Final Report to

North Alberta Development Council

Title:
Development of Early Maturing Flax for Northern Grown Regions

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Executive Summary

The objective of this study was to develop early maturing flax lines suitable for the growing conditions of northern Alberta. A portion of a population of 10,000 ethyl methanesulfonate (EMS) mutagenized M2 families was screened. This population was a CDC Bethune variety, which has been produced, maintained and provided by the University of Alberta. We randomly selected 5456 plants from the mutation population and planted them in the field at AITF's experimental farm in Vegreville. Based on field performance, 35 lines were selected for further assessments in a greenhouse setting. Indoor screening resulted in the selection of 10 lines for 2013 field seed increase/evaluation trial followed by the 2014 plot experiment that evaluated northern adopted characteristics of the selected flax lines. Three lines exhibiting early maturity were selected for further testing. This project initiated another alternative to obtain elite gemplasms that have characteristic of early maturation that are suitable for northern Alberta and especially the Peace Country flax growers.

1. Introduction

A reliable supply of flax for healthy food industries is essential to the future of the Canadian bioeconomy industry. According to the goals of Flax Canada, the flax acreage will be approximately 1.7 million acres by 2015 (Flax Council of Canada). The flax industry contributes approximately $300 million annually to the Canadian economy. Analysis of flax grown in the Peace Country (northern Alberta) showed higher α-Linolenic acid (ALA) and lignans. Gas chromatography analyses of fatty acids profiles of the brown flax Vimy from PC Milling grains of the Peace Country showed an ALA increase of 9.9% over CDC Bethune grown in central Alberta.. The secoisolariciresinol diglucoside (SDG) content in flax grown in the Peace Country was 15-66% higher as compared to the crop grown in central Alberta (Zhang and Cao, 2009).

The advantage of growing flax in Peace regions has not been fully realized due to the lack of suitable flax varieties adapted to the regional climate conditions. Flax Council of Canada reported that only about 9% of flax is produced in Alberta (http://www.flaxcouncil.ca/english/index.jsp?p=statistics2&mp=statistics). Most of Canada's flax production is concentrated in southeast Saskatchewan and southwest Manitoba. Since flax is one of the latest-maturing traditional crops, there is a significant increased risk of having insufficient frost-free days for flax maturation in the northern zone climate. The northern climate also increases the challenges of harvest delays and harvest difficulties due to indeterminate growth (continuous flowering), late maturity and stems that remain green after the bolls are mature.

Therefore, development of early maturing (early flowering has a good correlation with early maturity) flax varieties is urgent to promote high quality, Alberta-grown flax production. Traditional breeding takes longer (~10 years) than marker assisted breeding (MAB, ~5 years) to commercialize a new variety, so development of forward genetics approaches for an early flowering trait in flax is a pre-requisite for this project.

The demand for early maturing flax has attracted the attention of multinational companies like CPS (formerly Viterra) to Alberta for the development of flax breeding programs aiming to capture the opportunity in this prime flax growing region.

The development of the agronomic traits and nutrient value of crops like flax through genetic improvement can take advantage of effective genomics technologies. A population of 10,000 EMS mutagenized M2 families of the CDC Bethune variety has been produced and maintained at the University of Alberta. We applied both a forward and reverse genetics approach to identify early maturing flax lines and the genes involved in early maturing (early germination, early flowering), and possibly other improved qualities (cold tolerance). The development of an elite germplasm platform and post-genomic technology has made flax ready to embrace and create new opportunities through forward and reverse genetic for northern flax growth regions.

The overall objective of this project was to develop elite germplasm exhibiting characteristics of early maturation that would be suitable for northern Alberta and especially the Peace Country flax growers. We were using phenotypic screening of a non-genetically modified organism (non-GMO) mutant population.

Specific objectives included:

1) Phenotypic identification of EMS mutants of elite flax variety that shows early maturing characteristics
2) Evaluation of selected lines for agronomic performance in the northern regions.

2. Research Methodology

2.1. Location of the trials in 2014

The early maturing flax trials evaluating seeding date, seeding rate, genotype (EMS mutation lines), and flax field performance were conducted at Vegreville, Alberta (53°30'19.003" N; 112°05'52.004" W) in 2014 after the previous two years of screening. The Vegreville site is operated by Dr. Jan Slaski's group at Alberta Innovates Technology Futures's (AITF).

2.2. Site preparation for 2014

The agronomy trials were seeded into barley (Vegreville) stubble land. Soil samples were taken and soil fertility tests were performed in early spring for Vegreville. The tests revealed that the soil at Vegreville was depleted of nitrogen (16 ppm N at Vegreville) and deficient of phosphorus (9 ppm P at Vegreville) in the top 15 cm. Prior to staking out the experimental plots in Vegreville, barley straw residues were harrowed in different directions to create an even coverage across all trials.

2.3. Seeding practice for 2014

In 2014 the plots were seeded with a Fabro plot seeder (with 10 zero-till disc openers, 20 cm apart). All Fabro seeders were manufactured in Swift Current, Saskatchewan. Plot size at the AITF Vegreville site was 12 m^2 (2m x 6m). To circumvent the edge effect, guard plots flanked each seeded replicate. Lines were seeded including lines selected from the 2013 screening test plot. Plots were seeded directly into barley stubble on June 2. Mineral fertilization was based on the soil test results. At the Vegreville site, 72 lb/ac N (102 lb/ac of 46-0-0), 21 lb/ac K (35 lb/ac of 0-0-60) and 21 lb/ac S (87.5 lb/ac 20.5-0-0-24) for a 48 bu/ac yield were cross banded immediately before seeding and 34 lb/ac P (65.4 lb/ac 11-52-0) was applied with the seed.

2.4. Trial maintenance in 2014

In the 2014 Vegreville trial, Round-up Transorb (glyphosate) was applied at a rate of 2.5 L/ha as a pre-seed burn-off and Poast Ultra (0.2 L/ac) followed by Buctril M (0.4 L/ac) as in-crop weed control. Some hand weeding of the plots at early boll setting stage was also necessary.

2.5. Harvest management in 2014

Each tested line was hand harvested, the plants were bundled and brought to a header house for thrashing and clipper cleaning.

2.6. Observations and measurements of crop performance

Plots were inspected on a regular basis to monitor the effects of the applied treatments on crop performance. The following observations and measurements/ratings were made during the course of the growing season:

- Days to flowering (50% flowers in bloom)
- Days from seeding to physiological maturity (75% brown bolls)
- Lodging rating (if lodging is present) 1 = erect, 9 = completely flat
- Disease occurrence and severity (1-10 scale) where 1 = no disease; 10 = severe infestation

3. Results

The research team performed three years' of experiments to develop the elite flax gemplasms that have early maturing characteristics. The milestones and results are summarized below in Table 1.

Table 1: Milestones and Results

Task	Deliverable	Finish Date	
(a) Planting 5,000 mutation lines in AITF Vegreville centre; Phenotypic screening for early germination, early flowering and cold tolerance lines	Criteria for early germination, early flowering will be defined	Dec, 2012	Completed. 5456 lines were planted in AITF's Vegreville experiment farm
(b) Harvesting and store the chosen lines with targeted characteristic	Chosen mutation lines will be documented	Mar, 2013	Completed. 35 lines were selected
(c) Characterize the early maturing flax lines by December 2013	Agricultural trials	Sep, 2013	Completed
(d) Planting selected line in plot experiment in northern Alberta	Agricultural excellence	April 2014	Completed
(e) Document the plotting experiments		November 2014	Completed
(f) final report		March 2015	In progress

3.1 2012/2013 field Phenotypic Screening

To screen the mutation population, a well-defined field trial was carried out in spring of 2012. On May 28, 2012, 5456 plants randomly selected from an EMS mutation population were planted on AITF's experiment farm. Fertilizing and weeding were applied to the plots. Regular observations of the plots were conducted.

The observations were focused on flowering time, plants overall appearance and seed pod development (Figure 1). The growth stages were assessed and recorded. 35 plants showed

early flowering phenotype and were labelled. Harvesting was performed on Octctober 1, 2012. Further greenhouse screening of selected lines was carried out at the greenhouse facility at AITF Vegreville. The subsequent generation's seeds were harvested and were put through further screening.

Fig 1. Flax plants in fields. A. Labeled plant; B. early flowering plants

3.2. Field trail of selected lines in 2013

Ten lines with expected early maturing characteristicwere seeded on June 5, 2013 in Vegreville. Single rows of tested lines were placed next to experimental plots of six flax cultivars registered for cultivation in Canada including CDC Arras, CDC Bethune, CDC Sorrel, Hanley, Vimy and Prairie Grande. This placement permitted visual comparisons of the lines with performance of commercial cultivars. Prior to seeding on June 4, the plots were treated with herbicides (Start-up + MCPA ester 600) to control weeds. In-crop herbicide treatment involved application of Poast Ultra + Buctril M with the back-pack sprayer on June 13.

Based on visual evaluation we can conclude that the tested lines in general matured earlier or at the same time as that the commercial cultivars did. Clear identification of top performers

within the pool of 19 lines with regard to maturity was somewhat concealed by late seeding. Because the lines exhibit potential for selection of superior, early maturing genetics, we recommend re-testing them in the field. An early May seeding date should be used to screen the lines for fitness to the northern soil/climatic conditions. Such experiments were planned for the 2014 growing season in Vegreville as a part of a larger project, testing newly developed flax cultivars adapted to the northern prairies by Viterra.

The plants showed normal growth and development and produced normal seed (Figure 2).

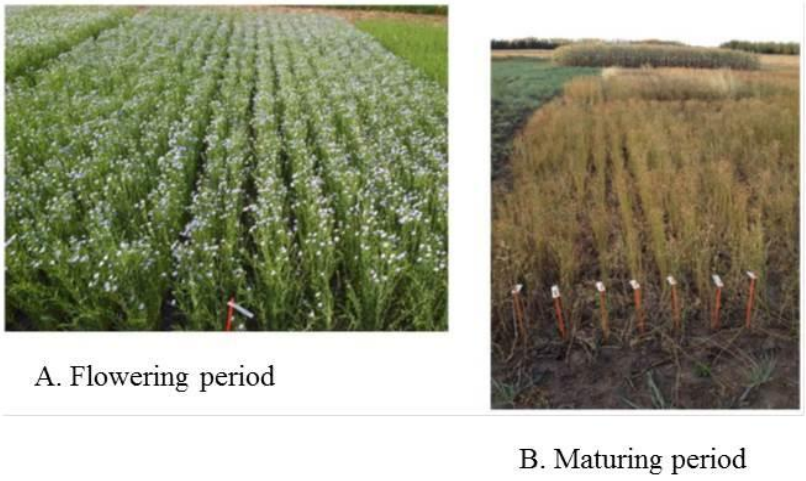

Fig. 2. 2013 Flax filed trail in AITF experiment farm

3.3. Agricultural performance trial in 2014

In addition to ample precipitation recorded in Vegreville in the first part of the summer, the 2014 season was characterized by lack of late spring and late summer frost as summarized in Table 2.

Table 2: Precipitation (mm) at Vegreville in the 2014 flax growing season

Month of the year	Vegreville
May	58
June	96
July	120
August	24
September	25
Total	260

3.4. Evaluation of field performance of flax based on visual observations

- Uniformly distributed and sufficient precipitation in spring (84 mm and 65 mm at Vegreville, for April and May, respectively), contributed to good germination and robust crop establishment.
- No infection with pasmo was observed, while some flax plots grown in close proximity developed moderate to severe disease symptoms.
- None of the tested lines exhibited late summer re-flowering (indeterminate growth).

4. Discussion

The development of the early maturing flax for the northern region from a mutation population was carried out in 2012 based on phenotypical characteristic screening. Early germination, early flowering and early maturation were identified as the screening consideration. The planted population exhibited a variety of phenotypes including plants with 1) tall stems; 2) early germination; 3) early flowering; 4) elevated number of branches; 5) tolerant to herbicide and others. The field trials carried out in the 2013 and 2014 growing seasons were aimed at evaluation of the comparisons with control variety, CDC Bethune and leading varieties of northern adopted lines that are currently under evaluation. The variety Prairie Grande was used as a control. Nine parameters evaluating field performance of flax were taken into consideration including stand density, days to flowering, and days to maturity, canopy height, seed and straw yield, harvest index, disease occurrence and severity and

lodging. Such a comprehensive study should provide information facilitating adoption of flax in the northern portion of the Canadian Prairies.

5. Conclusions

Based on three years field screenings and trials, the research team has identified some promising early maturing flax lines as germplasms to provide breeding material for northern Alberta regions. The following specific conclusions can be drawn:

1. The best lines obtained from the study are indicated in Table 3:

Table 3: Maturity Ranking of EMS flax lines in 2014 field trail

Flax lines	Maturation Ranking*
717-2	Early
2701-3	Early
2854-2	Early

*Prairie Grande was used as a benchmark. Observations made on Sep. 05, 2014

2. The mutation population holds key for development of new and elite flax varieties that can avoid need for application of genetic modification approaches.
3. Integration of selected flax lines for northern regions.
   With 3 early maturity ranking flax lines, a full scale flax trial will be required to carry out in the Peace regions. These lines will join Dr. Jan Slaski's northern adopted flax program for further evaluation depending on additional funding support.

6. References

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