperatures using chlorophyll fluorescence parameters. We measured survivals and chlorophyll fluorescence ( $F_v/F_m$ ,  $F_v/F_0$ ,  $F_m$ ,  $F_0$ , and  $F_v$ ) before and after cold treatment, including freezing shock and cold acclimation. We detected significant variation in freezing and cold acclimation among genotypes. Under both cold treatments, the values of the chlorophyll fluorescence parameters significantly decreased after 24 hours in non-hardy genotypes, leading to irreversible changes. Among the hardy genotypes, chlorophyll fluorescence was more correlated to freezing shock tolerance than to cold acclimation.

We successfully produced 17 populations; each of them derived from a cross between spring barley and winter barley lines. These populations are segregating for growth habit (spring versus winter type) and cold tolerance at seedling growth stage. Three  $F_{2.5}$  populations were selected based on spring type and then screened for low temperature tolerance at seedling growth stage under field conditions. The populations were derived from the following crosses: ♀ 02Ab431 X ♂ Bentley; ♀ 02Ab08-X05W061-208 X ♂ AC Metcalfe; ♀ AC Metcalfe X ♂ 02Ab08-X05W061-208. The agronomic traits are being evaluated in the 2016 field season with a funding support from the Brewing and Malting Barley Research Institute. An application for additional funding has been submitted to Agriculture Funding Consortium to cover activities related to malting quality and molecular analysis.

DNA of 96 varieties/breeding lines used to study limit dextrinase and beta-glucanase was hybridized and scanned using the 9K Barley Infinium iSelect SNP Assay. The software STRUCTURE (Pritchard et al. 2000) used to identify underlying population structure. Genome-wide association analyses were carried out with TASSEL (Bradbury et al, 2007). A total of 7864 SNPs were used for the genome-wide association analysis (GWAS). The GWAS analyses identified 39 positive SNP associations with the chlorophyll fluorescence of which 19 were under cold acclimation and 20 were under freezing shock treatment.

We concluded that the chlorophyll fluorescence method is a tool to accurately determine freezing tolerance of leaves. It is quick and inexpensive, and could potentially be used for large scale screening. This project 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 cold tolerance in barley. The project demonstrated that large genotypic differences exist in the low temperature tolerance of barley that can be exploited to develop new high performing and stable yielding varieties.

## **Section C: Project details**

### **1. Project team**

A multi-disciplinary research comprising plant physiologists, molecular geneticist, and barley breeder from AITF and Western Canadian Barley Breeding Program Centres was proposed.

**Dr. Capo-chichi** is a Molecular Geneticist and Plant Breeder. He led the functioning of the project, drafted progress reports and any abstracts submitted for presentation at the national and international conferences. Dr. Capo-chichi is responsible for final reporting of the project at the technical point of view as well as financial management and reporting. He performed all statistical analyses and their interpretations.

**Dr. Aaron Beattie** is an assistant professor at the CDC in charge of the barley breeding program. Dr. Beattie provided the 91 varieties/breeding lines) that he used to study limit dextrinase and

beta-glucanase. He has also made available the SNPs data used in the project. He has contributed in reviewing any abstracts we have submitted for presentation.

**Dr. Jan Slaski** is a plant physiologist, agronomist and a breeder recognized by the CSGA. Dr. Slaski worked together with Dr. Capo-chichi in physiological data collection and interpretation. He conducted extensive research on biochemical and genetic mechanisms of adaptation of cereals to abiotic stresses.

**Dr. Patricia Juskiw** is a barley breeder, Lacombe, AB, AARD- Dr. Juskiw provided commercial malting barley varieties and assisted in the process of making crosses between winter barley and spring barley.

**Dr. Joseph Nyachiro** is a barley breeder Lacombe FCDC in charge of the 6-rowed and hulless breeding program. Dr. Nyachiro has assisted in the process of making crosses between winter type and spring type of barley. He has reviewed abstracts and articles submitted for presentation at the national and international conferences.

## **2. Background**

The malting barley varieties account for 70% of Canada's annual barley production (Canada Malting Barley Technical Centre, 2012 - <https://www.grainscanada.gc.ca/barley-orge/harvest-recolte/2012/qbsm12-qosm12-eng.pdf>). However, only 25% - 30% of the malting barley grown in Canada is accepted for malting. The Agri-Food Industry growth strategy requires increased food and grain production to meet the demand of the expanding malting barley and livestock industries. Grain availability and quality are the major limitations for the continued growth of the agriculture industry in Canada. Although significant progress has been made in improving the genetic gain for barley yield and malting quality, the crop rarely reaches its full yield potential due to seasonal variation, including the low temperatures prevailing early and late in the growing season.

Cold stress is a major environmental factor that often affects seedling development and crop productivity, leading to substantial crop losses and yield reduction. The extent of damage depends on the temperature, length of exposure time, the growth stages of the plant, and the speed to which the low temperature was reached. Reports termed cold stress as chilling and freezing stresses (Tomashow, 1999; Larcher, 2001). Chilling stress results from temperatures cool enough to produce injury without forming ice crystals in plant tissues, whereas freezing stress results in ice formation within plant tissues. Plants differ in their tolerance to chilling (0-15°C) and freezing (<0°C) temperatures (Browse and Xin, 2001). Often, injury occurs when low temperatures coincide with sensitive plant growth stages.

Low temperatures early in the season can completely destroy the crop, while in fall low temperatures can reduce the yield and grade of the end product, resulting in higher green seed count and increase grain spoilage. Monetary losses for farmers are a result from reseed costs due to delayed seeding which can result in low grain quality at harvest.

Selection for cold tolerance/susceptibility in barley varieties can be associated with a reliable screening technique. Classical methods, such as, measurement of survival rates and plant re-growth are slow, lengthy and do not accurately quantify the level of cold tolerance (Novillo et al., 2004). Other methods focus on injury to the plasma membranes that result in leakage of electrolytes from plant tissues (Steponkus et al., 1990). The electrolyte leakage is easily quantified by conductivity measurements (Rohde et al., 2004). For such experiments, plants are subjected to freeze-thaw cycles with minimum temperatures typically ranging from -1 to -50°C, and electrolyte leakage caused by cooling is then measured. The parameter LT50EL is determined as the temperature at which 50% of maximum leakage occurs. The methods to quantify the cold tolerance of plants should be non-invasive and applicable at a high-throughput screening rate, so that the underlying genetic determinations can be effectively quantified and established (Mishra et al., 2011). Research suggests that chlorophyll fluorescence emission can effectively be used in high-throughput screening of plant's low temperature tolerance (Mishra et al., 2011).

Chlorophyll fluorescence responds to changes in PSII photochemistry, and therefore represents an efficient and rapid tool to evaluate the capacity of the photosynthetic machinery at low temperatures (Lichtenthalet and Rinderle, 1988). The photosynthetic apparatus function can be evaluated by measuring the ratio of chlorophyll variable fluorescence ( $F_V$ ) over the maximum fluorescence value ( $F_M$ ), which indicates the efficiency of the excitation capture by open photosystem II reaction centers (Frachebound et al., 1999; Rizza et al., 2001).

Chlorophyll fluorescence as a screening method for cold tolerance has already been investigated in several crop species including *Zea mays* (Schapendonk et al., 1989; Frachebound et al., 1999;), in *Oryza sativa* (Sthapit et al., 1995, *Arabidopsis thaliana* (Mishra et al., 2011). An analysis of variable component of chlorophyll fluorescence  $F_V$  at low temperatures in spring and winter barley, wheat and rye suggested that, in hardened leaves, the rate of variable fluorescence may reflect their expected freezing tolerance (Smillie and Hetherington, 1983). A good correlation between frost tolerance and the decrease of  $F_V/F_M$  during hardening and after freezing was found in winter wheat (Clement and Hasselt, 1996). A significant reversible decrease in  $F_V/F_M$  was found in all genotypes of oat (*Avena sativa* L.) during acclimation to low, nonfreezing temperatures, and  $F_V/F_M$  measurement was found to be highly correlated with field-evaluated frost damage (Rizza et al., 2001). Freezing on the functionality of the photosynthetic apparatus has been used to assess the cold tolerance of *Brassica napus* (Chengci et al., 2005). Little information is available on the effect of freezing on chlorophyll fluorescence in spring barley. The present study investigated the response of barley varieties at three- to four-leaf stages to freezing temperature using chlorophyll fluorescence quenching.

### **3. Objectives and Deliverables**

#### **3.1. Objectives**

- a. Determine the effects of low temperatures on seed germination and seedling performance among spring malting barley germplasm. We will define the minimum temperature for germination and seedling growth of malting barley.
- b. Validate new techniques to screen large spring barley populations for cold tolerance.

- c. Conduct association tests to identify markers linked to alleles for improved cold tolerance (the genotyping has already been done on the association mapping panel and will be used along with the data generated from objective 1 to run the association tests).
- d. Introgress cold tolerance from winter malting barley into spring malting barley
- e. Screen hybrid lines for germination and seedling cold tolerance.

### **3.2. Deliverables**

- a. Novel tools to screen spring barley varieties for cold tolerance.
- b. Ranking of the Canadian spring malting barley varieties for their ability to tolerate low temperatures
- c. Knowledge of genetic variation for cold tolerance in Canadian spring malting barley.
- d. Knowledge of minimum temperatures for germination and seedling growth of malting barley lines present in the panel.
- e. Development of hybrid lines segregating for germination and seedling cold tolerance.

## **4. Research design and methodology**

### **4.1. Plant materials**

Canadian barley breeding programs have developed numerous malting barley varieties and advanced breeding lines that have been in the western coop over the last two decades. This includes malting varieties currently registered in western Canada such as AC Metcalfe, Bentley, CDC Copeland, 'Newdale', 'CDC Kendall', 'CDC Kindersley', CDC Meredith, 'CDC Landis', 'Merit 57', 'CDC PolarStar', 'Cerveza', 'Major', 'Norman', and CDC Reserve.

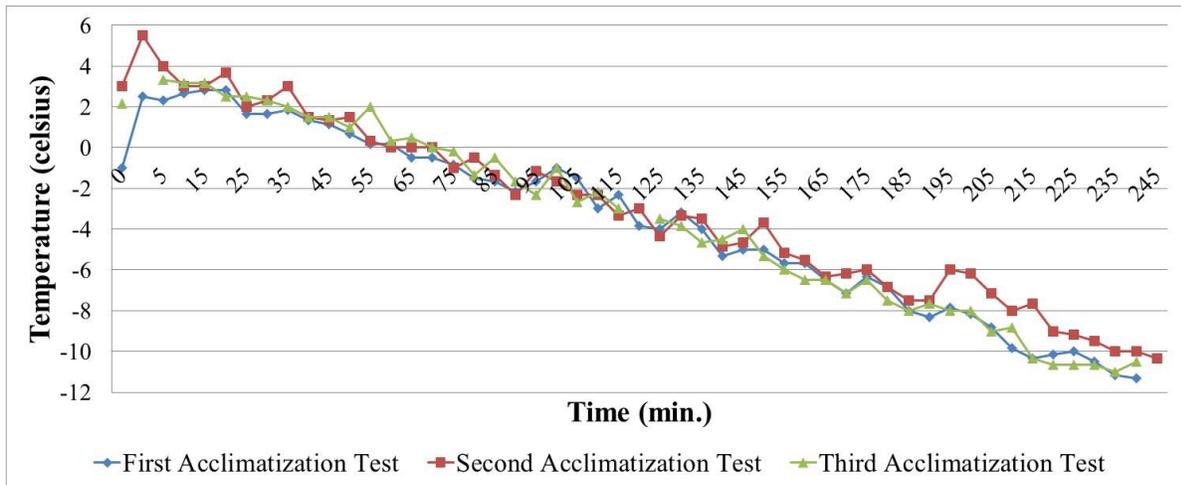
### **4.2. Methodology**

#### **4.2.1. Cold treatments**

Two experiments were designed to screen the genotypes, namely, a freezing shock experiment and a cold temperature acclimation experiment. Both experiments were initiated by germinating seeds in pots containing pasteurized field soil and incubated in a growth chamber with a daily regime of 12 h light at 20°C and 12 h dark at 15°C. Approximately 10 seeds of each genotype were deposited onto wet soil in an 8cm×8cm×7 cm pots with 2 replicates. An identical amount of soil was used to cover the seeds in each pot to insure even emergence. The seedlings were grown

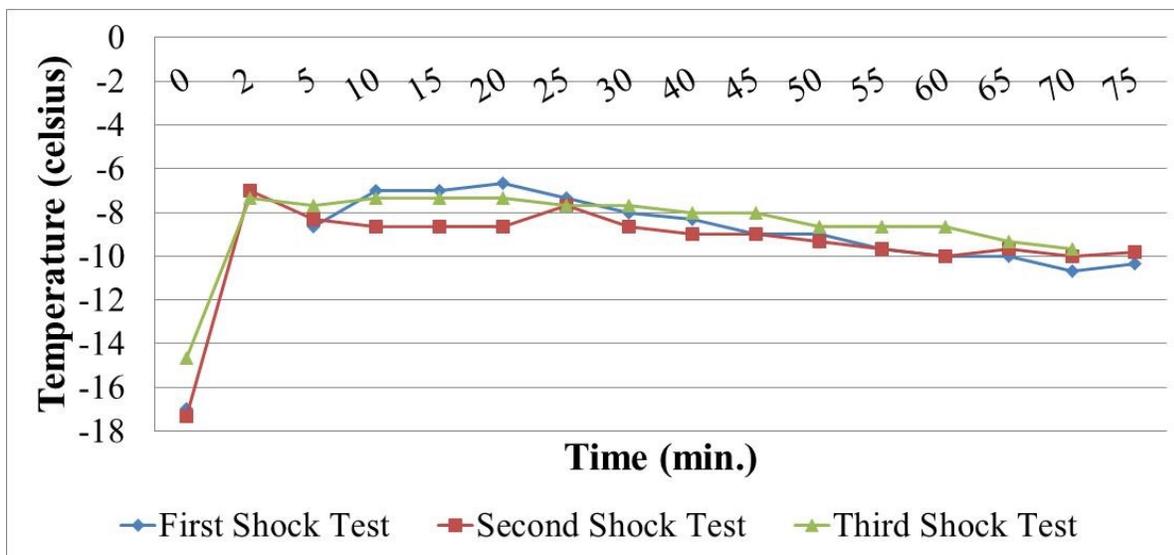
until they reached the full three leaf growth stage. Plants were thinned to five seedlings per pot before cold treatment.

The acclimation treatment consisted of placing plants at the three leaf stage in a chest freezer at  $-1^{\circ}\text{C}$ . The temperature was raised to between  $3^{\circ}\text{C}$  and  $5^{\circ}\text{C}$ , and then gradually decreased to  $-12^{\circ}\text{C}$  over a duration of 4 hours (Figure 1).



**Figure 1: Temperature variation during cold acclimation treatment of 95 spring barley lines and 4 winter barley lines at the three leaf stage.**

The freeze shock treatment consisted of placing the plants at the three leaf stage in a chest freezer at temperatures varying from  $-11^{\circ}\text{C}$  to  $-6^{\circ}\text{C}$  for 75 min (Figure 2). Measurements of chlorophyll fluorescence were used to differentiate genotypes. They were taken at 3 different times during the experiment: before the freezing treatment, two hours after the treatment, and 24 hours after the treatment. The plants measured in each pot were numbered from 1 to 5, so as to record chlorophyll fluorescence on the same five plants each time.



**Figure 2: Temperature variation during freezing shock of 95 spring barley lines and 4 winter barley lines at the three leaf stage.**

The chlorophyll fluorescence include measurements of the chlorophyll fluorescence parameter ( $F_v/F_m = (F_m - F_0)/F_m$ ) (Ghassemi-Golezani et al 2008), where  $F_0$  = Fluorescence occurring when all antenna sites are assumed to be open (dark adapted),  $F_m$  = Fluorescence intensity under exposure to excitation source, variable fluorescence ( $F_v$ ), and the photochemical efficiency  $F_v/F_m$  of photosystem II (PSII). The  $F_v/F_m$  ( $F_m - F_0)/F_m$ , the ratio of variable fluorescence to maximal fluorescence, is an indicator of maximum quantum efficiency and gives important information concerning the effect of environmental stress on the plant. The  $F_v/F_0$  ratio is used as a very sensitive indicator of the maximum efficiency of photochemical processes in PSII and/or the potential photosynthetic activity of healthy as well as stressed plants (Krause et al. 1982, Lichtenthaler et al. 1984, Babani and Lichtenthaler 1996). At the end of the stress period, plants were moved back to the growth chambers used for germination. Frost survival rate was counted one week later.

#### 4.2.2. Genome wide association study

Genotyping of the AM population was done at the USDA-ARS genotyping lab (Fargo, ND) using the 9K Barley Infinium iSelect SNP Assay. This assay is able to interrogate approximately 9,000 single nucleotide polymorphism (SNP) markers, including the 1,596 SNPs contained on BOPA1

### 4.2.3. Data analysis

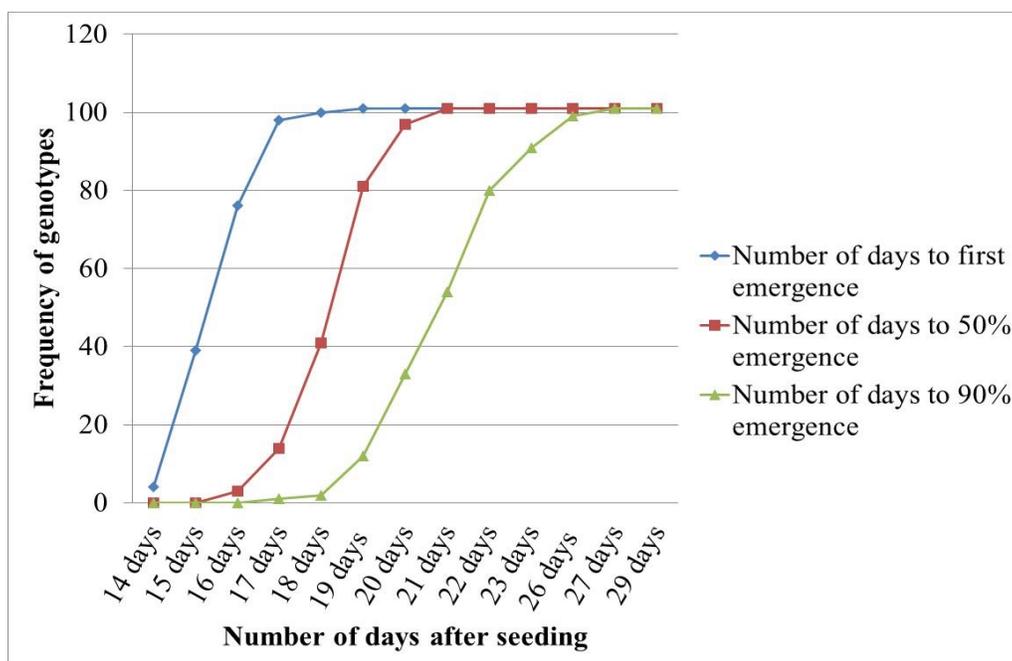
All genotypes were arranged as a completely randomized design with two replications. The analysis of variance was performed and a LSD ( $P= 0.05$ ) was calculated to identify significant differences.

## 5. Results and discussions

### 5.1. Results

#### 5.1.1. Germination

At 5°C, the time required to the onset of emergence ranged from 14 to 19 days for all genotypes (Figure 3). At Day 14, the emergence rates varied from 0 to 38%, while at Day 19, the emergence rates ranged from 4 to 100%. The time required for 50% emergence varied from 15 to 22 days, indicating that some genotypes have ability to emerge faster than others. We observed several lines that exhibited adequate performance at 5°C.



**Figure 3:** Frequency to first, 50% (T50) and greater than 90% (T>90) emergence of spring barley genotypes.

#### 5.1.2. Chlorophyll fluorescence under freezing shock

Prior to freezing treatments, the  $F_v/F_m$  values varied from 0.739 to 0.766, averaging 0.752 (Table 1). Two hours after freezing-shock, the  $F_v/F_m$  values recorded varied from 0.012 to 0.609,

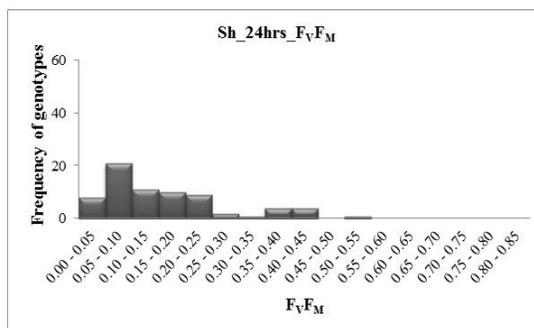
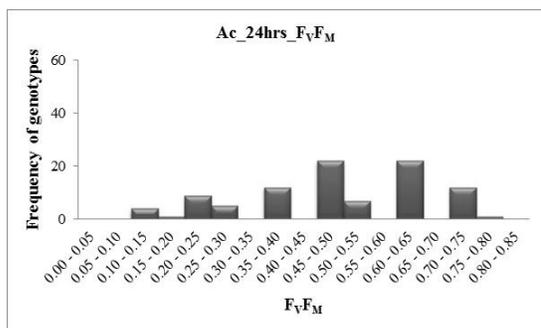
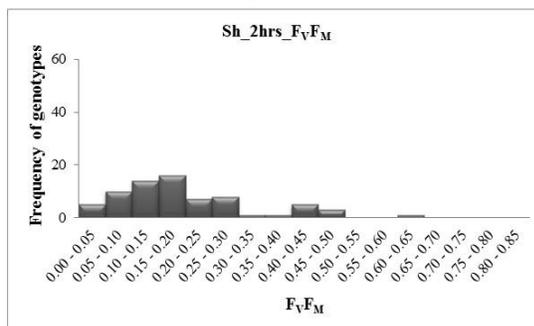
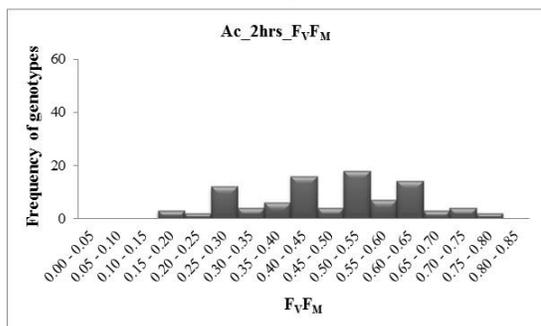
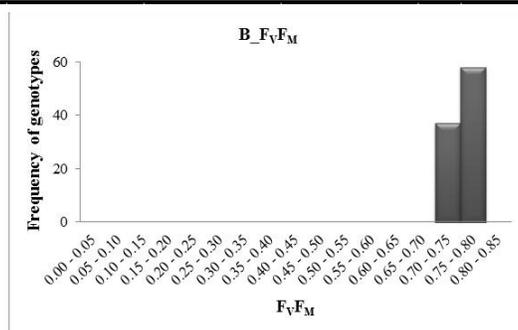
averaging 0.199 across the genotypes (Table 1). A measure carried out 24 hours after freezing shock varied from 0 to 0.517. The values recorded for  $F_V/F_0$  before freezing shock ranged from 2.847 to 3.291, averaging 3.190. Two hours after freezing shock, the  $F_V/F_0$  varied from 0.013 to 2.412, averaging 0.694 (Table 1). A measure carried out 24 hours after freezing shock ranged from 0 to 1.968. The freezing-shock treatment caused a large  $F_V/F_M$  and  $F_V/F_0$  decrease in non-hardy genotypes. We observed that this effect was irreversible during recovery in the growth chambers, depending on the threshold. In most cases, the effect is irreversible when  $F_V/F_M$  values were below 0.220. For hardy genotypes, the  $F_V/F_M$  values ranged from 0.477 to 0.609 after freezing treatment while the  $F_V/F_0$  varied from 1.832 to 2.413. The frequency distribution of the chlorophyll fluorescence parameters,  $F_V/F_M$  and  $F_V/F_0$  after freezing shock is shown in Figures 4 and 5.

### **5.1.3. Chlorophyll fluorescence under cold acclimation**

Two hours after cold acclimation, the  $F_V/F_M$  values recorded varied from 0.171 to 0.756, averaging 0.476 across the genotypes. A measure carried out 24 hours after cold acclimation varied from 0.125 to 0.752 (Table 1). The values recorded for  $F_V/F_0$  before cold acclimation ranged from 2.710 to 3.393, averaging 3.056. Two hours after cold acclimation, the  $F_V/F_0$  varied from 0.543 to 3.105 (Table 1). A measure carried out 24 hours after cold acclimation ranged from 0.502 to 3.040, averaging 1.881. The frequency distribution of the chlorophyll fluorescence parameters,  $F_V/F_M$  and  $F_V/F_0$ , before and after the freezing shock and cold acclimation treatments are shown in Figures 4 and 5.

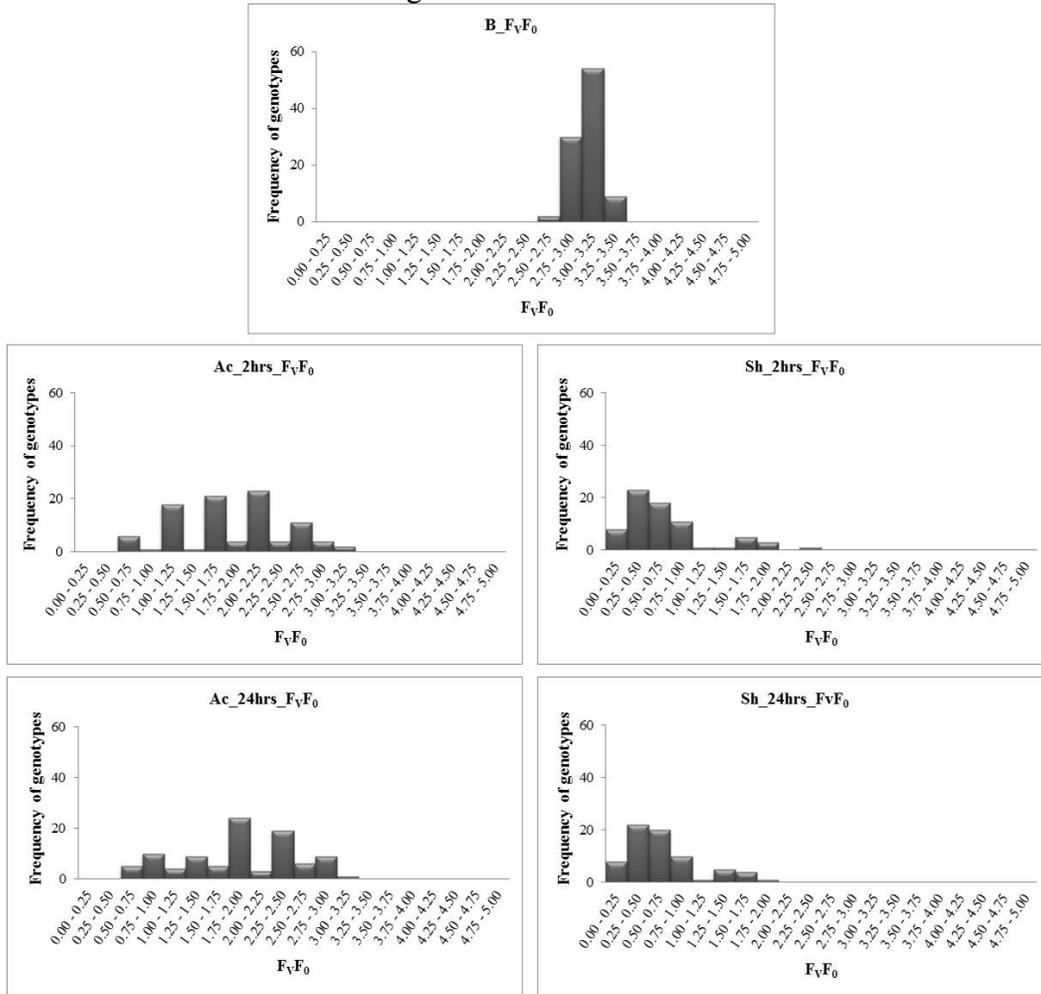
**Table 1:** Ratio of variable fluorescence to maximal fluorescence ( $F_V/F_M$ ) and variable fluorescence to fluorescence occurring when all antenna sites are assumed to be open ( $F_V/F_0$ ) of spring barley genotypes after freezing shock and cold acclimation treatments.

| Treatment            |                | F <sub>V</sub> /F <sub>M</sub> |       |       | F <sub>V</sub> /F <sub>0</sub> |       |       |
|----------------------|----------------|--------------------------------|-------|-------|--------------------------------|-------|-------|
|                      |                | Max                            | Min   | Ave   | Max                            | Min   | Ave   |
| Freezing shock       |                |                                |       |       |                                |       |       |
|                      | Before         | 0.766                          | 0.739 | 0.752 | 3.291                          | 2.847 | 3.190 |
|                      | 2 hours after  | 0.609                          | 0.012 | 0.199 | 2.412                          | 0.013 | 0.694 |
|                      | 24 hours after | 0.517                          | 0.000 | 0.165 | 1.968                          | 0.000 | 0.621 |
| Cold acclimatization |                |                                |       |       |                                |       |       |
|                      | Before         | 0.772                          | 0.727 | 0.753 | 3.393                          | 2.710 | 3.056 |
|                      | 2 hours after  | 0.756                          | 0.171 | 0.476 | 3.105                          | 0.543 | 1.788 |
|                      | 24 hours after | 0.752                          | 0.125 | 0.485 | 3.040                          | 0.502 | 1.881 |



**Figure 4:** Frequency distribution of F<sub>V</sub>/F<sub>M</sub> values measured on 95 barley genotypes before and after cold acclimation and freezing shock. B\_ F<sub>V</sub>/F<sub>M</sub> = before freezing shock, Ac\_2hrs\_ F<sub>V</sub>/F<sub>M</sub> = 2 hours after cold acclimation, Sh\_2hrs\_ F<sub>V</sub>/F<sub>M</sub> = 2 hours after

freezing shock, Ac\_24hrs\_  $F_V/F_M = 24$  hours after cold acclimation, Sh\_24hrs\_  $F_V/F_M = 24$  hours after freezing shock.

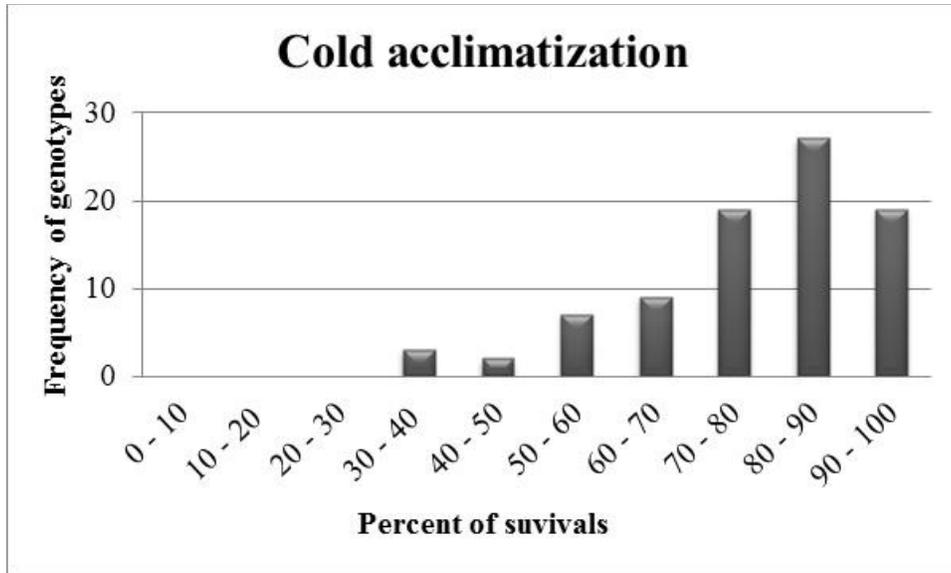


**Figure 5:** Frequency distribution of  $F_V/F_0$  values measured on 95 barley genotypes before and after cold acclimation and freezing shock. B\_  $F_V/F_0$  = before freezing shock, Ac\_2hrs\_  $F_V/F_0$  = 2 hours after cold acclimation, Sh\_2hrs\_  $F_V/F_0$  = 2 hours after freezing shock, Ac\_24hrs\_  $F_V/F_0$  = 24 hours after cold acclimation, Sh\_24hrs\_  $F_V/F_0$  = 24 hours after freezing shock.

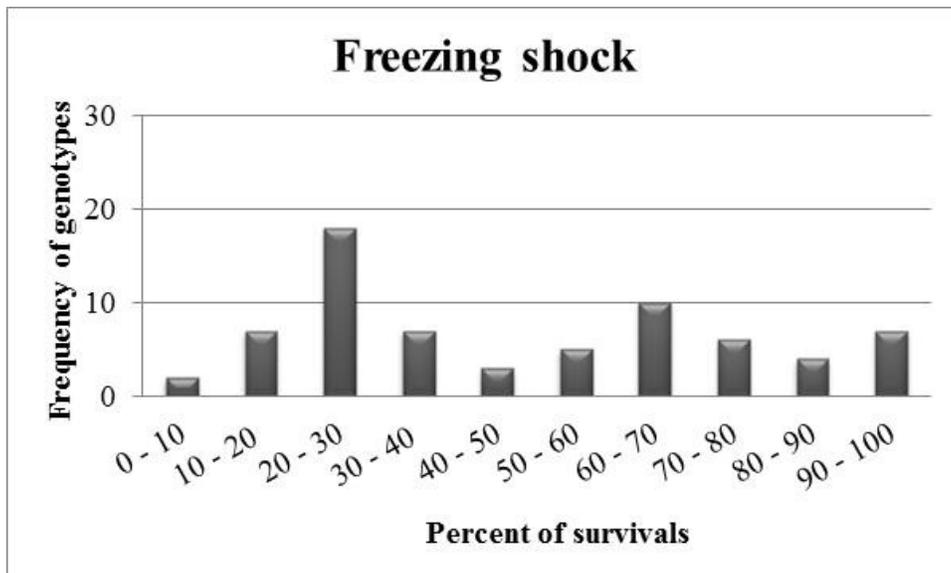
#### 5.1.4. Survival rates after freezing shock and cold acclimation

The variation in survival rate depended upon the cold treatment. Under cold acclimation, survival rates varied from 30% to 100% with the majority grouping between 80 and 90% (Figure 6) while for the freeze shock treatment, survival rates ranged from 0 to 100% with the majority of the genotypes between 20% and 30% survival (Figure 7). This suggests that the cold

acclimation treatment increased freezing tolerance more than the freezing shock treatment. The figure 8 shows two barley genotypes contrasting for cold tolerance.



**Figure 6:** Frequency distribution of survivals recorded on 95 genotypes of spring barley 7 days after cold acclimation. Plants were cold acclimatized at the three leaf stage.



**Figure 7:** Frequency distribution of survivals recorded on 95 genotypes of spring barley 7 days after freezing shock. Plants were freeze shocked at the three leaf stage.



**Figure 8:** Two genotypes of spring barley contrasting for cold tolerance at the three leaf stage after cold acclimation. A: cold tolerant genotype and B: cold susceptible genotype.

### 5.1.5. Introgression of cold tolerance from winter barley into spring barley

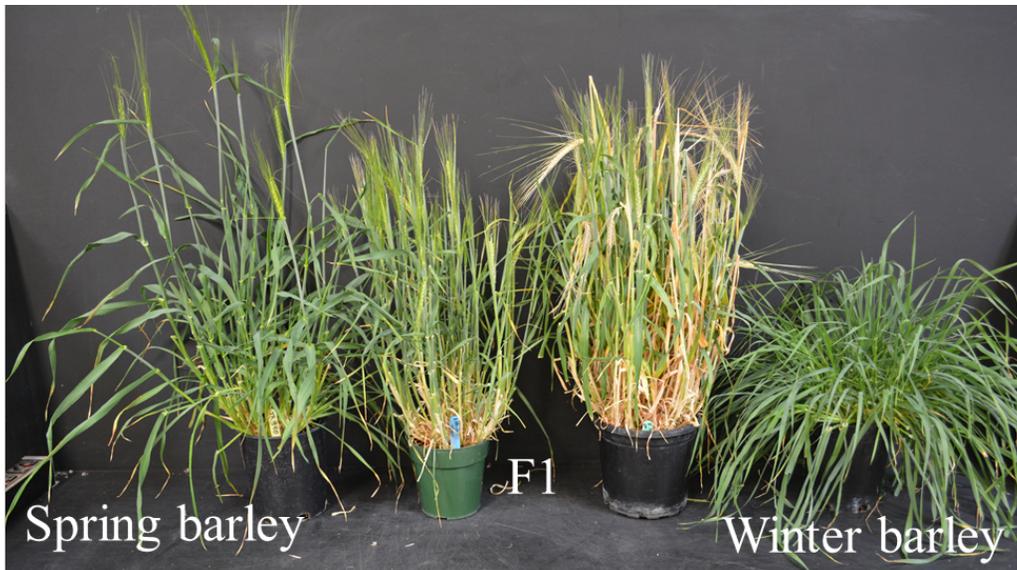
#### Plant materials and growth conditions

Four two-rowed winter barley lines ‘02Ab431’, ‘02Ab671’, ‘02Ab669’, and ‘2Ab08 X 05W061-208’ were obtained from the United States Department of Agriculture (USDA). The lines were selected based on their high percentage of winter survival and cold hardiness (ranging from 50 to 100% across several locations in the northern United States), excellent malt extract, and high seed yield. Vernalization of the lines was performed by placing two- to three-leaf stage seedlings in growth chambers at 5°C with 8 h of light. After ten weeks of vernalization, seedlings were transferred to a controlled environment at 20°C. Light conditions were set at 16/8 h light/dark and humidity was closely monitored. Upon flowering, seventeen crosses were made between spring barley genotypes and winter barley genotypes (Table 2).

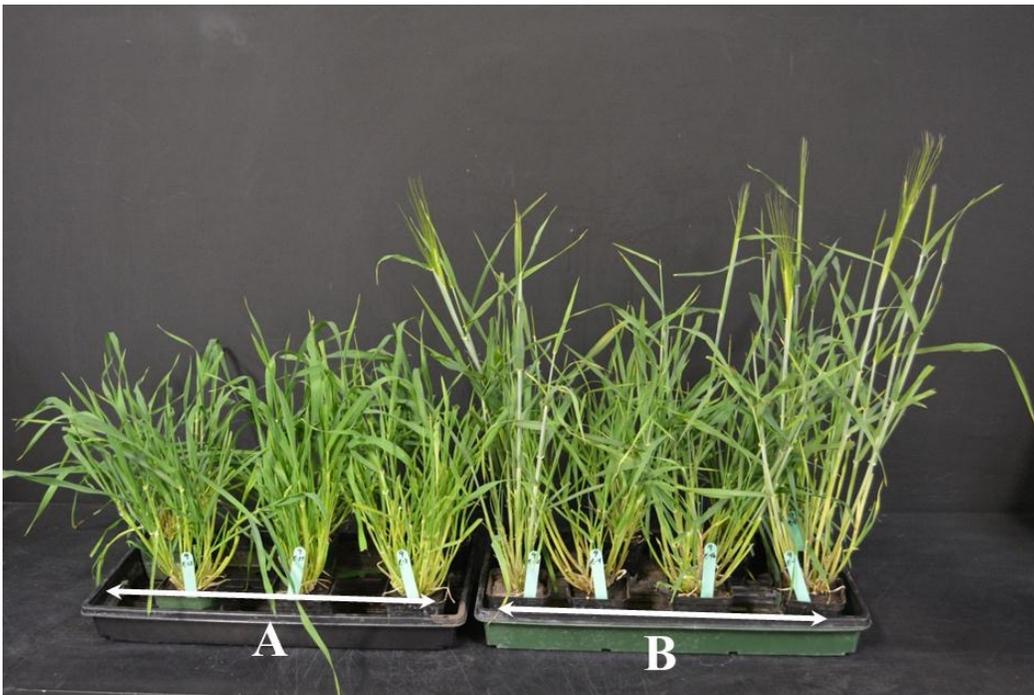
**Table 2:** List of crosses made between spring and winter barley types. Highlighted cells indicated barley parental varieties used to develop the three RIL<sub>2-5</sub> that enter in the field evaluation for cold tolerance and malting quality in 2016.

| Year | Cross # |   | <b>Spring<br/>genotypes</b> |   | <b>Winter<br/>genotypes</b> |
|------|---------|---|-----------------------------|---|-----------------------------|
| 2013 | 3       | ♀ | Meredith                    | ♂ | 02Ab431                     |
| 2013 | 4       | ♀ | AC Metcalfe                 | ♂ | 02Ab08-X05W061-208          |
| 2013 | 5       | ♀ | TR643                       | ♂ | 02Ab671                     |
| 2013 | 6       | ♀ | TR990                       | ♂ | 02Ab431                     |
| 2013 | 11      | ♀ | TR162                       | ♂ | 02Ab671                     |
| 2013 | 12      | ♀ | TR490                       | ♂ | 02Ab671                     |
| 2013 | 13      | ♀ | TR05912                     | ♂ | 02Ab08-X05W061-208          |
| 2013 | 14      | ♀ | CDC Reserve                 | ♂ | 02Ab431                     |
| 2013 | 15      | ♀ | Meredith                    | ♂ | 02Ab08-X05W061-208          |
| 2013 | 16      | ♀ | TR643                       | ♂ | 02Ab08-X05W061-208          |
| 2013 | 17      | ♀ | TR490                       | ♂ | 02Ab08-X05W061-208          |
| 2013 | 18      | ♀ | AC Metcalfe                 | ♂ | 02Ab431                     |
|      |         |   |                             |   |                             |
|      |         |   |                             |   |                             |
|      |         |   | <b>Winter<br/>genotypes</b> |   | <b>Spring<br/>genotypes</b> |
| 2013 | 1       | ♀ | 02Ab669                     | ♂ | TR990                       |
| 2013 | 2       | ♀ | 02Ab431                     | ♂ | TR5287                      |
| 2013 | 7       | ♀ | 02Ab08-X05W061              | ♂ | CDC Reserve                 |
| 2013 | 8       | ♀ | 02Ab431                     | ♂ | AC Metcalfe                 |
| 2013 | 9       | ♀ | 02Ab431                     | ♂ | Benthey                     |
| 2013 | 10      | ♀ | 02Ab08-X05W061              | ♂ | AC Metcalfe                 |

The F1 population was successfully produced (Figure 9). From the F1 populations, a single seed was planted per pot. Growth habit phenotypes were scored as either winter and spring type, representing genotypes with no flowers or all tillers fully flowered, respectively. Figure 10 shows F2 population that is segregating for growth habit. The genotypes with spring growth habit were selected and tested for cold tolerance.



**Figure 9:** Two F1 derived from a cross between 02Ab431 (♀- winter type) and Bentley (♂- spring type). We observed the presence of more tillers in the F1 populations compared to the spring type of the parental variety.

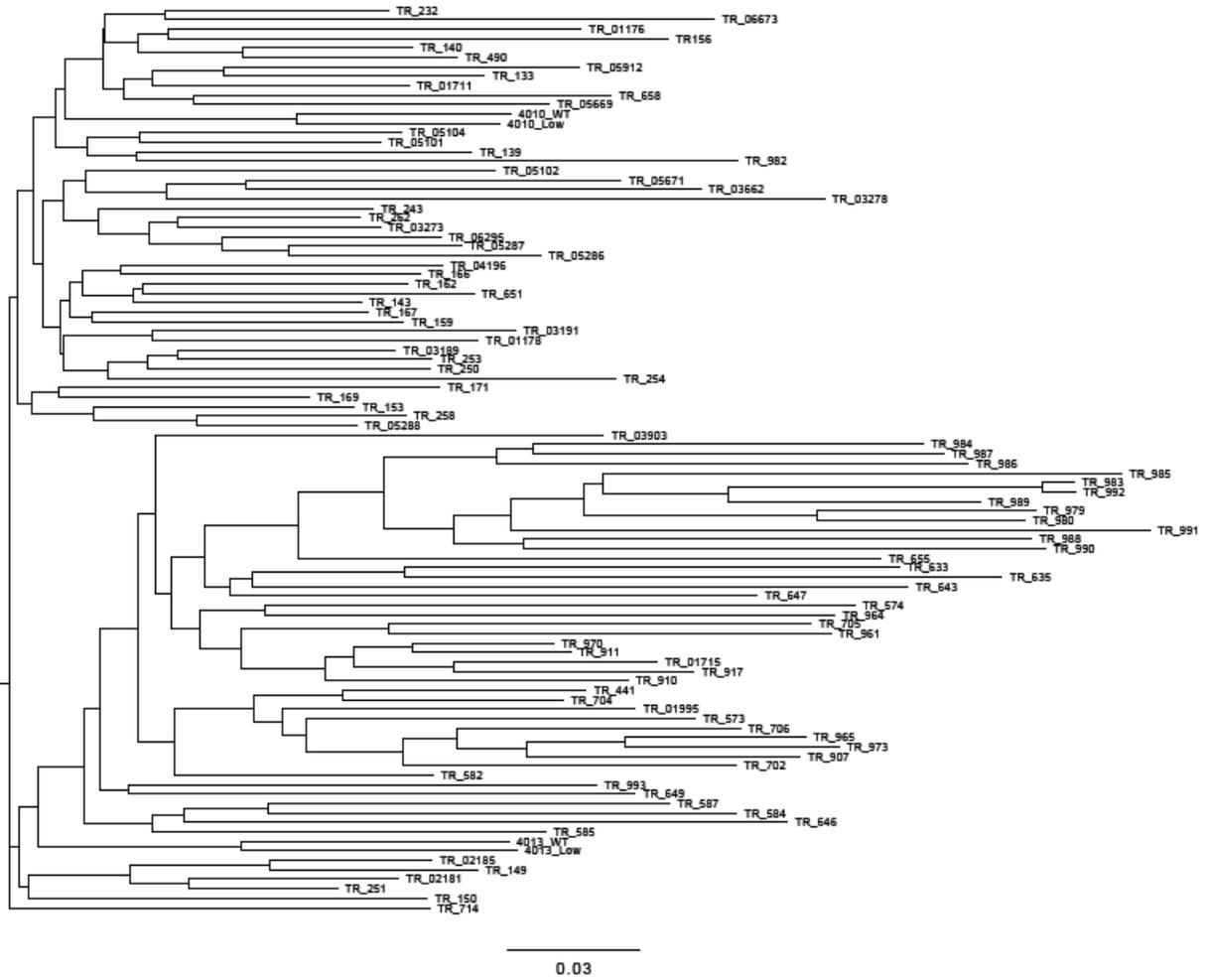


**Figure 10:** F2 population derived from a cross between 02Ab431 (♀) and Bentley (♂) and segregating for growth habit (spring versus winter type). The photo was taken 39 days after seeding. A: genotypes showing winter type. B: genotypes showing spring type with early flowering.

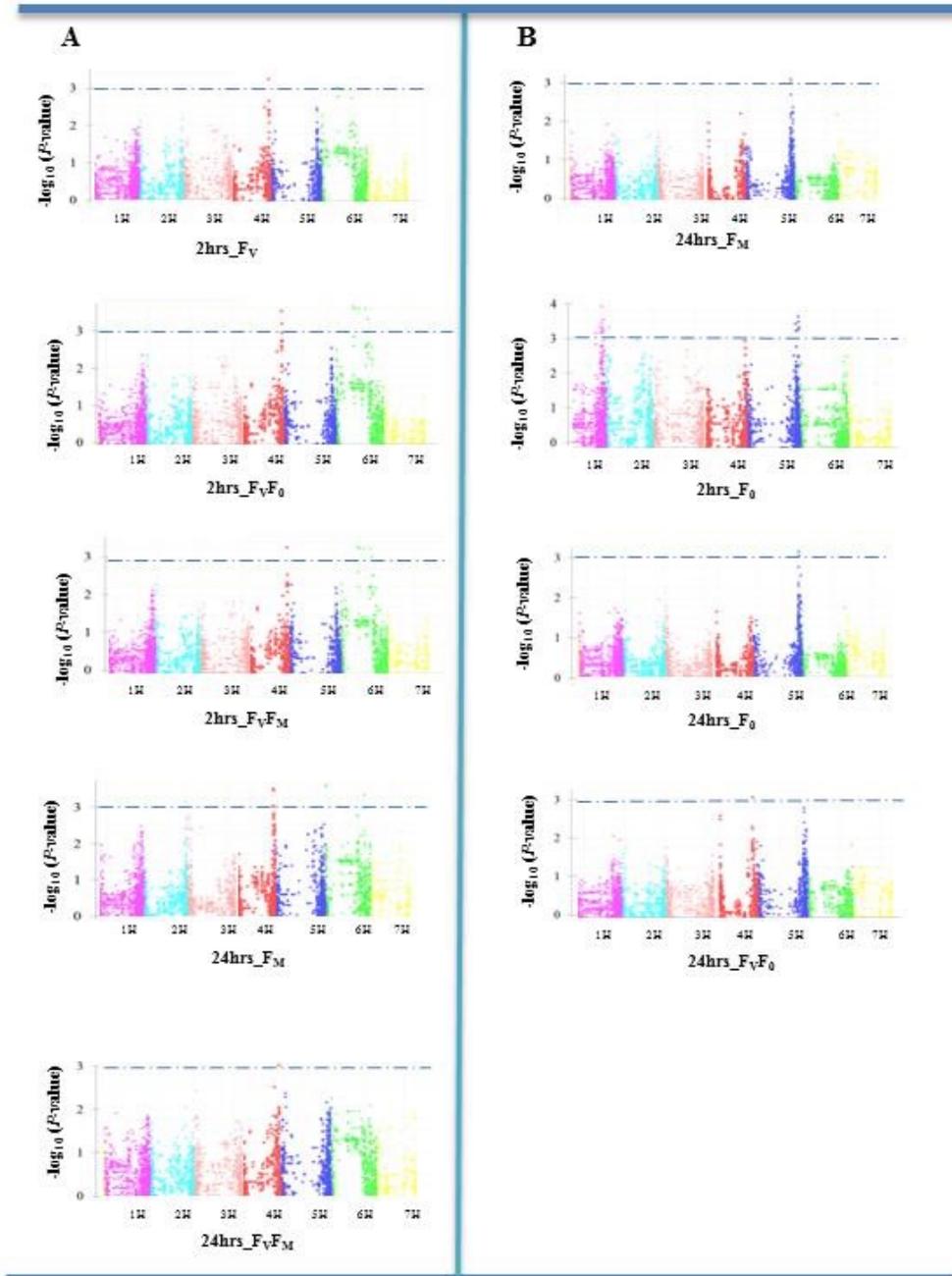
#### **5.1.6. Genome-wide association analysis: Population structure and association analysis.**

The neighbour-joining phylogenetic tree analysis, high resolution population structure using 7863 genome-wide SNPs differentiated all the lines from each other and clustered into two distinct populations (Figure 11). A total of 7864 SNPs were used for the genome wide association analysis (GWAS). The GWAS analyses identified 39 positive SNP associations with the chlorophyll fluorescence of which 21 were under cold acclimation and 18 were under freezing shock treatment (Figure 12). No significant SNPs were associated with traits related to chlorophyll fluorescence before cold treatment (Figure 13).

Under cold acclimation, the significantly associated SNP markers were located on chromosomes 4H and 6H (Figure 12). Seven SNP markers were found to be significantly associated with  $F_vF_M$  and chromosome 6H harbored the maximum number of markers associated with the trait (Figure 12). Seven markers displayed significant associations with  $F_vF_0$  while six markers were associated with  $F_M$  (Figure 12). Under freezing shock, the 18 positive SNPs were distributed on chromosomes 1H, 2H, 4H, and 5H with the majority found on 1H and 5H (Figure 12). Fourteen out of the 18 SNP markers were found to be associated with  $F_0$  and evenly distributed on chromosomes 1H and 5H (Figure 12).

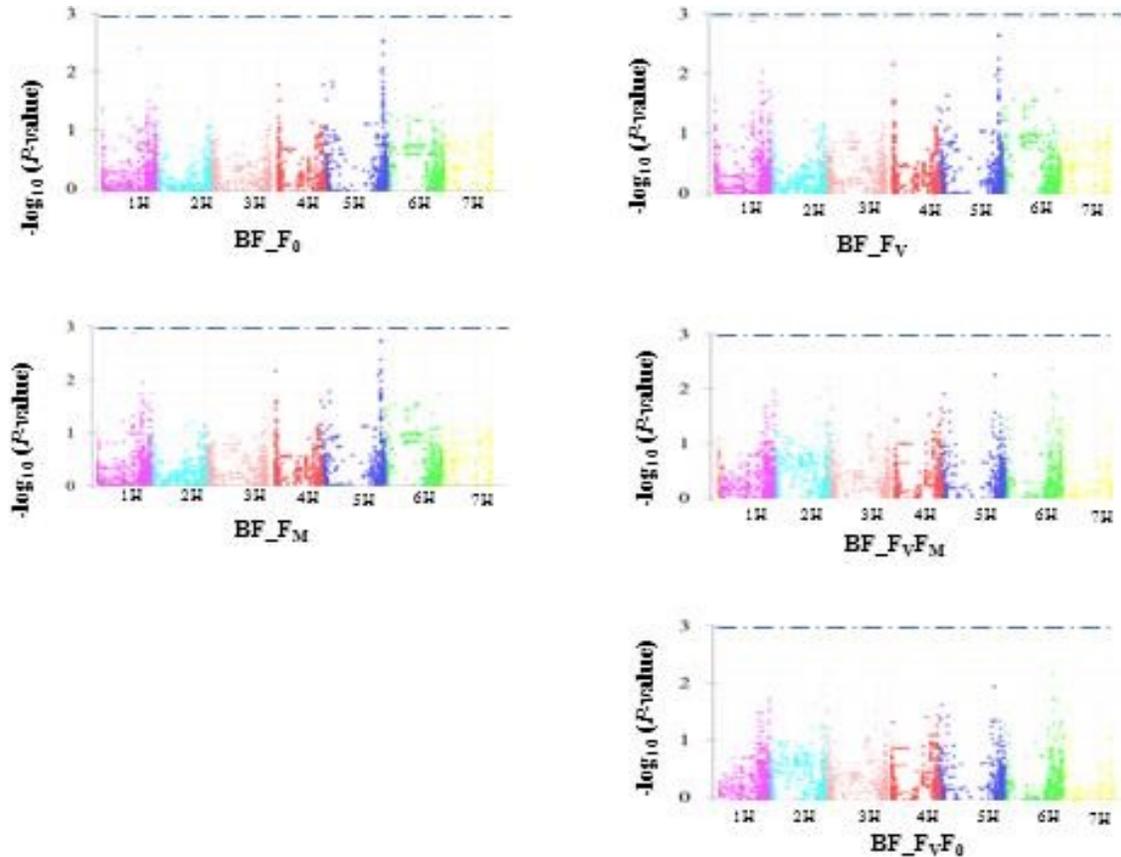


**Figure 11:** Phylogenetic tree depicting the genetic relations among the barley lines using 7864 SNPs.



**Figure 12:** GWAS showing significant  $P$ -values associated with chlorophyll fluorescence parameters using 7864 SNPs. The x-axis indicates the relative density of identified reference genome-based SNPs mapped on seven chromosomes. The y-axis represents the  $-\log_{10}$   $P$ -value for significant association with traits. The peaks above dashed lines indicate significance thresholds ( $P$ -value = 0.03). A = Cold acclimation; B = Freezing shock

2hrs\_ FvF<sub>M</sub> = FvF<sub>M</sub> 2 hours after cold treatment; 24hrs\_ FvF<sub>M</sub> = FvF<sub>M</sub> 24 hours after cold treatment; 24hrs\_ FvF<sub>0</sub> = 24 hours cold treatment; 2hrs\_ FvF<sub>0</sub> = FvF<sub>0</sub> 2 hours after cold treatment. H = chromosome



**Figure 13:** GWAS showing  $P$ -values associated with chlorophyll fluorescence parameters before cold treatment using 7864 SNPs . No significant SNPs were observed. Dashed lines indicate significance threshold. B = Before

## 5.2. Discussions

### 5.2.1. Chlorophyll fluorescence

The temperature at which cold or freezing injury occurs varies with the growth stage of the plant, moisture content, the length of time the temperature remains below freezing, and the amount of cold temperature hardening the plant has experienced. Frost cover (ice crystals) on a plant does not necessarily mean the plant has been damaged. Damage occurs when ice crystals form within the plant or the plant actually freezes, causing rupture of cell walls or physical disruption of the cell contents. The time interval in which freezing temperatures occur is important. A severe drop

in temperature which only lasts a very short time may not damage barley plants, while a light frost of a few degrees that lasts all night may cause severe damage.

Cold tolerance of barley at the seedling stage is difficult to measure in a single test because of the complexity of the resulting injuries and symptoms. In this study, cold tolerance was evaluated based on the visual symptom of the leaves and values observed of the chlorophyll fluorescence during the low temperatures treatment. The seedlings treated under this condition suffered leaf wilting as the duration of stress was prolonged.

Under both cold acclimation and freezing shock, the  $F_vF_M$  declines rapidly after cold treatment and can serve as an indicator of the freezing tolerance and subsequent loss of viability. The time to complete wilting of the leaves was shorter under freezing shock than under cold acclimation treatment. Previous investigations suggest that wilting was primarily caused by reduced water-uptake, and the stomatal response to water stress was not affected by lowered temperature. The depression in osmotic potential was accounted for an increase in sugars. Sugars are thought to function mainly as cryo-protectants. Previous results suggest that accumulation of these solutes is important not only for freezing tolerance but also for prevention of cell dehydration under low temperatures. Although it is possible that other photosynthetic parameters could be employed as an indicator of viability, the  $F_vF_M$  parameter is recommended for several reasons. First, as shown in Figure 4, any small decline in  $F_vF_M$  is easily noticeable and signifies clearly that loss of viability is imminent. The consistency of the  $F_vF_M$  parameter also increases the ease with which a threshold level can be defined. More importantly, unlike light-dependent parameters,  $F_vF_M$  is obtained from specimens in the dark-adapted state, negating the need for an extended period of illumination prior to measurement. Thus, as measurement of  $F_vF_M$  can be completed using a single saturating pulse, rapid screening of a large number of plants may be achieved.

The  $F_vF_M$  analysis competes with other well-established freezing tests. It is more sensitive and less subjective than plant survival analysis, which may have large experimental errors and involves considerable delay between freezing and the assessment of survival (Jenkins and

Roffey, 1974). Despite some limitations, freezing evaluation tests represent a useful tool for breeding purposes and for the evaluation of biodiversity. In this context, the  $F_vF_M$  analysis is an attractive test because it allows one to monitor the capacity to maintain a functional PSII after freezing, which may be associated with a crucial trait for the acquisition of the frost tolerance. To use the decline in  $F_vF_M$  as a means of determining viability after cold acclimation and freezing shock, it was necessary to identify a threshold  $F_vF_M$  level that would reflect a point at which recovery was no longer possible. As it is of course impossible to define an exact threshold level beyond which viability is lost, we identify a conservative threshold of 33% of control specimen measurements and showed that, in practice, decline of  $F_vF_M$  below this level no plants were viable upon re-watering.

### **5.2.2. Genome-wide association analysis**

In the present study we describe the application of whole genome association mapping in a panel of diverse spring barley lines for traits related to chlorophyll fluorescence under cold acclimation and freezing shock. We identified 39 positive SNP associations with the chlorophyll fluorescence parameters of which 21 were under cold acclimation treatment and 18 under freezing shock treatment. No significant SNPs were associated with traits related to chlorophyll fluorescence before cold treatment. Under cold acclimation, the significantly associated SNP markers were located on chromosomes 4H and 6H. Seven SNP markers were found to be significantly associated with  $F_vF_M$  and chromosome 6H had the maximum number of markers associated with the trait. Seven markers showed significant associations with  $F_vF_0$  while six markers were associated with  $F_M$ . Under freezing shock, the 18 positive SNPs were distributed on chromosomes 1H, 2H, 4H, and 5H with the majority found on 1H and 5H. Fourteen out of the 18 SNP markers were found to be associated with  $F_0$  and evenly distributed on chromosomes 1H and 5H.

## **6. Conclusions and Recommendations**

This project has generated substantial amount of information and tools that will be useful in breeding programs and other projects aimed at screening and developing barley varieties with improved cold tolerance.

The study has demonstrated that cold hardiness can be introgressed from winter barley into spring barley without the risk of transferring the genes related to vernalization requirement.

Among the F<sub>2</sub> populations, we identified few spring lines with early flowering compared to the parental lines. These lines would however need to be advanced to further generations and validated in field conditions before they can be deployed in breeding programs. We therefore suggest additional studies to refine and validate the cold hardiness of these lines.

The chlorophyll fluorescence is a useful tool to determine the degree of frost hardiness in spring barley. The measurement of the ratio  $F_v/F_M$  is reliable and can be rapidly done. For assessing frost tolerance in a controlled environment freezing tests, one has to be aware that supercooling has to be prevented, because it can affect the freezing temperature significantly.

If the cold hardiness present in the hardiest germplasm sources can be combined with other desired traits present in commercial cultivars, it should be possible to extend the range into colder regions and reduce the risk of cold injury.

## 7. Literature cited

- Babani, F. and H. K. Lichtenthaler. 1996. Light-induced and age-dependent development of chloroplasts in etiolated barley leaves as visualized by determination of photosynthetic pigments, CO<sub>2</sub> assimilation rates and different kinds of chlorophyll fluorescence ratios. – *J. Plant Physiol.* 148: 555-566.
- Bradbury PJ, Z. Zhang, D.E. Kroon, T.M. Casstevens, Y. Ramdoss, and E.S. Buckler. 2007. TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics* 23:2633-2635.
- Browse J. and Z. Xin. 2001. Temperature sensing and cold acclimation. *Current Opinion in Plant Biology* 4, 241–246.
- Canada Malting Barley Technical Centre, 2012 - <https://www.grainscanada.gc.ca/barley-orge/harvest-recolte/2012/qbsm12-qosm12-eng.pdf>).
- Chengci, C., J. Grant, N. Karnes, W. David, J. Gregory, and J. Duane. 2005. Determining the feasibility of early seeding canola in the Northern Great Plains. *Agronomy Journal*, Vol. 97, No. 4, pp. 1252-1262.
- Clement J.M.A.M. and P.R. van Hasselt. 1996. Chlorophyll fluorescence as a parameter for frost hardiness in winter wheat. A comparison with other hardiness parameters, *Phyton* 36 (1996) 29–41.
- Frachebound Y, Haldimann P, Leipner J, Stamp P. 1999. Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays* L.). *Journal of Experimental Botany* 50, 1533–1540.
- Ghassemi-Golezani, K., Khomari, S, Valizadeh, M. and H. Alyari. 2008. Effects of seed vigour and the duration of cold acclimation on freezing tolerance of winter oilseed rape, *Seed Sci. & Technol.*, 36, 767-775.
- Jenkins, G., and A.P. Roffey. 1974. A method of estimating the cold hardiness of cereals by measuring electrical conductance after freezing. *J. Agric. Sci. Camb.* 83:87-92.
- Krause, G.H., C. Vernotte, J.M. Briantais. 1982. Photoinduced quenching of chlorophyll fluorescence in intact chloroplasts and algae. – *Biochim. Biophys. Acta* 679: 116-124.
- Larcher, W. 2001. *Physiological plant ecology*, 4th edn. Berlin: Springer.
- Lichtenthaler, H.K., D. Meier, C. Buschmann. 1984. Development of chloroplasts at high and low light quanta fluence rates. – *Israel J. Bot.* 33: 185-194.

- Lichtenthaler, H.K. and U. Rinderle. 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. *CRC Crit. Rev. Anal. Chem.* 19 (suppl. 1 ), S29-S85
- Mishra, A., K.B. Mishra, K. B., I. I. Hoermiller, A.G. Heyer, and L. Nedbal. 2011. Chlorophyll fluorescence emission as a reporter on cold tolerance in *Arabidopsis thaliana* accessions. *Plant Signaling and Behavior*, 6: 301–310.
- McKenzie, R.H., A.B. Middleton, and E. Bremer. 2005. Fertilization, seeding date, and seeding rate for malting barley yield and quality in southern Alberta. *Canadian Journal of Plant Science.* 85:603-614.
- Novillo F, J.M. Alonso, J.R. Ecker, J. Salinas. 2004. CBF2/DREB1C is a negative regulator of CBF1/DREB1B and CBF3/DREB1A expression and plays a central role in stress tolerance in *Arabidopsis*. *Proc Natl Acad Sci USA* 101: 3985–3990
- Pritchard JK, M. Stephens, P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.
- Rizza, F., D. Pagani, A.M. Stanca, and L. Cattivelli. 2001. Use of chlorophyll fluorescence to evaluate the cold acclimation and freezing tolerance of winter and spring oats. *Plant Breed.* 120:389–396
- Rohde P, D.K. Hinch, A.G. Heyer. 2004. Heterosis in the freezing tolerance of crosses between two *Arabidopsis thaliana* accessions (Columbia-0 and C24) that show differences in nonacclimated and acclimated freezing tolerance. *Plant J* 38:790–799
- Schapendonk, A.H.C.M., O. Dolstra, O. Van Kootenz. 1989. The use of chlorophyll fluorescence as a screening method for cold tolerance in maize. – *Photosynth. Res.* 20: 235-247, 1989.
- Smillie, R.M., and S.E. Hetherington. 1983. Stress tolerance and stress induced injury in crop plants measured by chlorophyll fluorescence in vivo. *Plant Physiol.* 72:1043–1050
- Steponkus, P.L., D.V. Lynch, and M. Uemura. 1990. The influence of cold acclimation on the lipid composition and cryobehaviour of the plasma membrane of isolated rye protoplasts. *Philos Trans R Soc Lond B Biol. Sci.* 326: 571-583.
- Sthapit, B.R., J. R. Witcombe, and J.M. Wilson. 1995. Methods of selection for chilling tolerance in Nepalese rice by chlorophyll fluorescence analysis. *Crop Science* 35,90–94.
- Thomashow, M. F. 1999. Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. *Annu. Rev. Plant Biol.* 50, 571–599.

## 8. Benefits to the industry

### 8.1. Impact of the project results on Alberta's agriculture and food industry.

New breeding lines of malting barley with improved cold tolerance and malting quality towards the breeding of high and stable yielding varieties on the Canadian Prairies. The breeding lines developed will be owned by AITF. Benefits will be shared with any user of the breeding lines to develop new varieties. This would be subjected to negotiation.

#### Benefits for Alberta:

| <b>Benefit</b>   | <b>Time Horizon to Recognize Outcome</b><br>(Short: 0-3 years, Medium: 4-7 years, or Long: 8 years +) |
|--|---|
| New cold tolerant varieties/<br>germplasm developed  | Medium: 4-7 years   |
| Farmers would have a wider<br>window of opportunities for crop<br>production and timely weed control<br>in seeded spring barley. | Medium: 4-7 years, or Long: 8 years +   |
| New varieties/germplasm with<br>improved malting quality   | Medium: 4-7 years, or Long: 8 years +   |

### 8.2. Potential economic impact of the project results

- Alberta's Agri-Food Industry growth strategy proposes to grow the industry to \$20B/\$10B (value added/primary agriculture) by 2030. The development of new crop varieties is a key requirement.
- This project will develop spring barley lines with improved cold tolerance. Delayed seeding by several weeks can result in a 17.1 % decline in grain yield (McKenzie et al. 1982). This translates to an annual loss of billions of dollars to the Canadian economy (C-CIARN Archive – 2003).
- Farmers would have a wider seeding window and timely weed control in seeded spring barley.

- Cold-tolerant spring barley would improve quality for high-value markets and lower risk of crop failure. Growers could plant their crops earlier and maximize the utilization of initial soil moisture.
- Early seeding leads to earlier harvesting, generally under more favourable conditions; and thus improves grain quality.
- Cold tolerant spring barley varieties would have the opportunity to maximize yields.
- If a barley variety with 20% more yield under cold environment was seeded, this would have guaranteed grain yield of 4.1 million tones and \$81.3 million gain in revenue.
- While the production of new cold tolerant varieties of barley would cost several million dollars, this cost could be recovered in a single cold year given a good adoption rate of the new varieties.

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

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

- Five research scientists.
- One Senior Technician.

**10. Knowledge transfer/technology transfer/commercialisation (max 1 page)**

**Publications and communication activities**

- Capo-chichi, L.A.J., A. Beattie, P. Juskiw, J. Nyachiro, R. Lange, and J. J. Slaski. Association of SNP markers and chlorophyll fluorescence parameters with low temperature tolerance in spring barley. 12<sup>th</sup> International Barley Genetics Symposium. June 25 – 30, 2016. Minneapolis, Minnesota. USA.
- Capo-chichi, L.A.J., A. Beattie, P. Juskiw, J. Nyachiro, R. Lange, and J. J. Slaski. Low temperature tolerance of spring barley for improvement of early crop establishment. International Conference Botany, July 25 – 29, 2015. Edmonton, Alberta Canada.
- Capo-chichi, L.A.J., A. Beattie, P. Juskiw, J. Nyachiro, R. Lange, and J. J. Slaski. Genetic variations in low temperature seedling performance. Prairie Grains Development

Committee (PGDC), Prairie Grain Recommending Committee for Oat and Barley (PRCOB), and Brewing Malting Barley Research Institute (BMBRI), February 22, 2016. Saskatoon, Saskatchewan, Canada.

- Capo-chichi, L.A.J. Physio-Breeding for Performance Traits in Barley: Genetic Improvement of Yield and Yield Stability. Annual General Meetings/Alberta Barley Commission. December 12, 2014 Banff, Alberta, Canada.
- Capo-chichi, L.A.J. Presentation at the FarmTech 2013, 2014, and 2015. Edmonton, Alberta, Canada.
- **Popular press**

<http://www.rcinet.ca/fr/2015/08/20/prairies-des-plantes-qui-pousseront-malgre-la-secheresse/>  
<https://twitter.com/rcinet>

Aussi disponible sur Facebook à <https://www.facebook.com/rcinet>  
<http://www.rcinet.ca/fr/author/mjobin/>

## **11. Acknowledgements**

This study was funded by Brewing and Malting Barley Research Institute (BMBRI) and Alberta Crop Industry Development Fund (ACIDF). We are grateful to the staff of Field Crop Development Centre (FCDC), Lacombe and the Crop Development Centre (CDC), Saskatoon for providing materials used in this study. In-kind support provided by FCDC and CDC for making available the SNP markers used in this study. We are also grateful to the staff of FCDC Biotechnology Lab molecular data analysis.

## **Section D: Project resources (See separate document)**

### **1. Statement of revenues and expenditures:**

- a) 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.
- b) Provide a justification of project expenditures and discuss any major variance (*i.e.*,  $\pm$  10%) from the budget approved by the funder(s).

## 2. Resources:

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

| <b>Total resources contributed to the project</b> |                     |   |               |
|---|---------------------|---|---------------|
| <b>Source</b>                                     | <b>Total amount</b> | <b>Percentage of total project cost</b> | <b>Status</b> |
| Funder (BMBRI)                                    | \$48,898.08         | 17.85%                                  | Received      |
| Funder (ACIDF)                                    | \$30,000.00         | 10.95%                                  | Received      |
| Other government sources:<br>Cash                 | \$194,983.09        | 71.20%                                  | Received      |
| Other government sources:<br>In-kind              |                     | %                                       |               |
| Industry: Cash                                    |                     | %                                       |               |
| Industry: In-kind                                 |                     | %                                       |               |
| <b>Total Project Cost</b>                         | <b>\$273,881.17</b> | <b>100%</b>                             |               |

| <b>External resources (additional rows may be added if necessary)</b> |              |                |
|---|--------------|----------------|
| <b>Government sources</b>   |              |                |
| Name (only approved abbreviations please)                             | Amount cash  | Amount in-kind |
| Alberta Innovates – Technology Futures                                | \$194,983.09 |                |
|   |              |                |
| <b>Industry sources</b>   |              |                |
| Name (only approved abbreviations please)                             | Amount cash  | Amount in-kind |
| Brewing and Malting Barley Research Institute (BMBRI)                 | \$48,898.08  | \$0            |
| Alberta Crop Industry Fund (ACIDF)                                    | \$30,000.00  | \$0            |

## **Section E: The next steps (max 2 pages)**

- a) Is new research required to deal with issues and opportunities that the project raised or discovered but were not dealt with within the current project?

Selected barley genotypes at the seedling growth stage respond differently to cold acclimation and freezing shock. A few genotypes have shown superior ability to tolerate freezing shock than others, but less tolerance under cold acclimation. However, this mechanism is not fully understood. Success in breeding for traits related to cold tolerance can only come about when the limitation is better understood.

- b) Is there related work that needs to be undertaken to continue advancement of the project technology or practice?

Alberta Innovates – Technology Futures (AITF) in collaboration with the Crop Development Centre (CDC) in Saskatoon and the Field Crop Development Centre (FCDC) in Lacombe have developed barley recombinant inbred lines (RILs) that are segregating for cold tolerance at the seedling growth stages. This work was conducted under controlled environments. New research is required to evaluate these RILs for cold tolerance under field conditions and advance the populations to deliver high yielding breeding lines with improved cold tolerance and malting quality.

Specific objectives are:

- 1- Develop barley breeding lines (germplasm) with enhanced cold tolerance and superior malting quality attributes. These lines will then be given to the FCDC and CDC breeding programs for further evaluation under field condition and released as commercial cultivars or used as parents in the breeding programs.
- 2- Screen the RILs seed protein content and starch; and other malting quality traits required by the malting industry.
- 3- Validate SNPs markers for cold tolerance identified and molecular markers developed for malting quality. The FCDC have developed six molecular markers for malting quality for use in

marker assisted selection approach towards the breeding of elite varieties with improved cold tolerance, high yield, and traits related to malting quality.

c) Did the project identify any new technology or practice that needs to be developed? We applied the method of chlorophyll fluorescence developed from previous research to screen barley genotypes and identify molecular marker linked to the traits related to cold tolerance. This method used has been optimized and new protocol has been developed to screen barley varieties for cold tolerance.

**What suggestions do you have that increase commercial use of results by farmers and/or companies.**

- We hold an annual field day which attracts between 70 to 100 participants. The information at this field day is represented at the provincial field day as well. A farmer specific information package will be made available to all interested Alberta agriculture crop representatives.
- AITF utilizes its communication's team to contact publications such as the Top Crop Manager, Western Producer, and others, to publicize the results.
- Two minute video indicating the results of our study to be distributed through AITF communications to interested outlets.
- We also host special tours from the industry including seed growers, seed companies, beef producers, hog producers, and funding agencies. At least two tours are organized with producers, growers, and seed companies.
- We publish in many international journals depending on the topic. (e.g Journal of Cereal Science)
- We also collaborate with University professors and publish with them on feeding and animal science related topics (e.g. Journal of Cereal Science or Journal of Crop Science).
- We will continue to use a multi-format approach toward the transfer of the technology to the seed, grain and livestock producers.