Biochemical Profiling of Selected Lines/Varieties of Northern Adapted Flax and Full Utilization of Alberta's Flax Fiber

Northern Alberta Bioeconomy Initiative

Final Report

Report to:

Northern Alberta Development Council

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Executive Summary

The project titled “Biochemical Profiling of Selected Lines/Varieties of Northern Adapted Flax and Full Utilization of Alberta’s Flax Fiber- Northern Alberta Bioeconomy Initiative” has been carried out according to original plan. The biochemical analysis protocol for fatty acid has been verified and selected flax lines exhibiting early maturity traits were planted at several sites in northern Alberta and at the Vegreville centre under respective growth conditions.

Initiated by this project, a BioFiber and Food for Northern Alberta Working Group was formed including the Government of Alberta and Northern Alberta Development Council (NADC), InnoTech Alberta and relevant industry representatives. Well-coordinated meetings have taken place to collaborate among stakeholders. Initiatives such as a seminar series promoting the flax and industrial hemp industry have been implemented in northern Alberta.

Marketing feasibility of potential Alberta flax fiber exporting to new market and visits to Chinese textile fiber industries have been completed. Assessment of supplying alterative material source to textile industry in China has been identified.

Preliminary genomic research showed a clear difference from winter retted flax materials. This provided direction for further research for the flax fiber industry. The varietal incorporation into the flax breeding program using selected early maturing germplasm from this project has been initiated.

Project deliverables revealed:

- The biochemical analyses of fatty acid profiles of three early mature lines are comparable to the previous study which documented a northern advantage, and appeared to be three to four days early maturation than the reference flax line.
- Initial conversations with two Chinese textile manufactures indicate their genuine interest in exploring a possibility of sourcing Alberta grown flax fiber for their linen fabric production.
- Retting genomic research provided basic understanding of winter field flax retting process, and that northern Alberta could provide natural retting condition.

Based on the project results, we recommend:

- Coordinating the efforts of the relevant stakeholders to develop flax as a viable food and fiber crop driving economic development of the northern Alberta region.
- Selected early maturing flax accessions be incorporated into commercial Canadian flax breeding program lead by the Crop Development Centre.
- Further detailed research on northern Alberta conditioned retting application for the flax fiber industry should be continued.

Table of Contents

List of Figures

List of Tables

Introduction

Flax (*Linum usitatissimum* L.) is a commercially important oilseed and textile fiber crop. Flax oil has both industrial and food applications, and flax meal is used as ruminant feed. Flax fibers are used for the manufacturing of high quality linen fabrics and other industrial products. Flax seed has also increasingly been used as a nutraceutical in recent years since it contains exceptionally high concentrations of phytoestrogenic compounds known as lignins and dietary fibers, in addition to a high content of ω-3-fatty acid. Analysis of flax grown in the Peace country (northern Alberta) showed elevated ALA and lignin levels.

Stem residues of flax grown in Alberta after seed harvesting are a concern for the growers. Presently there is virtually no use for the flax straw. Straw left on the ground decomposes slowly and causes problems with the agronomic management of the next year's crop.

Flax is not well adapted to the northern prairies and, as a consequence, most of Canada's flax production is concentrated in southeast Saskatchewan and southwest Manitoba. Flax grown in Alberta is concentrated in the south and the Peace Region. Since flax is one of the latest-maturing traditional crops, there is a significantly 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 (reflowering), late maturity and stems that remain green after the bolls are mature.

Taking advantage of northern vigor for producing good quality flax is dependent on the availability of early maturing flax varieties. 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 forward and reverse genetics approaches to identify early maturing flax lines and the genes involved in early maturing (early germination, early flowering), and possibly other improved qualities (cold tolerance). Based on the previous

three-year field screening conducted by AITF and the University of Alberta, we have obtained three lines; 717, 2701 and 2854, for this project.

In order to improve the flax agronomic characteristic especially for trait like early mature, InnoTech Alberta carried out preliminary research on winter retting of flax straw and Fiber processing in our decortication facility. These activities attracted the attention of the Chinese linen industry which was interested in utilization of unexploited post-harvest residues. The collaboration between NADC and InnoTech Alberta takes advantage of InnoTech Alberta's research and connection with the Chinese linen fabric industry to add value to Alberta's flax fiber and to address the concerns of flax growers with respect to straw management. The germplasms identified from this project are ready to be incorporated into the breeding program led by Crop Development Centre for flax.

There is growing interest, trend and demand for supply among the local, provincial and international bio fiber food industry. Canada is the leader for flax seed production and is sold to multinational markets.

Flax is a great opportunity for agricultural sustainability and diversification for our northern Alberta economy. There is a need to recognize the full value of the opportunity for bio fiber and food in local, national and international markets.

The rough estimates for the potential financial return are based on licensing the new germplasms to flax breeder who will breed early maturity flax for growers. Flax is currently grown on approximately 3 million acres in North America. According to Flax Canada 2015 Vision, the flax acreage will be increased.

Recognition of higher quality commodity such as flax from northern Alberta will create an opportunity to Alberta farmers to obtain better pricing and higher value for their products.

The initiative of introducing Alberta's flax fiber to be a part of Chinese linen supplier will not only benefit to the province's bio economy's strategy, but also will help flax growers to eliminate straw left over and promote more flax planting in the province, especially in northern Alberta.

The research team visited Jianyan, Rugao, Jiangsu province of China near Shanghai in 2017. The president and CEO of relevant companies met the team and provided tours of very impressive facilities. The conclusion is that the socio-economic impact for the northern Alberta region of becoming an alternative, high end fiber supplier to the textile industry in China will be very important.

Overall Objectives:

The objectives of this project were to document the biochemical profile of selected lines and varieties of northern adapted flax and lay foundation for full utilization (grain and straw) of northern Alberta flax.

Scientific Objectives:

1. Analysis fatty acids profile of elite lines that showed early flowering or northern adapted characteristics in field trials.
2. Document and report the fatty acids composition of selected lines.
3. Detail research of flax retting under northern Alberta environments.

Business Objectives:

1. Provide fundamental nutritional data for northern Alberta and Canada flax industry to provide evidence to the Alberta flax industry for promoting and marketing its product to full potential.
2. Verify performance of the selected elite flax lines developed for early maturity under northern Prairies conditions.
3. Indicate development of markets for Alberta's flax fiber and build the foundations for full utilization Alberta bio-fiber potential. The initiation of a feasibility of marketing access research on the linen textile fiber supply chain in Jiangsu province of China.

Materials and Methods

Plant materials:

The best performing flax lines obtained from previous studies were selected for this project. Lines 717, 2701 and 2854 from 600 M3 EMS mutagenesis lines are used in this study and CDC

Bethune was used as check line for the whole project. All three lines exhibited earlier flowering and maturity characteristics by approximately 10 days. The planting and location of trials are described below.

Field Locations

The trials were carried out at northern Alberta locations:

1. Mackenzie Applied Research Association (MARA), 5901 River Road, Fort Vermilion, Alberta Canada, T0H1N0 (www.mackenzieresearch.ca), 58°3854565; 116°0234012.
2. Smoky Applied Research And Demonstration Association - SARDA , 701 Mainstreet,T0H 1M0, Falher, AB (www.sarda.ca), 55°7286767; 117°1923529.
3. InnoTech Alberta, Vegreville, AB T9C1T4 (www.innotechalberta.ca), Greenhouse facility,. 53.5027434,-112.0964649

The flax field performance evaluations were conducted and operated by Dr. Jan Slaski's flax research group.

Controlled Environment Experiments

The growth chamber experiments were designed based on the selected northern Alberta climate conditions to accommodate plant performance evaluation. The experimental condition is detailed in Appendix 2.

Fatty Acid Analysis

Fatty acid composition of total acyl lipid from mature seeds harvested from field experiments from northern Alberta was determined following the International Organization for Standardization method reference number ISO 5508:1990 (E), "Animal and vegetable fats and oils—Analysis by gas chromatography of methyl esters of fatty acids". Between 50 and 100 mg of seeds were homogenized in 1 mL of petroleum ether in a 5 mL polypropylene vial using a steel rod. After allowing the meal to settle, 0.5 mL of the supernatant was transferred to a glass tube containing 1.2 mL of methylating solution (2% sodium methoxide in methanol). After thorough mixing, the solution was incubated at room temperature for 30 minutes. One mL of ddH2O was added to the solution, mixed well and left

for 10 minutes at room temperature for the phases to separate. After separation, 200 µL from the upper layer was diluted with 300 µL of petroleum ether in a GC autosampler vial and 2 µL was injected into a GC column.

Separation of fatty acid methyl esters (FAMEs) was performed on a flame ionization gas chromatograph (model 6890, Hewlett Packard, Mississauga, ON) fitted with a 30-m X 0.25mm (i.d.) column (HP-INNOWAX, crosslinked polyethylene glycol) with helium as the carrier gas at a flow rate of 28.0 mL/minute. The oven temperature was increased from 180°C to 230°C at a rate of 5°C/minute and then held at 230°C for 13 minutes. Peaks were assigned by comparing retention times to those in the FAME standards (Nu-Chek Prep, Elysian MN, USA) and relative proportions of FAMEs were determined as percentages of summed peak areas (see Appendix 1 for details).

Evaluation of flax performance

Field evaluation (visual observations)

Plots were inspected on a regular basis to monitor flax plant performance. The following observations were made during the course of vegetative season:

1. Crop emergence – the number of days from seeding to plant emergence when rows were clearly visible was recorded.
2. Seedling vigor – the vigor of the stand was assessed, where 1 is less vigorous than the check, 2 is same as the check and 3 is more vigorous than the check.
3. Maturity – the number of days from seeding to physiological maturity (75% brown bolls)

Growth chamber plant evaluation

Flax plants were grown in growth chambers and experiments were designed based on the selected northern Alberta climate conditions to accommodate plant performance evaluation. The experimental conditions are detailed in Appendix 2. 10 inch pot was used for planting and fertilizer were applied once every 2 weeks, daily observation was carried out to evaluate plant growth and performance.

Plants were inspected on regular basis for observations of performance. The following parameters were evaluated:

1. Seedling vigor – the vigor of the stand was assessed, where 1 is less vigorous than the check, 2 is same as the check and 3 is more vigorous than the check.
2. Maturity - days from seeding to physiological maturity (75% brown bolls).

Feasibility market entry assessment of the linen textile fiber supply for China market

Visits to linen textile fiber production companies in Jiangsu province, China was carried out in July, 2017. Initial discussions with potential end users in Jiangsu province including the owner of Jiangyan City New Style Textile C. Ltd. of northern Alberta flax fiber have been initiated. Feasibility market entry possibility has been assessed. A processing plant to support a 1,000-3,000 tonnes of flax fiber will be required in northern Alberta in order to supply the fiber material for the identified partners in Jiangsu province.

Retting Genomics test

Flax and soil samples were collected from the InnoTech Alberta research plots on October 28, 2016 for flax. Both crops had be harvested and left in the field to ret, the samples were taken just prior to their collection from the field but after the retting process were well underway.

The flax samples had been grown and harvested in smaller replicated plots with significantly shorter rows, so we selected three rows of one fiber variety and three rows of one oil variety and collected the retted material, the soil under the retted material, and the standing material that showed no sign of retting for each of the three rows.

Samples were immediately taken into the lab and frozen at -80℃, and processed the next day by first grinding all the samples in a bead mill for five minutes, and then processing 300 μg of each sample using the method for DNA extraction from environmental soil samples developed by the Foght lab and used extensively for difficult to extract bitumen and core samples (based on Foght et al. (2004) Microbial Ecology 47:329-340). Samples were quantified via nanodrop (Table 1) then diluted to 80ng/uL and sent to Genome Quebec, McGill for high throughput amplification and sequencing using current standard 16S and ITS primer sets (16S primers: 341F – CCTACGGGNGGCWGCAG and 805R – GACTACHVGGGTATCTAATCC, and ITS primers :

ITS1F – CTTGGTCATTTAGAGGAAGTAA and ITS2 - GCTGCGTTCTTCATCGATGC). The amplicons were tagged for each sample and run using an Illumina MiSeq PE250 run.

Results

Plant performance evaluation

Field performance of three experimental lines, 717, 2701 and 2854, was compared against the check variety CDC Bethune. Visual observations revealed that line 2701 flowered three to four days earlier than CDC Bethune, while line 717 commenced flowering two to three days later that the check (Fig. 1). Similar differences in maturity were also reported at harvest time. Interestingly, at harvest all experimental lines were shorter than CDC Bethune, with line 2701 appearing to be the shortest of all. This trait is appreciated by flax seed growers who prefer cultivars generating lower volumes of underutilized straw.

Figure 1. *Differences in maturity at the flowering stage (Left to right - 717, 2701, 2854 and CDC Bethune)*

Similar differences in maturity were reported when new lines were grown in growth chambers under conditions imitating northern Alberta climate. All three lines: 717, 2701 and 2854 exhibited early maturation ranging from 96 to 105 days (Fig. 2).

Figure 2. *Flax plants at 100 days after planting in growth chamber.*

Market feasibility analysis

Drs. Jan Slaski and Jian Zhang visited two major mills located in Jinagsu province, which is a hub for the textile industry in China. Jiangyan City New Style Textile C. Ltd factory (Fig. 3) owned by Mr. Gao (Fig. 4), established in 1985, manufactures yarn-dyed fabrics combined with spinning, dyeing, weaving and finishing. The company owns over 100 million yuan of assets, 25,000 spindles, 200 sets of rapier and looms and 36 whiff looms. The company obtained ISO 9001:2000 for the quality system and ISO14001:1996 for the environmental system. The current production capacity exceeds 12,000,000 linear meters of yard-dyed fabric per year (Fig. 5). Main products include yarn dyed 100% linen, 100% cotton, linen/cotton, linen/ramie,

bamboo Fiber and other fabrics. All products are exported to over 30 countries in Europe, Asia (Japan, South Korea, Hong Kong) and North America (USA).

Figure 3. *Spinning line of Jiangyan City New Style Textile C. Ltd.*

Figure 4. *Canadian visitors with Mr. Gao, the owner of Jiangyan City New Style Textile C. Ltd factory (second from the left).*

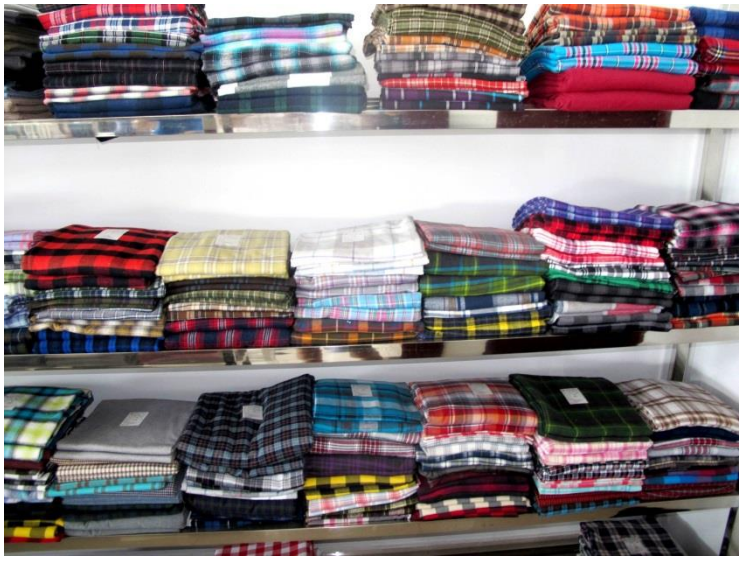

Figure 5. *Display of linen containing fabrics manufactured by Jiangyan City New Style Textile Company. Ltd*

Due to increasing market demands the company is currently expanding production of linen fabrics and hence is in the market for significant volumes of flax fiber. They intend to explore the possibility of sourcing flax fiber grown in Canada.

Figure 6. Production line of Jiangsu Rugao Textile Company

The Jiangsu Rugao Textile Company located in Rugao, Jiangsu that was visited by the research team has a production capacity (100,000,000 meters) over eight time larger than Jiangyan City New Style Textile Company. Lt. (Fig. 6). At present they do not incorporate flax fiber in their products, but are actively exploring international market needs for fabrics incorporating natural fibers.

Retting genomics for flax

Sequences from each of the sample sets (Flax 16S, and Flax ITS) were analyzed using CLC genomics workbench 10.0.1, the "Data QC and OTU clustering 2.0 Workflow" from the Microbial Genomics module, was used to do the initial analysis and Operational Taxonomic Unit (OTU) identification using the SILVA 16S v128 97% database for bacteria and the UNITE v7.1 97% database for fungus. One sample, unretted hemp biological sample 1, was rejected for both 16S and ITS analysis due to low number of reads that passed the QC step (Table 1 and Table 2).

***Table 1**: DNA concentration for Hemp and Flax Retting Samples and number of reads that passed QC*

Sample name	**Sample ID**	**DNA extracted ng/uL**	**16S reads after QC**	**ITS reads after QC**
Soil Linen Flax Row 1	SoL1	519.66	127060	126636
Soil Linen Flax Row 2	SoL2	554.84	92960	138836
Soil Linen Flax Row 3	SoL3	839.37	105364	141750
Retted Linen Flax Row 1	RL1	343.84	124854	173465
Retted Linen Flax Row 2	RL2	525.01	83812	152599
Retted Linen Flax Row 3	RL3	248.09	91172	193129
Unretted Linen Flax Row 1	UL1	421.02	146560	143845
Unretted Linen Flax Row 2	UL2	418.06	114812	168660
Unretted Linen Flax Row 3	UL3	394.88	141751	177428
Soil Oil Flax Row 1	SoO1	811.45	103000	178817
Soil Oil Flax Row 2	SoO2	570.23	115183	162955
Soil Oil Flax Row 3	SoO3	424.29	101685	168810
Retted Oil Flax Row 1	RO1	633.98	102315	169947
Retted Oil Flax Row 2	RO2	578.72	105378	133521
Retted Oil Flax Row 3	RO3	382.79	109499	150231
Unretted Oil Flax Row 1	UO1	880.43	155229	141285
Unretted Oil Flax Row 2	UO2	424.31	126603	142330
Unretted Oil Flax Row 3	UO3	603.29	138230	160533

***Table 2**:* *Total read counts for each sample set*

Sample set name	**Total sequences prior to QC**	**Total sequences analyzed after QC**	**Total OTUs found**
Flax 16S	7,000,518	6,205,958	2,734
Flax ITS	6,663,440	5,046,178	1,192

Flax 16S Community Analysis

The rarefaction graphs for the 16S Flax sample show, less of a complete community sequence compare to the soil samples. The flax soil samples are better covered than the hemp samples, and the unretted samples have more community coverage than the retted samples; though both the retted and unretted samples are sufficient with the amount of sequencing done (Fig. 7).

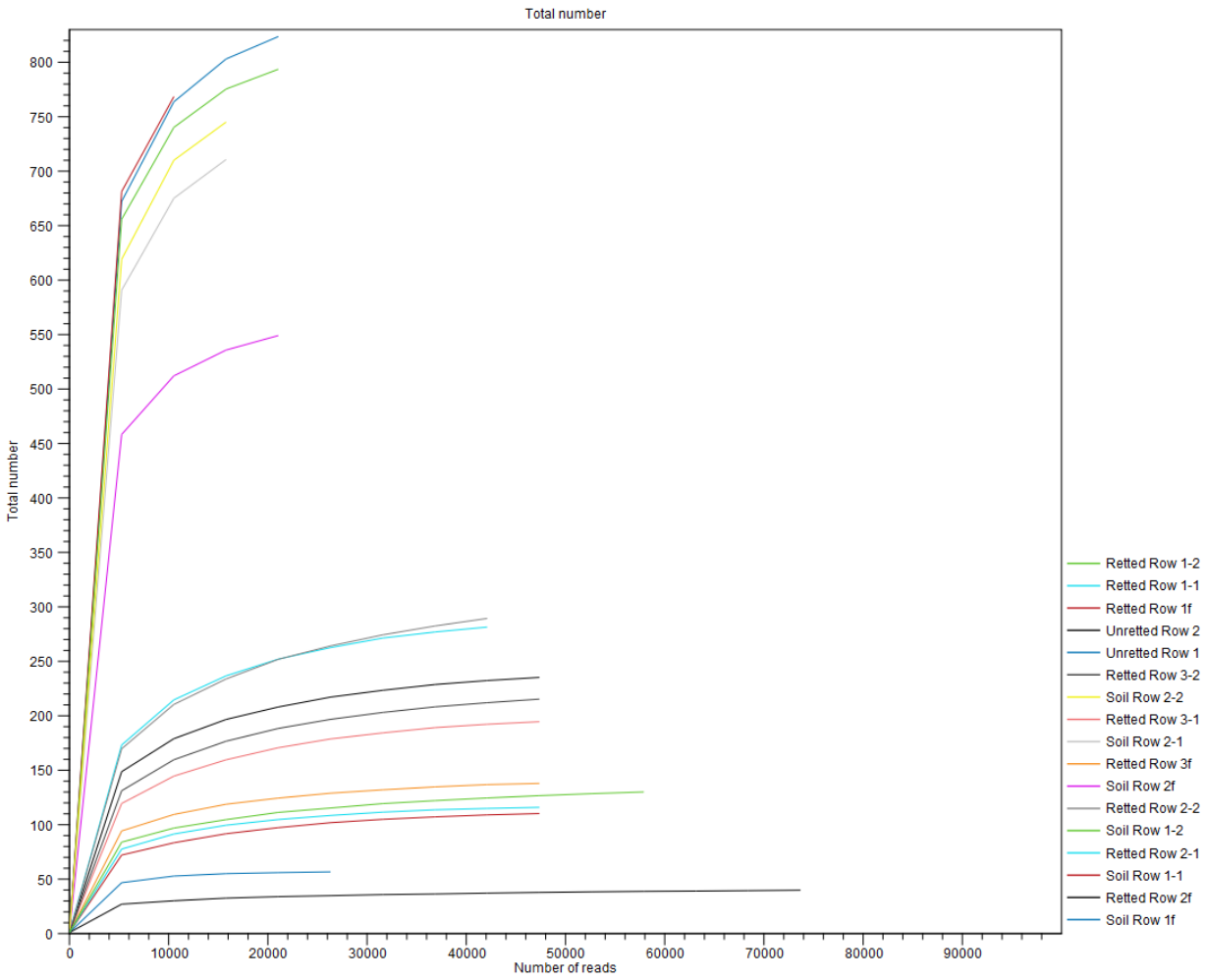

Figure 7. *Flax 16S Rarefaction (Alpha-Diversity reads per OTU)*

The PCoA Bray-Curtis analysis shows less of a distinct grouping between all three conditions examined (Fig. 8). However, if the oil and the linen flax are separated this overlap between the conditions is removed with each group clustering distinctly between the retted, unretted, and soil samples. Therefore the statistical analysis needs to distinguish between the oil and fiber varieties.

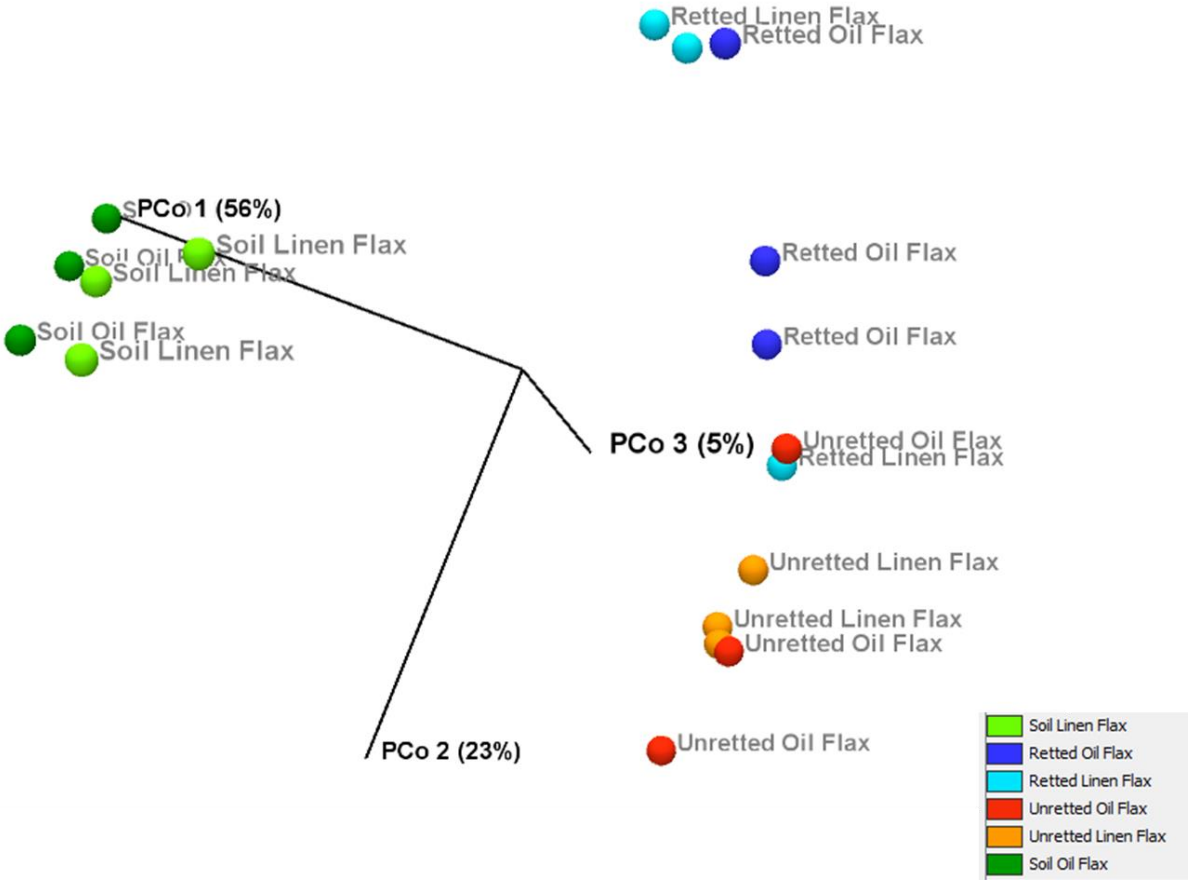

Figure 8. *Flax 16S PCoA Bray-Curtis Analysis*

The Permanova Bray-Curtis analysis again shows a statistically significant difference between all three conditions (p-value 0.00001) and a statistical significant difference between each sample pair retted vs soil, retted vs unretted, and soil vs. unretted (Bonferroni p-values 0.00003, 0.00174, and 0.00006 respectively).

The differential abundance analysis of the Flax 16S sample shows six unique OTUs, with a statistical change between the retted and unretted samples, whereas three other statistically significant changes found between the retted and unretted samples are also found between the samples and the soil. (Fig. 9)

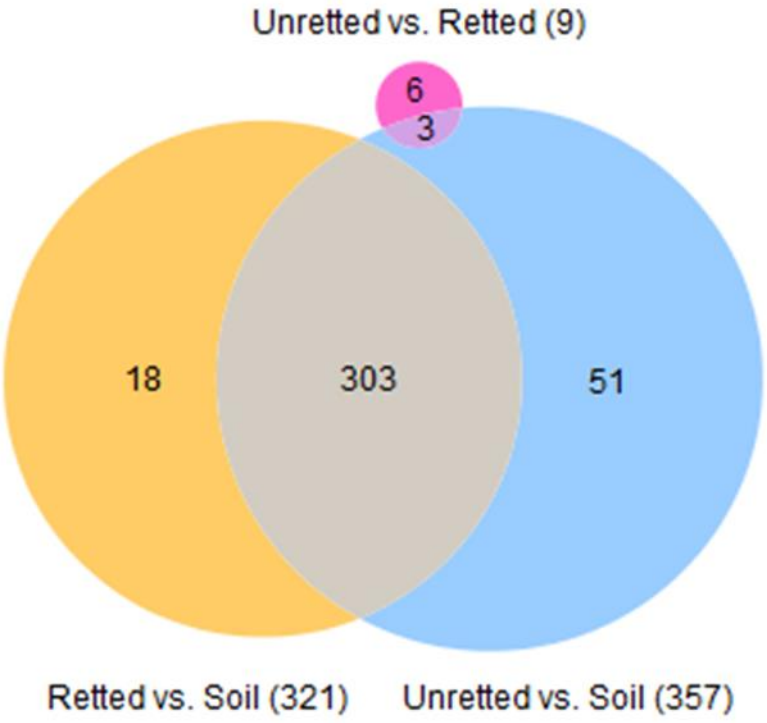

Figure 9. *OTUs showing a statically significant difference in any of the three conditions. Flax 16S Differential Abundance Analysis – Bonferroni <0.05, Fold change minimum 1.5*

The six unique OTUs that show a statistically significant change in the retted compared to the unretted samples are *Arthrobacter sp. THG-DN3.14*, an unidentified Beutenbergiaceae, an unidentified Rhodococcus, an unidentified Pseudarthrobacter, an unidentited Kineococcus, and an unidentified Pseudoclavibacter. NCBI blast identification (using the same blast parameters as above) identified these OTUS as an unidentified Pseudarthrobacter sp., *Salana multivorans*, *Rhodococcus corynebacterioides*, *Pseudarthrobacter defluvii*, and unidentified Kineococcus sp., and *Pseudoclavibacter terrae*. The Pseudarthrobacter sp., *Salana multivorans*, the Kineococcus sp. and *Pseudoclavibacter terrae* are all found in soil samples according to the NCBI database. The *Rhodococcus corynebacterioides* is found in a biofilm of a hydrothermal spring, and the *Pseudarthrobacter defluvii* is listed as a ground nut leaf methlotrophic bacteria.

The three OTUs that show a statistically significant change between the retted compared to the unretted but also show a statistically significant change in the unretted samples compared to the soil are *Arthrobacter globiformis*, an unidentified *Geodermatophilus* sp. and an unidentified

Marmoricola sp. These identifications are confirmed via the NCBI search. An unidentified Geodermatophilus sp. and an unidentified *Marmoricola* sp. were both found in the soil samples; but the *Arthrobacter globiformis* is found in oil contaminated soil and might again indicate a community active in degrading an oil component during retting.

Table 3: *Identification of non-unique fungal OTUs showing a significant difference between retted and unretted samples*

UNITE ID	NCBI ID	NCBI Description	Study accession is related to
Monographella cucumerina	KF493966.1	Uncultured Plectosphaerella	Roots of Heinz 2401 tomato grown on farm soil, Surveying the Mycobiome of Tomatoes Showing Symptoms of Vine Decline
unidentified *Nectriaceae* sp.	KJ620972.1	Fusarium sp. CF13	Isolated fungal flora from Chamblyal soil
Gibberella intricans	HG936944.1	Uncultured Fusarium	Spatial Distribution of Fungal Communities in an Arable Soil, Zea mays field bulk soil, July 2010
unidentified *Nectriaceae* sp.	KY430581.1	Uncultured Fusarium	Phylogenetic diversity and composition of belowground endophytic fungal communities associated with wheat (Triticum aestivum) from organic and conventional farming systems, Triticum aestivum voucher TUB 021598 fine roots
No database identification OTU1	KR094465.1	*Alternaria infectoria*	Secondary Metabolites from Fungal Endophytes of *Echinacea purpurea* Suppress Cytokine Secretion by Macrophage-Type Cells
Microdochium bolleyi	GU566262.1	*Microdochium bolleyi*	Spatial Distribution of Fungal Communities in an Arable Soil, *Zea mays* roots, September 2010, rhizosphere

unidentified *Sordariomycetes* sp	HG935577.1	Uncultured *Verticillium*	Spatial Distribution of Fungal Communities in an Arable Soil, Zea mays field bulk soil, September 2010
No database identification OTU2	KY103437.1	*Filobasidium magnum*	Rainwater
No database identification OTU3	KY430484.1	Uncultured *Cryptococcus*	Phylogenetic diversity and composition of belowground endophytic fungal communities associated with wheat (*Triticum aestivum*) from organic and conventional farming systems, *Triticum aestivum* voucher TUB 021567 fine roots
Filobasidium wieringae	KY103450.1	*Filobasidium wieringae*	Seasonally dynamic fungal communities in *Quercus macrocarpa* phyllosphere differ among urban and rural environments, phyllosphere
unidentified *Leotiomycetes* sp	KT999357.1	Uncultured fungus	Apple Phyllosphere fungal and bacterial microbiota: impact of the farming system, *Malus domestica* phyllosphere
Cryptococcus victoriae	KY430500.1	Uncultured Cryptococcus	Phylogenetic diversity and composition of belowground endophytic fungal communities associated with wheat (*Triticum aestivum*) from organic and conventional farming systems, *Triticum aestivum* voucher TUB 021548 fine roots

Flax ITS Community Analysis

Flax ITS sample shows sufficient community OTU coverage based on the rarefaction analysis. (Fig. 10)

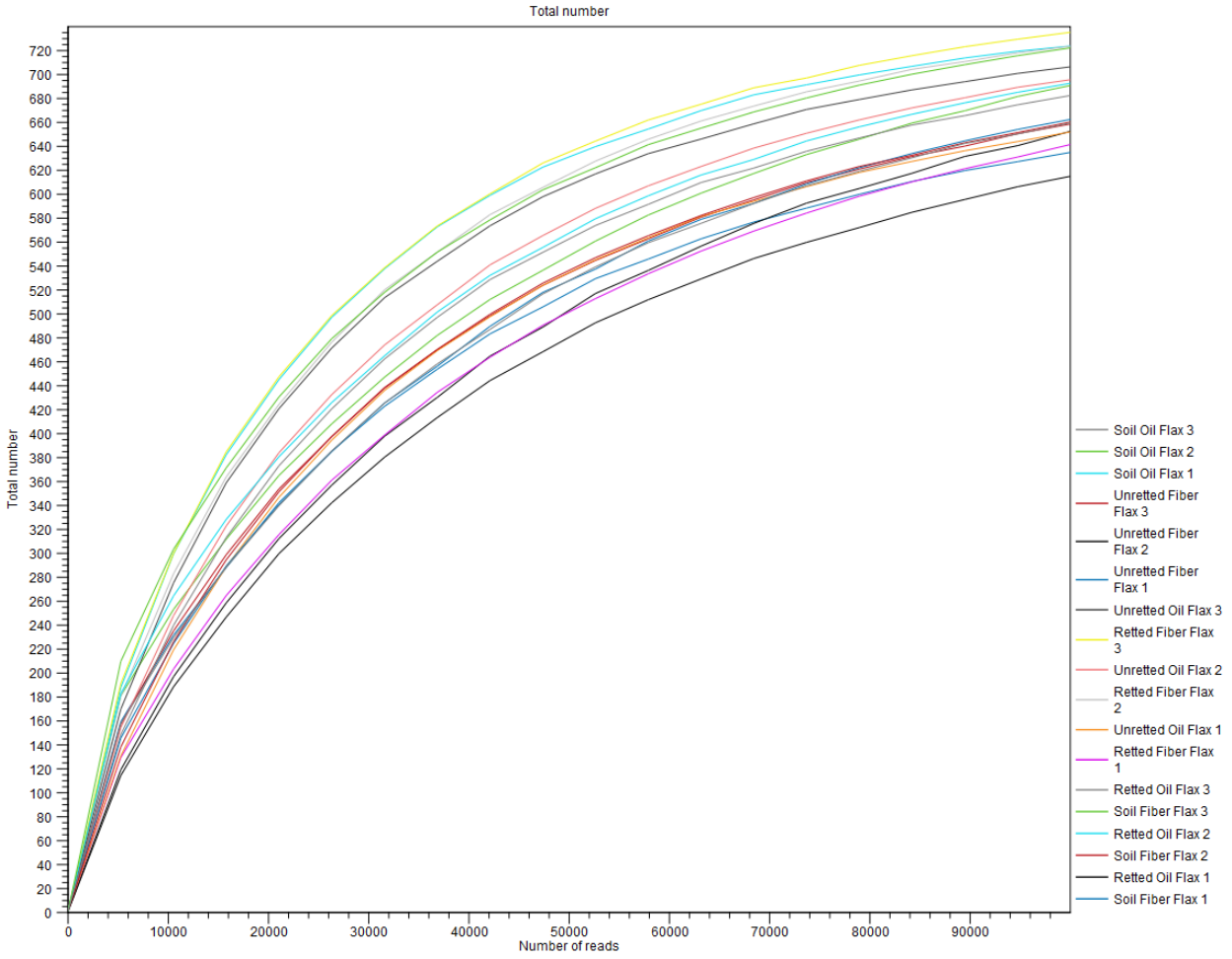

Figure 10. *Flax ITS Rarefaction (Alpha-Diversity reads per OTU)*

The PCoA Bray-Curtis analysis is not as clear as either the Hemp ITS or the Flax 16S and the retted and unretted fiber flax show only a very small difference between the sample sets. The oil flax shows a more distinct difference between the retted and unretted samples, but even so, the groups are fairly close together. The soil is a distinct group from the retted and unretted groups in both flax sample group. (Fig. 4). This could be due to the fact that the flax samples were cut down much later and the retting time that occurred for them was significantly less than the hemp samples, due to an early snow.

The study showed evidence that winter retting here in Vegreville could provide a base microbe population for flax fiber retting (Fig. 11).

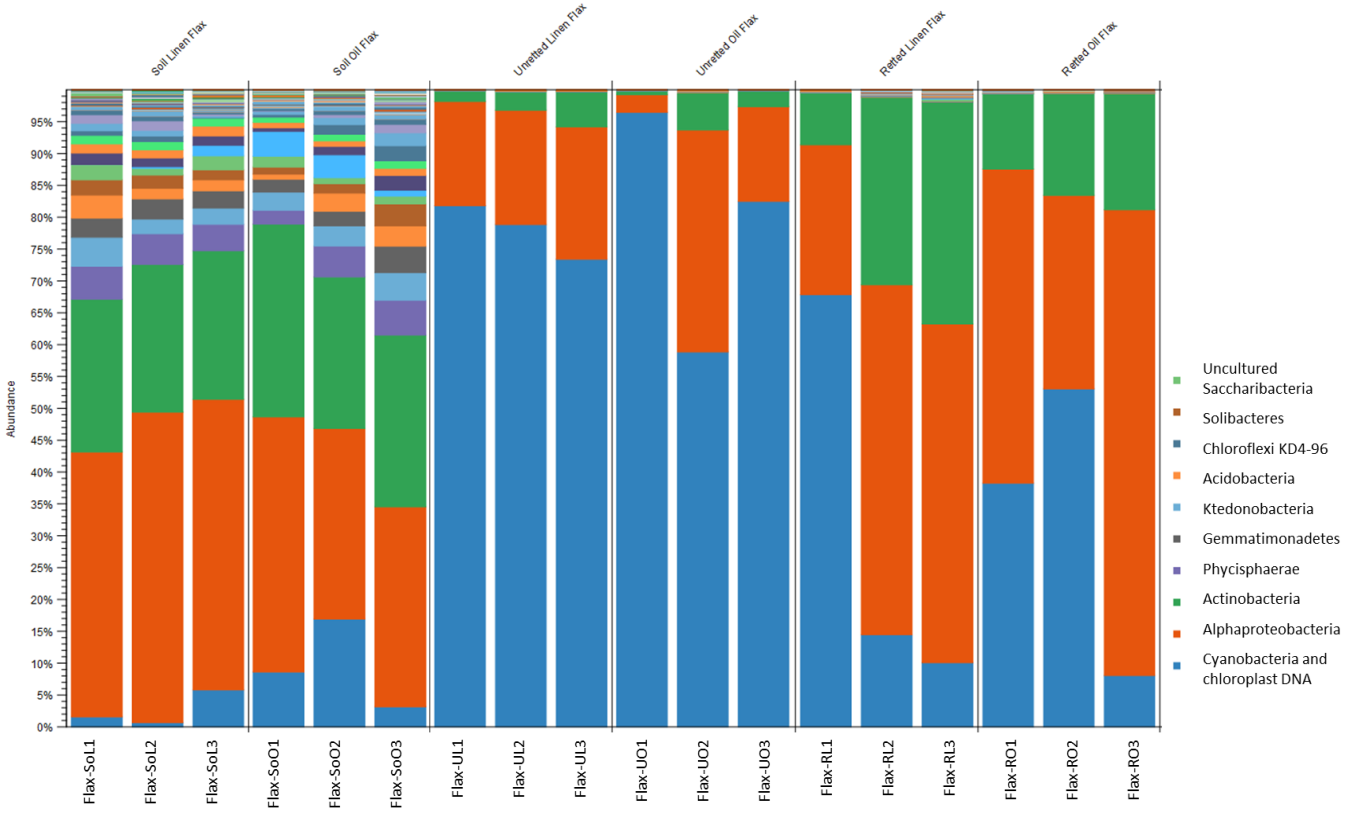

Figure 11. *Population of bacteria showed a significant increase in retted flax samples*

Conclusions and Recommendations

Data and information gathered during the course of this project, primarily focused on genomics and agronomy of flax fiber under northern Alberta climate, supports the following conclusions:

- Among experimental flax accessions, line 2701 appeared to be three to four days earlier than CDC Bethune.
- The biochemical analyses of fatty acid profiles of lines 717, 2701 and 2854 revealed no substantial differences between them and the reference flax line, CDC Bethune.

- Initial conversations with two Chinese fabric manufactures in Jiangsu Province indicate their genuine interest in exploring a possibility of sourcing Alberta grown flax fiber for their linen fabric production.
- The BioFiber and Food for Northern Alberta Working Group was established including representatives from different levels of government, researchers and relevant industries. The group set up objectives to focus on northern Alberta's flax and hemp industry.
- Retting genomic research provides basic understanding of winter field flax retting process and that northern Alberta could provide a natural retting condition.

Based on the project outcomes, recommendations are as follows:

- Selected early maturing flax accessions identified in this study (lines 717, 2701 and 2854) should be incorporated into commercial Canadian flax breeding programs.
- Since this project can be considered as an enabler of the flax retting genomic program, further detailed research with additional significant resources should be conducted to determine the most efficient microbe species and suitable conditions for the benefits of biofiber industry in Western Canada in general, and northern Alberta in particular.
- Explore possibilities to build a processing plant for northern Alberta fibers that would absorb the flax straw and potentially hemp fiber production.

Appendix. 1: Fatty acids analysis of early mature flax lines

```
Injection Date  : 21/07/2016 3:03:46 PM                Inj :   1
                                                  Inj Volume : 1 µl
Sequence File   : C:\CHEM32\1\SEQUENCE\20160721.S
Method          : C:\CHEM32\1\METHODS\FA_FINAL  [20120208 FLAX].M
Last changed    : 29/07/2014 10:25:05 AM by margarita
Method Info     : FA_FINAL.M-Short Run(20140723)
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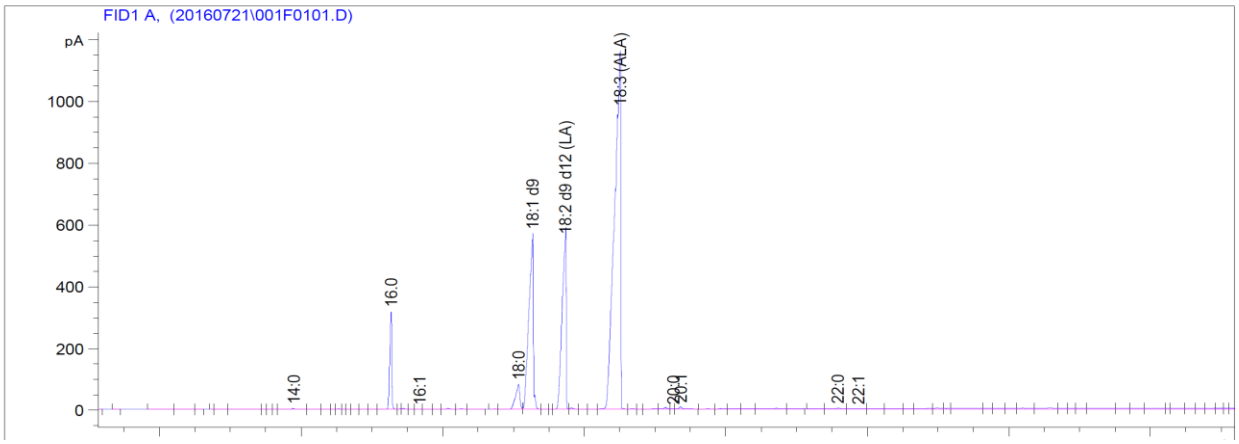


```
Acq. Operator   : LIMIN                          Seq. Line :   2
Acq. Instrument : Instrument 1                    Location : Vial 2
Injection Date  : 20/07/2016 11:53:08 AM               Inj :   1
                                                  Inj Volume : 1 µl
Sequence File   : C:\CHEM32\1\SEQUENCE\20160720.S
Method          : C:\CHEM32\1\METHODS\FA_FINAL  [20120208 FLAX].M
Last changed    : 29/07/2014 10:25:05 AM by margarita
Method Info     : FA_FINAL.M-Short Run(20140723)
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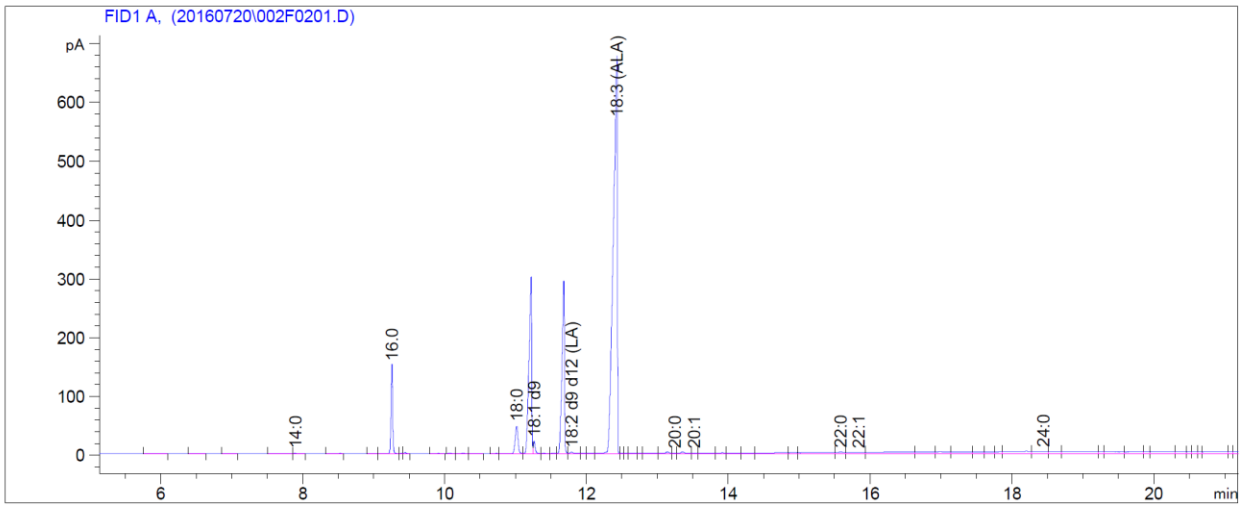

Appendix 2: Growth conditions of Northern Alberta

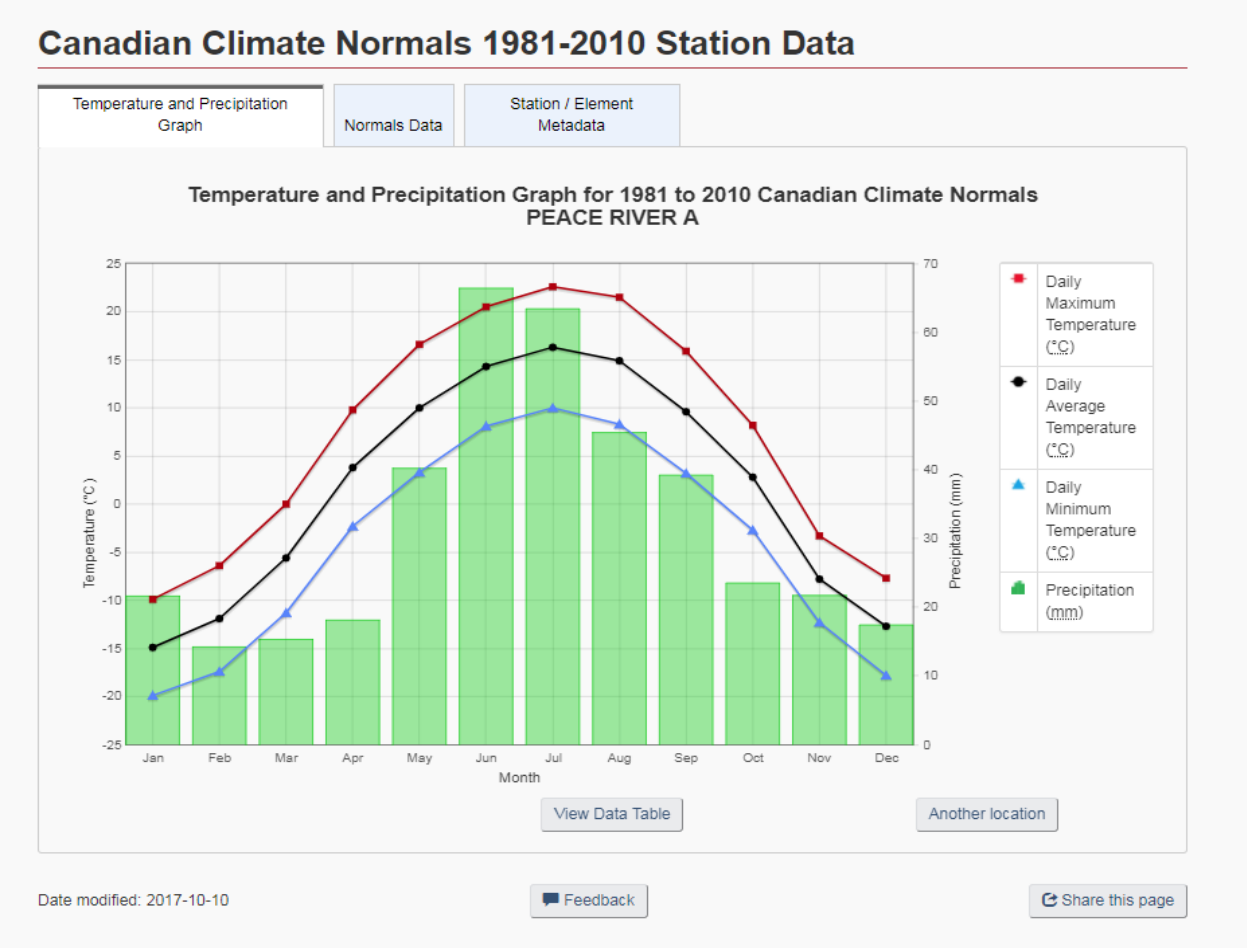

Figure 12. *Climate data set for Peace River used in the growth chamber experiment*

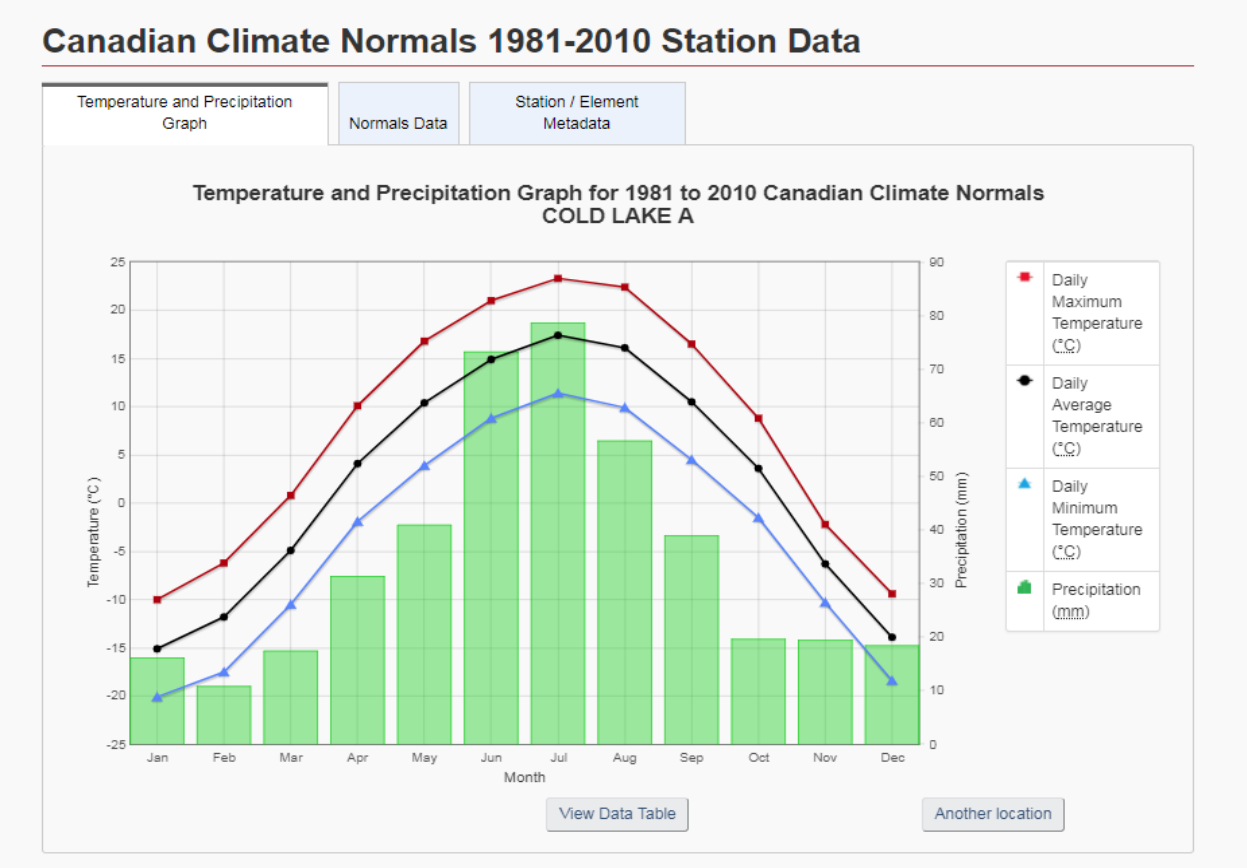

Figure 13. *Climate data set for Cold Lake used in the growth chamber experiment*

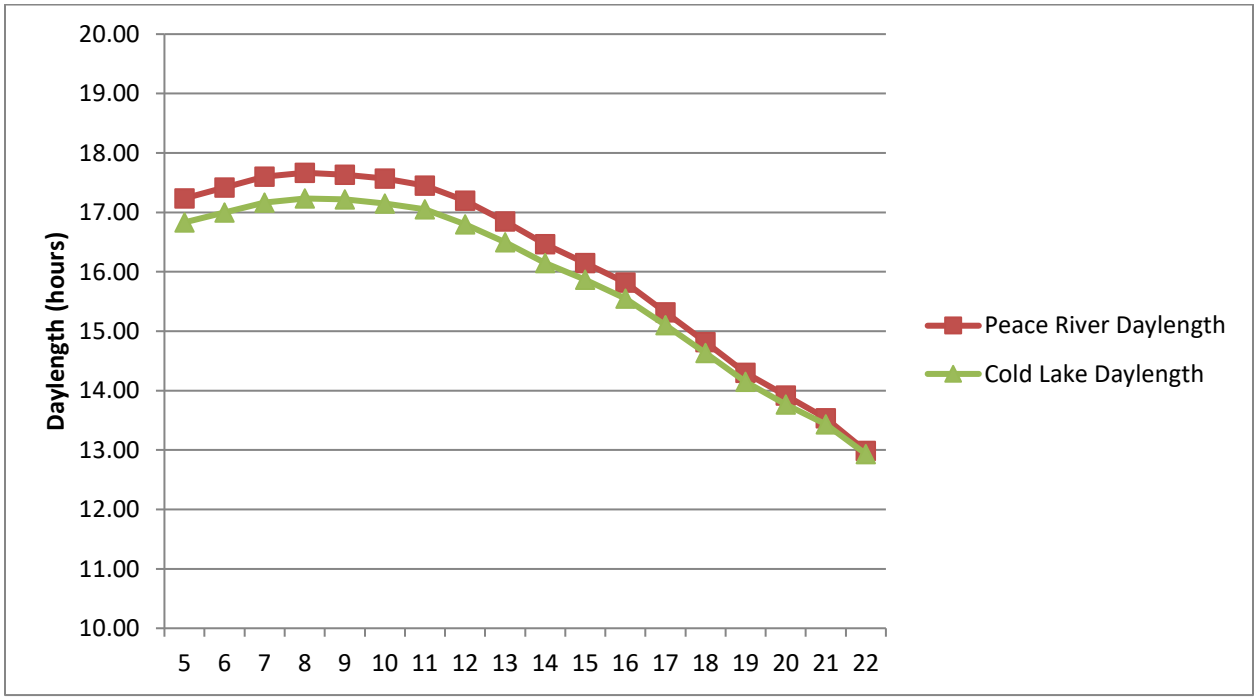

Figure 14. *Day length schedule used in the growth chambers for selected flax lines*

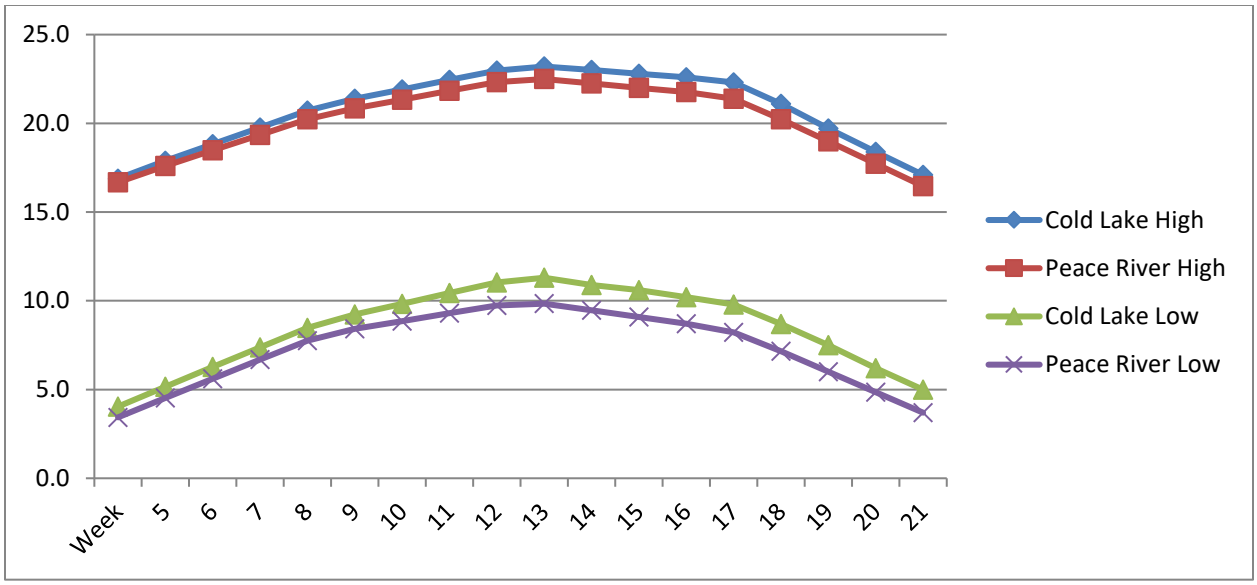

Figure 15. *Temperature schedule used in the growth chambers for selected flax lines*

Growth duration with northern Alberta climate condition; growth chamber = minimum 4°C; experiment started week 6 for temp and week 4 for day length