Grant Proposal
North Central Region Canola Research, FY 2010

Development of spring canola lines for biodiesel applications in the North Central Region

Proposal # 4 – Phil McClean, $47,987 (year 1 request, year 2 request is $52,259)

1. Introduction

Background. Oilseeds is one of the fastest growing agricultural markets. It is driven by 1) the emerging and rapidly growing biodiesel industry, which uses canola and other oilseeds as feedstock; 2) shifts in demand for healthful oil traits in the United States and elsewhere; and 3) the combined effects of increased demand for oils in foods world-wide. Oilseeds are strategic crops of great importance to North Dakota. We are the largest producer of canola, a crop with a number of important attributes. The oil content per acre of canola is high and exceeds that of soybeans and other competing crops. Its healthy attributes make it desirable for the food industry, and its cold-flow properties and lubricity properties make it a valuable feedstock for biodiesel production. The major factor limiting the growth of these sectors is the supply of canola as a feedstock.

Specifics. Canola-based biodiesel is a growth industry in the Northern Plains of the United States with new processing plants under construction or in planning stages. Expansion of this industry will reduce US dependency on foreign fossil-based fuels. The production of canola (*Brassica napus*), a favored feedstock for the biodiesel industry, is centered in this region. Currently production does not meet the demand from these plants. Additionally, demand for canola for human consumption is increasing. The estimated short-term demand for canola feedstock by the biodiesel and human oil industries will require 5 million acres annually, a five-fold increase in production. Canola production in the Northern Plains also overlaps with other energy crops.

Canola is one of the most important oilseed crops cultivated in many parts of the world. It ranks as the world's second largest oilseed producing crop next to soybean. Global demand on oil production is increasing rapidly, and the present production is unable to meet the required demand. Canola seed contains higher oil per acre compared to soybeans, 80 gallons vs. 60 gallons. Low level of saturated fat in canola oil improves its cold flow properties. Recently, canola oil is being used for the production of biodiesel for transportation industries. This formulation generates fewer emissions, reduces CO_2, particulate matter, and volatile organic matter in the atmosphere, and provides better lubrication and low sulfur emissions in a large number of vehicles. This later aspect is particularly important for the biodiesel market development for which the production cost must be as low as possible (Coltrain, 2002). For the crushing industry, the main value of canola is linked to the oil content of the harvested seed. Therefore, increasing seed oil remains an important objective of canola breeding (Delourme et al., 2006). Quantitative genes with additive and epistatic gene action control the oil and protein contents in seed (Grami and Stefansson 1977).

Although seed oil is controlled by genetic factors, environment factors are also important (Leon and Becker 1995). Because of the environmental conditions, a high correlation between selection and target environments must be significant for selection to be efficient (Fehr, 1987). It has been reported that oil content is a highly heritable character in *Brassica* seed (Becker et al. 1999). Different research groups identified various numbers of genes that control the oil content in Brassica seed (Zhao et al. 2005, Zhao et al. 2006). These findings strongly support the concept that content can be increased by conventional plant breeding methods.

Plant selection is an important criterion for the development of high oil cultivars. For example, a 4% kernel oil contained maize line was improved to 20% kernel oil contained line by using 28 cycles of selection (Alexander 1988). In the United States, soybean acres are being replaced by corn in the southern region which opened an opportunity for canola in the northern regions. The Northern Plains is the major canola growing region, and biodiesel processing plants have been established in this area. These bring a large economic value to the economy.

Keeping all these aspects in mind, we have taken a four year breeding scheme to evaluate canola lines in the field by maximizing the exposure of the breeding lines to more than 11 environmental conditions. We will use winter nursery facilities in Chile to expedite progress. This will allow for the production of seed for field testing early in the inbreeding process. This will also allow us to evaluate the performance of lines in other environmental conditions.

Current work. Breeding work was initiated by the Center of Excellence for AgBiotechnology, Oilseeds (COE), during the winter of 2006-07 by crossing spring canola lines selected in 2006. Three hundred spring canola lines (offered for evaluation by Monsanto, our corporate sponsor) were evaluated for grain yield, standability, plant height, days and growing degree units (GDU) from planting to 10 and 90% flowering, flowering window (days and GDU), relative maturity compared to 357RR Magnum (check), and oil and protein content. Field trials were conducted at Prosper, Minot, Carrington, Langdon, and Williston, North Dakota. Around 100 lines were pre-selected based on seed yield and seed oil content. From them, 35 lines were selected based on yield of oil per acre and agronomic traits as parents of the segregating populations that were developed in order to combine desirable traits. On the basis of oil production/acre, six superior lines were identified and intra-crossed to develop superior lines from segregating populations. Also, double-crosses between selected F_1 hybrids and crosses with public rapeseed germplasm are being developed to attain the same objectives. These crosses are being made to increase the variability of the germplasm in the program.

In addition, a disease nursery was designed in cooperation with Dr. Luis del Rio (Canola research, Dept of Plant Pathology, NDSU). Research focused on: 1) the development of artificial inoculation methods to evaluate resistance to blackleg. Besides determining the level of resistance of the lines, we will be evaluating the impact of blackleg infection on seed yield, seed oil content and oil/acre; and 2) evaluation of germplasm for resistance to Sclerotinia Stem Rot (SSR). The first evaluation will be made in the greenhouse using the PIT methodology. The experimental units will be screened for physiological resistance reactions, and superior canola genotypes will be identified. The second evaluation will be conducted under field conditions in the summer of 2009 at the Prosper Research Extension Center (Prosper, ND). Testing and quality evaluation of canola lines for composition and biodiesel use will be performed by the NDSU Dept. of Ag. and Biosystems Engineering, in the framework of the recently-funded Center of Excellence for AgBiotechnology, Oilseeds.

Project personnel. Dr. Phillip McClean and Dr. Mukhlesur Raman are the co-principal investigator. Dr. McClean is associate director of the Center of Excellence for Oilseed Development. His responsibilities are in the area of plant breeding and line development. Dr. Raman is an employee of the center as the principal plant breeder. He manages the day-to-day activities of the breeding program. These two researchers collectively set the long-term goals of the program and will implement these goals in a manner that ensures success.

It is important to note that this grant is requesting funds to augment the funds currently allocated to the center. The research that is being proposed is outside the activities that are currently supported by the center.

2. Objectives

i. Develop high oil content spring canola germplasm

ii. Create canola germplasm with properties suitable for biodiesel applications in the North Central region of the United States

3. Rationale and Significance

Increasing the seed oil content in canola is an important breeding objective because of the economic value of the oil. In North Dakota, the construction of at least two new plants devoted to the production of biodiesel from canola oil will increase the demand of canola up to three-fold in the next years (Eric Mack, Archer Daniels Midland Co., personal communication). Increasing oil content in the seed by 1% would be worth an addition of $8/mt of seed price. This in turn will provide better financial return to the growers and the biodiesel industry (Eric Mack and corroborated by market research studies). The increasing demand for canola oil can be fulfilled by: 1) importing canola from Canada; 2) increasing the acreage devoted to canola in North Dakota; 3) increasing canola seed yields in North Dakota; 4) increasing the percentage of oil in canola commercial cultivars; or 5) by combining the last three options. From a breeding and genetics perspective, improvement of canola for seed yield and seed oil content to meet the needs of both farmers and oilseed industry implies an expensive challenge. This project will meet the challenge by releasing improved spring canola lines to be used as open-pollinated cultivars by farmers, used to develop inbred lines (A lines and B lines) for hybrid cultivar development, or as parents in other breeding programs. The funds from this proposal will complement the budget that the NDSU Canola Breeding Program obtained from the Center of Excellence for AgBiotechnology, Oilseeds. Specifically, a greater number of lines will be developed and evaluated, and the breeding process will be accelerated by planting and evaluating winter nurseries.

4. Approach:

Year one (2009-2010). New line development was initiated during the winter of 2007 by crossing spring canola lines selected in 2006. Selection criteria included high seed oil content, seed yield, and other important agronomic traits. Segregating populations developed to combine desirable characteristics are being grown at greenhouse facilities at NDSU. New crosses were developed during the winter of 2008 among selected lines from the preceding summer trial. Two generations of inbreeding is being achieved during the winter of 2008-09. In the year 2009, a total of 40 F_2 populations were planted, evaluated, self-pollinated and advanced to the $F_{2:3}$ level of inbreeding in the summer nursery. Traits evaluated were days to flowering, standability, resistance to blackleg and sclerotinia stem rot (SSR) under field condition, and relative maturity compared with standard checks. Selection was performed among and within populations. Seed from each selected F_2 plant was sent to a winter nursery location in Chile and are growing in individual rows. Three to five plants per row will be self-pollinated and advanced to the $F_{3:4}$ generation and will be planted in individual rows in the summer nursery of 2010. The remainder of each row will be bulk-harvested, and the seed will be used to conduct early generation testing (EGT) in three locations of North Dakota in 2010.

Year two (2010-2011). Lines collected from the year one winter nursery will be evaluated for oil content and then evaluated in an early generation testing (EGT) nursery that will be grown at three locations in North Dakota: Prosper, Minot, and Langdon. These trials will use appropriate field designs and a number of replications according to the number of entries for each experiment. Experimental units will consist of 6-row plots (48” width by 15’ length) with 8” distance between rows. The experimental units will be seeded to attain a plant density between 10 to 15 plants per square foot, swathed at ripening, and then harvested. The lines will be evaluated for seed yield, seed moisture at harvest, seed oil content and fatty acid composition, days to flowering, relative maturity, standability, resistance to blackleg and SSR, and other agronomic traits. Three to five plants per row will be self-pollinated and advanced to the $F_{4:5}$ generation. These plants will be planted in individual rows in the winter nursery of 2011. Three to five plants per row will be self-pollinated and advanced to $F_{5:6}$ generation and in turn planted in individual rows in the summer of 2012. The remainder of each row will be bulk-harvested, and the seed will be used to conduct preliminary field trials (PFT) in 2012. Field trials will be conducted at six locations in North Dakota: Prosper, Minot, Langdon, Carrington, Hettinger and Williston using appropriate field designs and number of replications according to the number of entries of each experiment. The experimental units will consist of 6-row plots (48” width by 15’ length) with 8” distance between rows. The experimental units will be seeded to attain a plant density between 10 to 15 plants per square foot, swathed at ripening, and then harvested.

Experimental data for each line will include seed yield, seed moisture at harvest, seed oil content and fatty acid composition, days to flowering, relative maturity, standability, resistance to blackleg and SSR, and other agronomic traits.

Year three (2011-2012). Seed from individual plants of selected lines will be grown in the winter nursery, purified, and bulked to conduct advanced field trials in 2012. Evaluation will be conducted as described for year 2 at six locations in North Dakota and the lines identified as potentially improved will be entered in canola variety trials. A simplified scheme of the procedures to attain the objective is shown in the Project Timetable.

Project Timetable. This project will be completed under the timetable found in Table 1. The table lists the season a specific aspect of the project will be completed, the plant breeding generation that will be tested during that season, the location of the test, and the specific traits that will be selected in that particular generation.

Table 1. Canola breeding project timetable.

Season	**Generation**	**Place**	**Trait selected**
Fall 2008	Line A x Line B	GH	
Spring 2009	F_1	GH	
Summer 2009	F_2	Field	BL, SSR, OIL, AGR
Fall 2009	$F_{2:3}$	WN	
Summer 2010	EGT ($F_{3:4}$)	Field	YIELD, MOIST, BL, SSR, OIL, AGR
Fall 2010	$F_{4:5}$	WN	
Summer 2011	PFT ($F_{5:6}$)	Field	YIELD, MOIST, BL SSR, OIL, AGR
Fall 2011	$F_{6:7}$	WN	
Summer 2012	AFT	Field	YIELD, MOIST, BL, SSR, OIL, AGR
Fall 2012			Final selection and increase of potential releases

Abbreviations: GH: greenhouse; **WN**: winter nursery; **EGT**: early generation testing; **PFT**: preliminary field trials; **AFT**: advanced field trials; **BL**: blackleg; **SSR**: Sclerotinia stem rot; **OIL**: seed oil content and fatty acid composition; **AGR**: agronomic traits.

5. Outreach/Extension Activities

The demand of canola feedstock is increasing and for biodiesel production in North Dakota. Indeed, most germplasm had been developed elsewhere, and as a result the crop is underperforming relative to its potential in North Dakota. Therefore, the research commitment to the development germplasm for these areas will have a positive impact to increase the potentiality of the crop in growers' field. The breeding effort will improve the genetics of the crop for high seed yield, high oil content, and disease resistant cultivars. Moreover, we will be developing materials that have unique agronomic adaptations (warmer and drier environments) and improved agronomic traits (reduced shattering). All these activities will be presented to growers and the appropriate commodity group. This information will assist them as they plan for the growing year.

References

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6. Personnel support

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Estimate of the time commitment involved: 80%

Budget

Student salary and fringe benefits - $7,475
Domestic Travel - $812
Foreign Travel - $3,500
Materials and Supplies - $1,200
Winter Nursery facilities rental - $35,000
Total $47,987