FINAL PROJECT REPORT
Canola Agronomic Research Program (CARP)

The Final Report should fully describe the work completed for the year and note the personnel involved. It should also note any deviations from the original plan and next and/or corrective steps as may be required if deviations are noted. The report should also provide an update on the status of the Project including forecasted date of completion. A complete statement of expenses should be included. In the event major changes are anticipated within the budget supporting notes along with a proposed budget should also be included. The report should also capture a complete summary of activity for the year.

Project Title: Field evaluation of a valuable germplasm resource designed to dissect complex traits in *Brassica napus* (the Nested Association Mapping population)

Research Team Information

Lead Researcher:		
Name	***Institution***	***Project Role***
Sally Vail	AAFC-Saskatoon	Lead
Research Team Members (add rows as required)		
Name	***Institution***	***Project Role***
Isobel Parkin	AAFC-Saskatoon	Co-Lead
Steve Robinson	AAFC-Saskatoon	Co-Lead
Henry Klein-Gebbinck	AAFC-Beaverlodge	Collaborator

Project Start Date: April 1, 2017 **Project Completion Date:** March 31, 2019

Reporting Period: April 1, 2018 **to** March 31, 2019

CARP Project Number: 2017.33

Instructions: This Final Project Report shall be completed and submitted on or about March 31st of the fiscal year that the agreement is in effect (upon completion of the project). The Lead Researcher of the project in question shall complete and submit the report on behalf of his/her complete research team.

This Report is a means by which to provide a detailed account upon completion of the project.. Final project financial reporting should be provided at this time.

The following template is provided to assist you in completing this task. Please forward the completed document electronically to the CCC contact listed below.

In addition to the Final Project Report, a one page Research Abstract including rationale, objective, methodology, summary and conclusions (with a summary graph/table or supporting image for the project), acknowledgement and references is due upon completion. The Research Abstract is intended for use in publications such as the *Canola Digest* and the CCC Research Hub and is intended to support messaging to all audiences.

Please include the funding acknowledgements outlined in your research agreement in all deliverables (publications, presentations, etc.) from this project.

1. Date of Completion:

March 31st, 2019

2. Status of Activity: (please check one)

_____ Ahead of Schedule __X__ On Schedule _____ Behind Schedule _____ Completed

Comment:

3. Completed actions, deliverables and results; any major issues or variance between planned and actual activities.

Considerable resources have been invested over the past four years to develop the spring *B. napus* Nested Association Mapping (NAM) population, which is now completed with over 2500 Recombinant Inbred Lines (RILs) which represent recombination of 50 diverse Founder Lines (FLs) with a reference line. The developmental *B. napus* NAM project encompassed one field season of evaluation of the large RIL population in 2016 at a single location/environment; however, extensive evaluation over several years under multi-environments is necessary to fully realize the complex trait combinations of this unique germplasm resource.

Project Objectives

The objectives originally proposed for this research project were to:

1. Comprehensively characterize the developed NAM RIL population for agronomic, phenological, yield predictive parameters and seed quality characteristics over two additional contrasting environments;
2. Provide field plots for sampling or data collection, basic data from field plots, or seed samples to multi-disciplinary collaborators to study various traits of interest to the industry;
3. Populate the NAM Centralize Database with mine-able data.

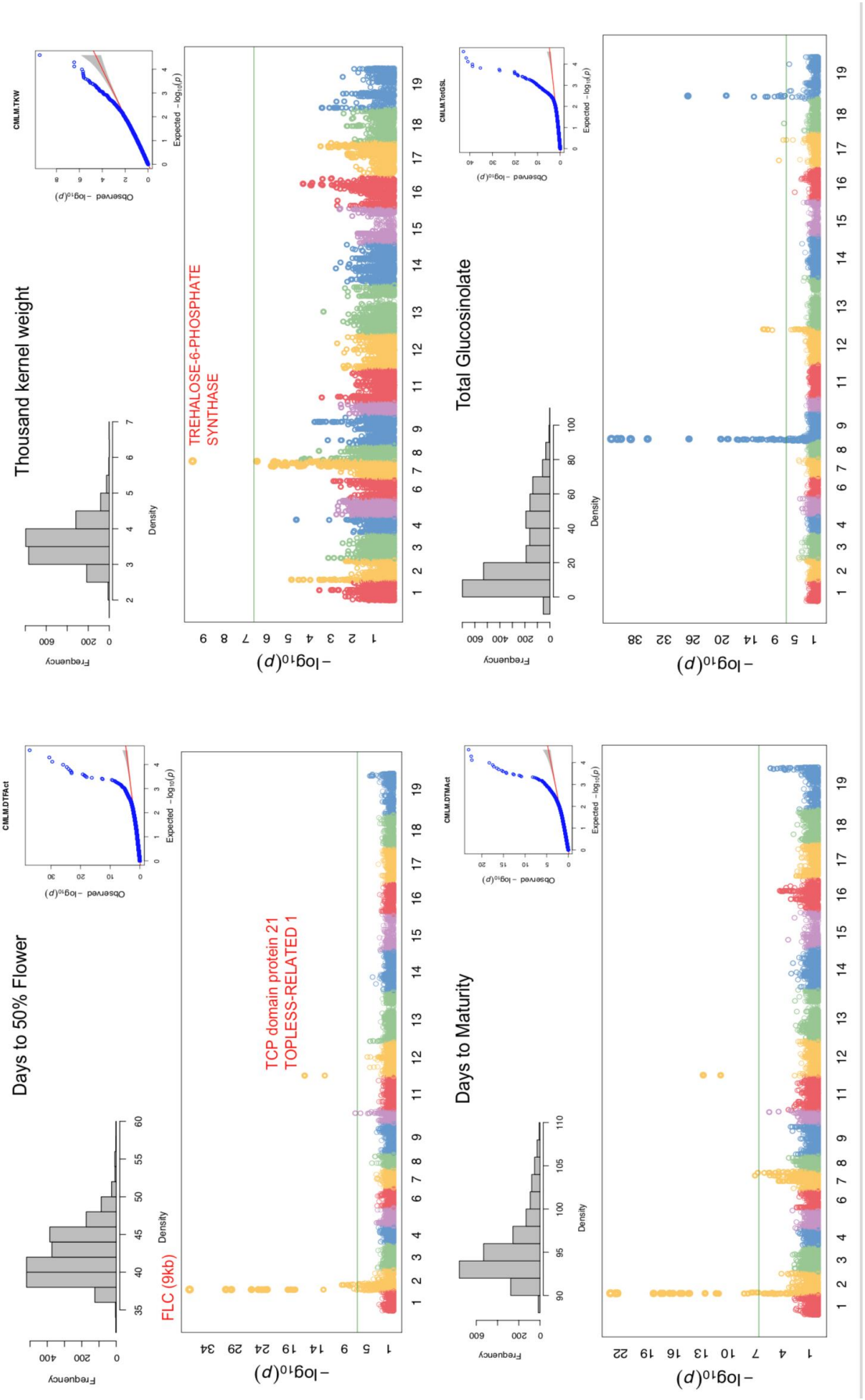
Please note that originally 2 years of field testing of the entire RIL population (2017 and 2018) were proposed for this project; however only a smaller 2017 field season was funded but 2017 was expanded to include sites in Manitoba.

Results

2016 Field Trials

Due to the early snowfall experienced in the fall of 2016, the initial evaluation of the whole NAM RIL population was compromised, resulting in the need to hand-harvest the majority of the trial. This in turn led to delay in analyzing of the seed samples for quality traits, curation and analysis of the data. Thus, over the course of this current project, processing of the data through a statistical pipeline to obtain adjusted means occurred followed by a Genome-Wide Association Study (GWAS) analysis of key phenotypes. Figure 1 shows GWAS results for a few selected adaptive as well as seed quality traits. Genomic associations for Flowering Time (days to 50% of the plants within a plot having an open flowers) and Days to Maturity showed strong associations to genomic regions harboring known genes associated with flowering time (FLC; Plant J 28(5):545) and circadian oscillations (TCP domain protein 21 and TOPLESS-related 1; Science 323(5920):1481, PNAS 110(2):761) in the related species *Arabidopsis*. The commonalities between Flowering Time and Days to Maturity associations are expected given maturity in canola is largely driven by the initiation of flowering. An association with seed size (Thousand kernel weight) co-localized with a gene involved in embryo development and maturation (Trehalose-6-phosphate synthase). The known major loci controlling seed glucosinolate content were also identified in the GWAS analysis (A9, C2, and C9) plus minor associations for other seed glucosinolate on C7 and C8 (New Phytol 193:96; Theor Appl Genet 116:1035).

Figure 1: Genome-Wide Association Study of Spring *Brassica napus* Nested Association Mapping Population Recombinant Inbred Lines for Flowering Time, Days to Maturity, Thousand Kernel Weight and Seed Glucosinolate Content



2017 Field Trials

In addition to the trials discussed in detail below, the NAM FLs were assessed in the field in 2017 at two sites in southern Manitoba. One trial was conducted by the University of Manitoba (in collaboration with Dr. Rob Duncan) with the specific objective to assess protein fraction in seeds produced in different environments (2015 and 2016 in Saskatoon, 2016 and 2017 in Winnipeg). Also, with an industry collaborator, the NAM FLs were assessed in a nursery in the 2017 growing season. It is anticipated that these results will be eventually incorporated into a mineable database alongside all the other results from NAM field trials.

Re-Growing the NAM FL in Beaverlodge, AB

Field testing of the NAM FLs in 2015 across several field sites in Saskatchewan and Beaverlodge (BE) demonstrated the uniqueness of the extreme environment at northern latitude in the Peace River Region. Agronomic or adaptation-related traits showed lower correlations between sites when BE was contrasted with sites in Saskatchewan (including Melfort, Saskatoon, Scott and Outlook under dryland and irrigated conditions). This was especially the case for the number of days to flower after seeding and the days to maturity. For the current project, we wanted to see if these differences in flowering and maturity times were consistent when examined across field seasons. Thus, the trial from 2015 was repeated in Beaverlodge in 2017 and flowering and maturity times on the NAM FLs were also available from a parallel study in Saskatoon and a nearby site just west of Saskatoon in 2017 for comparison.

Correlation analysis showed that Flowering Times (DTF) were more similar between the two sites near Saskatoon (r=0.90) compared to the correlations between SK sites and Beaverlodge (Figure 2) in 2017, which aligned with observations from 2015. That being said, the decrease in correlation for Days to Maturity (DTM) between Beaverlodge and Saskatchewan sites observed in 2015 was not observed in 2017 as Beavelodge and the site west of Saskatoon (ML) had an equal correlation to ML and Saskatoon (Figure 2). When DTF was compared between Beaverlodge and Saskatoon across field seasons (Figure 3), it was evident that inter-site correlations were similar across growing seasons, however the lowest correlation was between 2015 and 2017 in Beaverlodge, suggesting that the more varied seeding and early season conditions at this location are affecting flowering time more than photoperiod response. The lower correlation for DTM observed between sites in 2015 (r=0.56) was not observed in 2017 (r=0.73) suggesting the initial observation was due to factors other than photoperiod-driven flowering time differences as originally postulated, likely unpredictable maturation environment in Beaverlodge especially for later-maturing NAM founder lines. When DTF values and differences between site-years were examined, only one out of five (2015) and four (2017) FLs with significantly different flowering times within their respective site-years was common between the trials.

Figure 2: Correlation of Means of Days to Flower (DTF) and Days to Maturity (DTM) in 2017 on the NAM Founder Lines grown in Beaverlodge AB (BE), Saskatoon SK (SA) and a Site West of Saskatoon (ML)

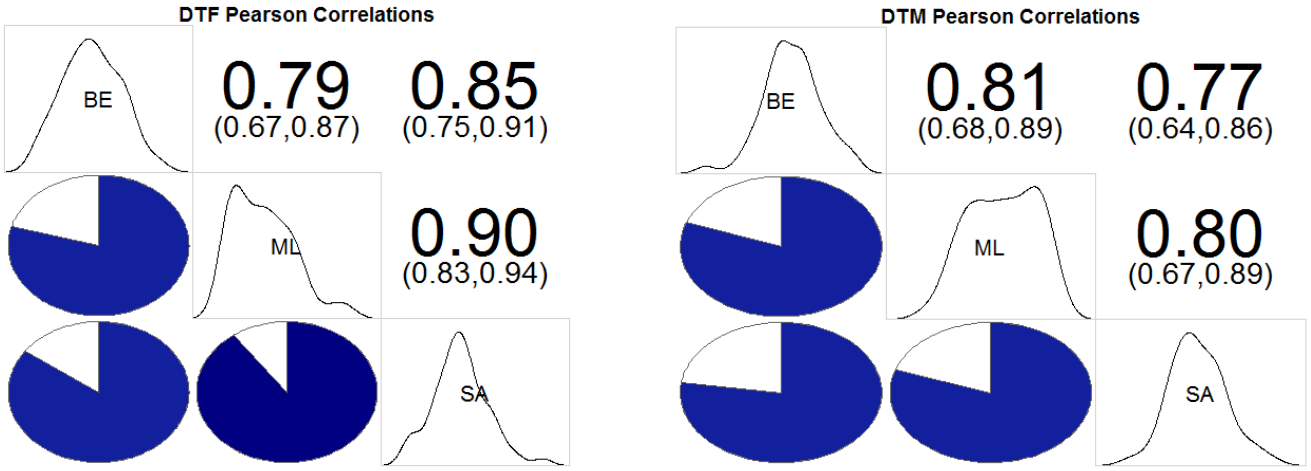
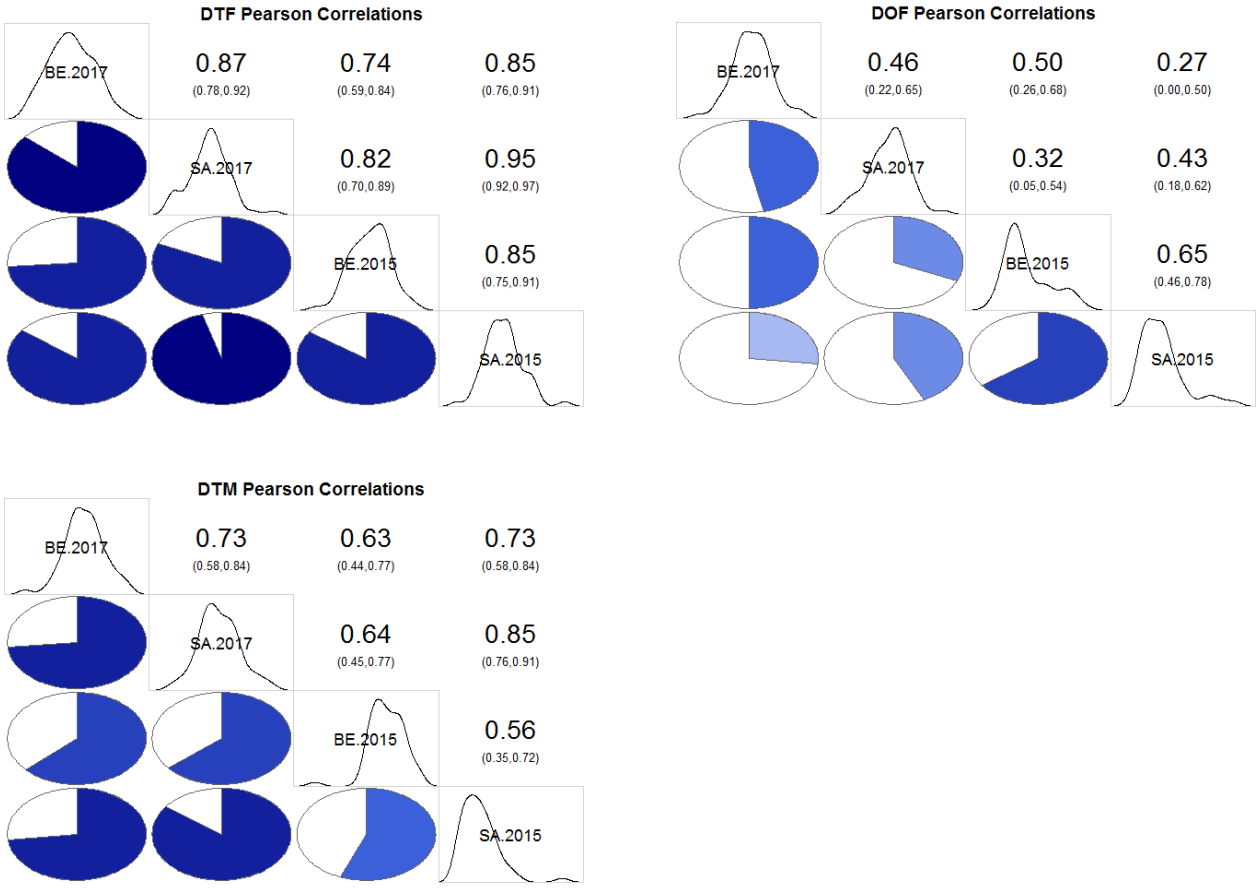


Figure 3: Correlation of Means of Days to Flower (DTF), Duration of Flowering (DOF) and Days to Maturity (DTM) in 2017 on the NAM Founder Lines grown in 2015 and 2017 seasons in Beaverlodge AB (BE) and Saskatoon SK (SA)



Photoperiod response is a physiological characteristic that not well understood in *B. napus*. It is known that there are differences in genotype in sensitivity (eg. Russian Journal of Genetics, 44:326; PLoS ONE, 9: e102611), however a comprehensive understanding of genetic control and how to specifically design plants for long-day/short-season environments is missing within the body of published literature. Working with the AAFC site in Beaverlodge (BE) Alberta offers a unique opportunity to work alongside the commercial canola hybrid industry to generate a body of knowledge on how to better optimize commercial hybrids for the Peace River Region. Studying response to daylength in field trials, rather than under controlled conditions, such as with growth cabinets, would be preferred for the following reasons: i) Accessing controlled conditions to evaluate segregating populations (ie. hundreds of plants) is both expensive and difficult due to limited availability of suitable bench space; and ii) Due to the semi-determinant growth habit of *B. napus*, agronomic factors such as plant stand are known to affect flowering and maturity times thus the translation of results under controlled conditions may not translate to the field under varied conditions year to year. Based on the results of 2015 as well as observations from multi-site yield trials of breeding lines within the AAFC Canola Breeding Program, it was postulated that the underlying genetics of photoperiod response in *B. napus* may be deciphered by growing segregating families of NAM RILs at higher latitude in Beaverlodge and comparing flowering times to those in Saskatchewan and Manitoba. Based on the results of this project, it is clear that using these methods would require multiple seasons of results as well as the construction of statistical models that would include factors such as growing degree-day accumulation and plant stand to normalize for confounding factors that are known to affect Flowering Time beyond photoperiod response.

Testing of Agronomic Selections of RILs in 2017 at Saskatoon, SK

From the 2016 nursery of NAM RILs, approximately 450 NAM RILs were selected for agronomic characteristics and increased for further field assessment. In 2017, these selections were re-evaluated in the field alongside the FLs and checks. In order to manage the size of the nursery, a Type II Modified Augmented Design (MAD) was employed where un-replicated entries are adjusted based on regularly reoccurring checks. Mini-plots consisted of three rows approximately 1 m long for a $1m^2$ plot. Basic agronomic characteristics, such as emergence counts, days to flowering, duration of flowering and days to maturity, were manually recorded using standardized protocols. Multiple quantifications of vegetative indices, such as NDVI, were taken prior to flowering and flowering intensity of the plots was monitored using UAV as well as ground-based phenotyping methods in Saskatoon. Seed yield was determined by harvesting with a plot combine and seed quality traits were determine by NIR (Oil, Protein and Fiber Fraction contents) and Gas Chromotography (Fatty Acid and Glucosinolate profiles). Resulting field and seed-data were analyzed by a MAD analysis pipeline in R to obtain adjusted means that were used to determine which NAM RILs were worthy of further assessment in yield trials.

From the 420 RILs assessed, 93 (or about 4% of the entire NAM RIL population) showed an ideal combination of agronomics (Days to Flowering and Maturity), seed quality profile (acceptable oil%, low glucosinolate and low erucic) and yield greater than the reference line (Table 1 and Figure 4). From the 93 RILs selected, the origin of the FLs mostly represented Canadian and European genepools with 35 RILs tracing to a Canadian FLs, 32 to a European FLs and an additional 6 tracing to a FL tracing to Argentina and another 4 from a cross with an Australian FL. One RIL was selected from a cross with a FL containing approximately 50% winter *B. napus* background while only 15 selected RILs traced to FLs from Korea. The lack of diverse pedigrees represented in the selections demonstrate the necessity of targeting specific phenotypes associated with stress tolerance followed by additional rounds of intercrossing in order to use the NAM resource to widen the genetic basis of canola-quality lines with flowering and maturity times suitable for the Canadian prairies. When RILs possessing suitable agronomics, oil and yield were examined that possessed low glucosinolate OR low erucic acid, 32 additional RILs were identified that were derived from crosses with 23 FLs, 11 of which were not represented in the pedigrees of the 93 RILs selections discussed above. From these, another 8 RILs were derived from crosses with European or Australian founders, with the balance of the RILs being from more diverse crosses with founders from across Asia (Korea, Bangladesh, India, etc.).

Figure 4: Venn diagram representing selection of 93 NAM RILs from 420 NAM RILs re-tested in 2017 for optimal combination of Days to Maturity (DTM), Yield greater than the reference line, low seed Erucic Acid (c221), low seed Glucosinolates (GLS) and commercially acceptable Oil content

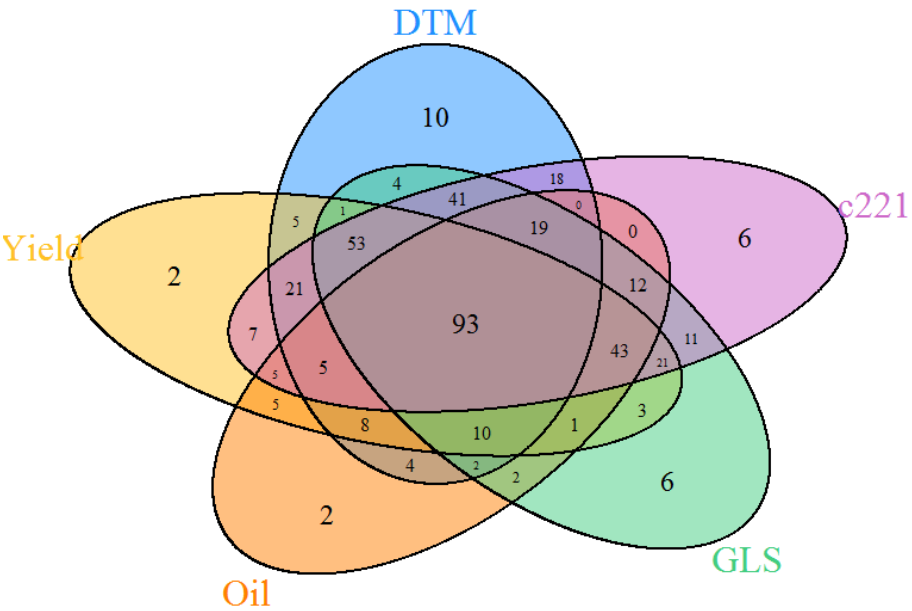


Table 1: Top 28 selections of NAM RILs from the 2017 nursery relative to commercial hybrid checks and the reference line NAM-0

Name	FL Origin	Days to Maturity[a]	Yield (% of Hybrid Checks)[b]	Oil (%)[c]	Protein (%)[c]	Glucosinolates (µmol/g seed)[d]	Erucic Acid (%)[e]
NN8F-029	European	89	85	44.50	28.30	10.60	0.08
NN6R-051	Canadian Adapted	88	82	46.45	26.99	10.75	0.05
NN11R-062	North Korea	90	82	44.80	26.77	10.77	0.05
NN7R-061	European	91	80	44.67	26.73	8.04	0.08
NN34R-040	European	87	80	44.63	28.29	14.23	0.06
NN11R-041	North Korea	87	78	45.39	26.93	11.28	0.11
NN35F-035	European	85	78	46.31	27.60	11.13	0.13
NN42R-059	Canadian Adapted	85	78	45.84	27.47	9.21	0.08
NN41R-055	Canadian Adapted	89	78	44.03	29.74	8.13	0.15
NN34F-022	European	90	77	44.65	26.54	11.22	0.05
NN9R-037	Australia	88	77	44.30	27.81	9.11	0.04
NN11F-008	North Korea	89	77	45.89	26.65	10.59	0.11
NN42R-048	Canadian Adapted	88	75	45.79	26.51	13.62	0.11
NN11F-029	North Korea	88	74	45.53	27.79	11.98	0.12
NN41R-037	Canadian Adapted	88	74	45.69	27.10	10.15	0.37
NN34F-028	European	91	74	45.70	27.50	11.49	0.09
NN41F-022	Canadian Adapted	89	73	45.31	27.35	10.35	0.10
NN11F-017	North Korea	87	73	43.90	27.13	7.72	0.06
NN10F-029	Canadian Adapted	86	73	46.05	26.86	11.05	0.09
NN49F-015	European	89	73	44.80	28.45	11.95	0.15
NN5R-069	European	91	72	44.48	26.99	11.98	0.22
NN6R-057	Canadian Adapted	86	72	44.93	28.30	16.08	0.11
NN16R-040	European	89	71	44.13	27.66	8.15	0.11
NN7F-034	European	90	70	45.27	26.49	9.26	0.13
NN41F-015	Canadian Adapted	91	70	44.10	28.44	11.87	0.16
NN42F-007	Canadian Adapted	85	68	46.05	27.07	9.24	0.05
NN6R-072	Canadian Adapted	86	67	44.33	28.13	16.53	0.05
NN42R-049	Canadian Adapted	90	67	46.72	26.79	7.82	0.17
Hybrid Check 1		90	103	44.51	26.51	11.21	0.08
Hybrid Check 2		91	100	45.53	26.93	18.16	0.08
Hybrid Check 3		115	88	44.68	27.54	18.10	0.05
Hybrid Check 4		89	97	46.03	26.91	8.75	0.05
Hybrid Check 5		88	75	47.62	25.10	14.06	0.09
Hybrid Check 6		89	101	45.87	25.51	8.90	0.04
NAM-0		89	58	45.28	27.49	9.05	0.16

[a] Days after planting to 50% seed color change half way up the main raceme
[b] Average yield of all plots of Hybrid Check 1 through 6
[c] % of the Seed
[d] Total seed glucosinolates; Lines with glucosinolates > 12µmol/g seed highlighted in yellow, but still within WCC-RRC requirements of being less than the mean of the checks plus 2 µmol/g seed
[e] % of the total fatty acids

Deliverables

The deliverables from the specific trials encompassed in this project were:

i) *A final report*
   - Complete

ii) Scientific publications on association of phenotypic traits with genotypic data to decipher genetics underlying the traits and design breeding tools for multi-genic or complex traits across environments
   - A publication summarizing results from the genotyping of the RILs and the 2016 field data is underway, however without repeating the field trial in 2016 with the entire set of 2500 RILs, publication of the data may prove to be challenging.

iii) Contribution of data to a centralized database of phenotypes and genotypes
   - This deliverable was not started due to the truncated project based on funding. Data from 2017 field trials has been analyzed and will also be available to add to the database once built.

iv) Information specifically on phenological response to environments with contrasting day-length and season length
   - Repeating the field trial in Beaverlodge, AB in 2017 revealed the complexity of differential response of a few NAM FLs to the longer daylength at higher latitude (Figures 2&3).

Field plots within the 2017 field trials were used by collaborators in the following ways:

i) In-field sampling or non-standard data collection
   - The RIL trial in 2017 was imaged regularly with GrowPro (RGB) and aerial platforms (RGB and Multi-spectral). Once the Plant Phenotyping and Imaging Research Centre (P2IRC) image analysis pipeline is available (mid-2019), these images will be segmented by plot and analyzed for various digital phenotypic traits.

ii) Providing source seed, seed samples and accompanying data
   - Through this project, we were able to provide seed to the University of Manitoba for both seeding local trials in 2017 and harvested seed from contrasting environments across Canada to assess protein profiles.
   - Once methods are available for high throughput determination of protein fractions (ie. an NIR calibration), reserve seed from 2017 trials (FLs at Beverlodge and RILs in Saskatoon) will be quantified for this new seed trait or phenotype.

Acknowledgements

Under the supervision of Isobel Parkin, Yogendra Khedikar completed means adjustment analysis on the 2016 field data and conducted the GWAS analysis.

4. Significant Accomplishments

- Identification of several NAM RILs with high yield potential that will be tested in yield trials in future seasons.
- A large database of images with associated ground-based phenotype data to be mined (outlined in Table 2).
- Analysis techniques and data-flow pipelines to determine unique digital phenotypes (in collaboration with P2IRC researchers at the University of Saskatchewan) will be available in 2019 and the manual notes from the 2017 RIL trial will be used to further validate the newly developed phenotyping processes and models.
- Data to support continued multi-site evaluation of RILs.
- Data to support development of genomic selection models. Resulting phenotypic values will be used independently or in conjunction with unique digital phenotypes to perform analyses to associate genomic regions controlling different traits and epistatic relationships between loci and traits.
- NAM FLs, RILs and NAM-hybrids (generated from crossing each of the FLs with two industry, male-sterile

testers to generate) have been grown and assessed for agronomic traits and yield in more than 50 trials across Canada since 2013 (Table 2).

Table 2: Complete list of field trials to-date testing NAM Founder Lines (FL), NAM Tester Hybrids and NAM RILs

Year	Germplasm	Design	# of Locations (Loc Codes)[a]	Standard Phenotypes[b]	Yield[c]	Other Phenotypes[c]	P2IRC Phenotypes
2013	Diversity/NAM FL (n~49)	Split-Plot, 4 rep	1 (LL)	X	X	N-Content, Physiological	
2014	NAM Founder Lines	RCBD, 4 rep	2 (SA, LL)	X	Nursery	IR Photos, Seed Diameter	
	NAM Founder Lines	RCBD, 4 rep	1 (LL)			Pod Shatter, Pod Angle	
2015	NAM Founder Lines	RCBD, 2 rep	7 (SA, LL, ME, SC, OUI, OUD, BE)	X	X	Roots and in-depth lodging (ME, OU), Light sensor or leaf area meter	
	NAM Hybrids (n~120), NAM FL & Inbreds (n~60)	TypeIIMAD	3 (LL, SA, SV)	X	X	Light sensor	IR Photos (SA and SV)
	NAM Founder Lines	Unreplicated	1 (LL)			Pod Shatter, Light sensor	
2016	NAM RILs & FL	TypeIIMAD	1 (LL)	X			4-5 UAV flights
	NAM Hybrids (n~110), NAM FL & Inbreds (n~60)	RCBD, 4 rep	1 (LL)	X		Pod Shattering	1 UAV flight, 8 time-lapse cameras (post-maturity)
	NAM Founder Lines	RCBD, 4 rep	1 (ML)	X	Nursery	Pod Shattering, Raceme Images (rep1)	
	NAM Hybrids (n=18), NAM FL & Inbreds (n=9)	RCBD, 4 rep; OU split-plot	7 (SV, SA, OU(+/-Irr), NO, ME, CH, LL)	X	X	IR Photos (OU)	
	NAM Hybrids (n~100), NAM FL & Inbreds (n~60)	TypeIIMAD	6 (SV, SC, SA, ME, LL, BE)	X	X	Light sensor (some sites)	1-2 UAV flights (LL)
	NAM Founder Lines	Rectangular Lattice, 3 reps	1 (LL)	X	X		X
	NAM Founder Lines	RCBD, 4 rep	2 (SA, LL)	X	Nursery	Precocious germination and secondary dormancy	
	NAM Founder Lines	RCBD, 4 rep	2 (SA, LL)	X	Nursery	Sclerotinia resistance	
	NAM Founder Lines	RCBD, 4 rep	1 (UM)	X	X	Protein fractions	
2017	NAM Founder Lines	Rectangular Lattice?, 3 reps	3 (LL, SC, ME)	X	X		X
	NAM Hybrids (n=32), NAM FL & Inbreds (n=16)	Rectangular Lattice?, 3 reps	1 (LL)	X	X		X
	NAM Hybrids (n=27)	RCBD, 4 rep	1 (NO)	X	X		
	NAM Founder Lines	RCBD, 2 rep	1 (BE)	X	X		
	NAM Hybrids (n~60), NAM FL & Inbreds (n=32)	RCBD, 4 rep	1 (SV)	X	?	Pictures	
	NAM Founder Lines	RCBD, 4 rep	2 (SA, ML)	X	X	Precocious germination and secondary dormancy, AAFC UAV	
	NAM RILs (n=1000) & FL	TypeIIMAD	1 (SA)	X	Nursery	AAFC UAV	GrowPro
	NAM RILs (n=500) & FL	TypeIIMAD	1 (LL)	X	Mini-Plots	AAFC UAV	GrowPro, UAV
	NAM Founder Lines	RCBD, 4 rep	1 (UM)	X	X	Protein fractions	
2018	NAM RILs (n=500) & FL	TypeIIMAD	2 (LL, OU)	X	Mini-Plots	AAFC UAV	GrowPro/ProTractor
	NAM Founder Lines	Rectangular Lattice, 3 reps	1 (LL)	X	X		X
	NAM Hybrids (n=32), NAM FL & Inbreds (n=16)	Rectangular Lattice, 3 reps	3 (LL, SC, ME)	X	X		X

[a] LL= Llewellyn Road Farm, SA=Saskatoon, ME=Melfort, SC=Scott, OUI=Outlook Irrigated, OUD=Outlook Dryland, BE=Beaverlodge, SV=Southern Manitoba, UM=Southern Manitoba, ML=Moon Lake, NO=Normandin;

[b] Days to Flower, Duration of Flowering, Days to Maturity, Height, Lodging, Early Vigour and Agronomic Scores, Yield, Seed Quality (Oil, Protein, Glucosinolates, Fatty Acid profiles and Fiber Fractions);

[c] X=Full Yield plots

5. Research and Action Plans

Development of a NAM Centralized Database is a critical step going forward. The population of the NAM Centralize Database with data could be used for the following applications:

i) Aid in the design of future experiments targeting specific traits or genotypes with specific or contrasting alleles across plant science disciplines;
ii) Be mined for phenotypic data to explore allelic effects in candidate genes of interest with molecular biology collaborators.

Potential Applications and Continuing Research

- The initiation of flowering and maturity time will be further examined by GWAS with 2017 RIL data where greater depth of data exists including flowering duration as well as the digital phenotypes for flowering metrics (flowering rate and counts). Also inclusion of other factors known or potentially involved in flowering and maturity time such as plant stand and pre-flowering biomass accumulation will help further understanding of these important adaptive agronomic traits.
- Furthermore, determining photoperiod response in the field is still critical, however pairing the approach with advanced statistical models and emerging imaging options for flowering metrics in a trials also testing seeding date x seeding rate interactions would benefit this endeavor.
- Seed glucosinolate loci identified through GWAS will be used to develop markers for breeding populations where non-canola quality crosses are made in introgress traits of interest (for example, scleortinia tolerance).

Seed Increase

One of our three commercial consortium partners agreed to contribute in-kind support to increase the RILs in the greenhouse in 2017-2018 (~1/3 of the lines) and a contract with a commercial plant breeding services company enabled increase of another ~1/3 of the lines in a greenhouse. Once the remaining ~1/3 of the lines are increased, this seed will be used to plant field-increases, ideally in mini-cages, which will generate enough seed to repeat field trials of the NAM RILs in future growing seasons as well as a reserve for further seed increase.

6. Final Project Budget and Financial Reporting

Please see the attached report.

Please forward an electronic copy of this completed document to:

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