APPLICATION CHECKLIST

Use this checklist as a tool to ensure that you have all of the components of the application package. Please note, this checklist is for your use only and does not need to be included in the package.

X☐	Application
X☐	Transmittal Letter
☐	$100 Application Fee SENT VIA MAIL
X☐	Tax Liability Statement
☐	Letters of Support (If Applicable)
☐	Other Appendices (If Applicable)

When the package is completed, send an electronic version to the Industrial Commission at ndicgrants@nd.gov. Send payment to:

North Dakota Industrial Commission
Attention: Renewable Energy Program
State Capitol – 14th Floor
600 East Boulevard Ave Dept 405
Bismarck, ND 58505-0840

For more information on the application process please visit:
https://www.ndic.nd.gov/renewable-energy-program/rep-applicant-council-information

Questions can be addressed by calling 701-328-3722.

Renewable Energy Program

North Dakota Industrial Commission

Application

Project Title: Use of Bioengineering to Enhance the Agronomic Potential of Camelina for Use as a Source for Biofuel Feedstock and Meal for Livestock

Applicant: CamBioGene

Principal Investigator: Eric J. Murphy

Date of Application: 2 October 2023

Amount of Request: $500,000

Total Amount of Proposed Project: $1,151,250

Duration of Project: 3 years

Point of Contact (POC): Eric J. Murphy

POC Telephone: 701-213-1510

POC Email: eric.murphy@cambiogene.com

POC Address: 3902 15th Ave. S. Grand Forks, ND 58201

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Transmittal Letter

North Dakota Industrial Commission
Attention: Renewable Energy Program
State Capitol – 14th Floor
600 East Boulevard Ave Dept 405
Bismarck, ND 58505-0840

2 October 2023

Dear Sirs:

Please find herein our application for a Renewable Energy Program grant from the North Dakota Industrial Commission. We are requesting $500,000 with an anticipated match of $543,250 and additional in-kind match of $108,000, for a total budget of $1,151,250.

CamBioGene is leveraging over 20+ years of experience in plant sciences and is a new startup company in Grand Forks, ND. We have no tax liability owed to the State of North Dakota. This is an exciting project to bring a sustainable source of oil seed feedstock to our biorefineries in the State of North Dakota. This would put North Dakota in a leadership position as we address the food vs fuel debate around use of soybean oil as a feedstock for fuels.

I look forward to your consideration of our proposal.

Sincerely,

Eric J. Murphy

CEO, CamBioGene

ABSTRACT

Objective:
There are three objectives to this project:
1). Expand existing high lauric/myristic acid camelina for field trials and commercialization.
2). Expand existing lines of camelina with mutated acetolactate synthase (ALS) that imparts class-2 herbicide tolerance for field trials and commercialization.
3). Transform camelina with purple acidic phosphatase-2 (PAP2) to increase seed size, seed number per plant, and net oil content and move transformed plants into field trials.

Expected Results:
For Objectives 1 and 2, we expect to expand 4-5 existing plants lines for the high lauric/myristic acid camelina and 4-5 existing lines for mutated ALS containing camelina in growth room located in Helsinki, then transferring the seed stock to the U.S. for field trials. As we routinely expand seed stocks, this expansion is not anticipated to be a problem. Application for field trials will be done through APHIS and EPA as necessitated by federal regulations. It would be ideal, based upon approval by federal regulatory agencies, to perform field trials at several field trial locations that are managed by North Dakota State University (NDSU). Based upon field trials data, applications for approval of commercial launch in the U.S. will be initiated.

For Objective 3, there is existing data suggesting that enhancing the expression of PAP2 in camelina will increase agronomic performance. Based upon our 20+ years of experience in transforming camelina, we do not anticipate a problem transforming camelina with PAP2. This process will take 1.5 to 2 years to establish fourth generation plants. We don't anticipate any problems with transformation or lethality of overexpression of PAP2 in the plant. Upon satisfying the requirements of U.S. regulatory agencies, we will move into field trials to commence in spring of 2026.

Duration:
Duration of this project is 3 years, with an anticipated start in late 4Q23 to early 1Q24.

Total Project Cost:
$1,151,250

Participants:
CamBioGene is based in Grand Forks, ND and Agragen, OY in Helsinki, Finland. Ongoing negotiations are in the final stages to transfer ownership of Agragen, OY to CamBioGene and the exclusive licensing of Agragen, LLC's existing intellectual property to CamBioGene. The founder and CEO of CamBioGene, Eric J. Murphy, currently serves as Chairman of the Board for Agragen, OY and Senior Vice-President of Research and Development of Agragen, OY and Agragen, LLC.

Additional participants are anticipated to be NDSU for field trials.

PROJECT DESCRIPTION

Objectives:

There are three objectives to this project:

1). Expand existing high lauric/myristic acid camelina for field trials and commercialization.

2). Expand existing lines of camelina with mutated acetolactate synthase (ALS) that imparts class-2 herbicide tolerance for field trials and commercialization.

3). Transform camelina with purple acidic phosphatase-2 (PAP2) to increase seed size, seed number per plant, and net oil content and move transformed plants into field trials.

Methodology:

Use our existing, patented transformation techniques to transform *Camelina sativa* (camelina) using agrobacterium to insert a construct containing the target gene into the genome of camelina. Stable transformants, as confirmed by expression of the target gene, are grown and the seeds harvested. These seeds are planted to generate the T2 generation of plants, and this process is repeated to ensure that no negative impacts of the transformation occur in successive generations and that the transformation is stable. Stable plant lines are then prepared for seed expansion and analysis as required by federal compliance agencies for field trials.

Field trails will be conducted in collaboration with NDSU. Initial trials will be small plots, 10 x 10 m, and then expanded to larger plots in the second year. Plant performance and phenotype will be assessed with an emphasis on target gene expression, yield, seed oil lipid composition and content, and protein amino acid composition and content. Upon the completion of two years of field trials, varieties will be selected to move into application for commercial release with APHIS and EPA as needed.

For camelina containing mutated ALS, application of class-2 herbicides will be used to determine dose-dependent tolerance to imidazolinone and to chlorsulfuron. Although the major objective of class-2 herbicide tolerant camelina is to prevent crop failure due to residual class-2 herbicides in the soil, it is possible that future use of class-2 herbicides to control weeds in fields will be advantageous.

Anticipated Results:

We anticipate that the phenotype for each camelina will be maintained during the field trials. Hence, both the ALS and high lauric/myristic acid camelinas will continue to produce the biochemical phenotype displayed in the growth room during field trials.

Because there is one paper in the literature demonstrating the positive attributes of overexpressing PAP2 in camelina, we anticipate seeing the same results in our hands. Hence, we anticipate generating a camelina with greater seed size and seed yield and successful generation of this camelina in the laboratory will result in plant varieties for field trials.

Facilities:

CamBioGene has a growth room in our Helsinki location and will expand our laboratory space for transformation of camelina. This will require a capital investment by CamBioGene for the necessary

equipment to carry out these transformations. The equipment previously held by Agragen, OY was sold in a financial reorganization and much of the equipment was antiquated and required replacement. Since that time, Agragen, OY has been using the growth room to expand seed stocks and to conduct camelina breeding.

Unfortunately, at this time there are no laboratory resources in the greater Grand Forks regions suitable for a plant sciences laboratory. As such, the plan is to continue operations in Helsinki for 2-3 years, when we anticipate was can raise sufficient capital for building a laboratory facility or laboratories are made available in a partnership with the University of North Dakota.

Resources:
Our major resource is the intellectual infrastructure that exists in Helsinki. Both Dr. Kuvshinov and Svetlana Kuvshinov pioneered the techniques for transforming camelina. Dr. Kuvshinov also pioneered the techniques used to effectively breed camelina. We have assembled a low cost approach to expand camelina in an inexpensive, controlled environment, essential for this project.

NDSU has worked with camelina at several of its Research Extension Centers. The REC at Williston, Hettinger, Dickinson, and Carrington will be considered for the field trials and a key consideration is ability to comply with APHIS requirements.

Financial resources for CamBioGene include an investor from the Cincinnati area investing $250,000 and an expected match of $250,000 from the federal COVID economic development funds in angel matching program at the North Dakota Development Fund, North Dakota Department of Commerce.

Techniques to Be Used, Their Availability and Capability:
The techniques used are well established by CamBioGene personnel who pioneered the transformation of camelina. As such, the intellectual infrastructure is readily deployable and has full capability to complete seed expansion and transformation as required in Objectives 1-3.

NDSU has worked with camelina in the past at multiple REC. As such, there is little doubt that they have the infrastructure in place to complete the field trials. However, engaging NDSU for seed trials will more than likely commence in late fall of this year as we determine seed stock expansion in Finland. If sufficient seed stock expansion has not occurred, we may use greenhouse expanded seed stock and initiate field trials in spring of 2025.

Environmental and Economic Impacts while Project is Underway:
There are no anticipated environmental impacts for this project. The transformed camelinas will be assessed for suitability to be released for commercial use by APHIS and other relevant federal agencies. Hence, that is the environmental impact assessment.

Economic impacts are perceived to be positive, giving North Dakota producers greater cropping choices, especially producers in the more arid regions of the state.

Ultimate Technological and Economic Impacts:
The ultimate technological impact is to produce varieties of camelina that have enhanced class-2 herbicide tolerance, that have enhanced seed yield and seed size, which reduces seed loss during harvesting, and that have a fatty acid profile that enhances the quality of the feedstock for traditional biofuel production as well as for production of non-oxygenated biofuels.

Commercialization of these bioengineered camelinas provides an additional cropping choice for North Dakota farmers, especially those producers in the more arid regions of North Dakota. Using a closed loop cropping system, producers uncouple the production from the traditional commodity pricing system. As the long-term goal of CamBioGene is focused on plant made pharmaceuticals, successful producers will have an opportunity to participate in growing camelina used in production of biotherapeutic proteins, which has a very high potential for enhanced payments to producers.

Why the Project is Needed:
The food to fuel debate is important one in selection of an oil to be used as a feedstock for biofuel production. Unfortunately, soybean oil comprises a tremendous amount of feedstock used in biofuel production, removing this a widely used oil from our food supply. As such, it is important to consider if this oil should be used to make fuel or whether alternative feedstocks from alternative crop oil feedstocks should be considered. Camelina fulfills this need as it is a non-food oil.

Additional advantages for camelina are that it grows on less productive land than soybeans, requires less inputs than canola, and its moisture requirements are low. This makes it an ideal crop for western North Dakota farmers and serves as an alternative to wheat. In fact, experience in Montana demonstrates that in a two-crop rotation, wheat yields following camelina are greater, presumably due to greater residual soil moisture, an added advantage for North Dakota producers.

In addition, camelina performs poorly for traditional biodiesel due to the high degree of fatty acid unsaturation in its oil and a higher gel point. By adding medium chain saturated fatty acids and reducing the unsaturation of the oil, the iodine number (degree of unsaturation) should be reduced, and the gel point will also be reduced, making it a much better oil feedstock for traditional biofuel. Hence, the rational for transforming camelina to synthesize high lauric and myristic acid (12:0 and14:0, respectively) is to reduce the degree of unsaturation while also changing the physical chemical properties of the oil, thereby reducing the gel point.

In our experience, significant crop failures for camelina have occurred due to residual class-2 herbicide levels in fields. While the emergence of these crops was outstanding, the seedlings quickly died. Forensic agronomy indicated that in fields experiencing this failure, there were high levels of residual class-2 herbicides accumulated in the soil. This coupled with the high susceptibility of camelina to these herbicides resulted in crop failure. We have engineered the active site of ALS to increase herbicide tolerance.

Hence, camelia provides a higher value cropping solution for the more arid regions of North Dakota that removes the traditional dependency on commodity pricing due to a closed loop farming system where CamBioGene, or a future partner, provides seed and purchases the crop for downstream processing into oil for biofuel production and meal for livestock feed.

STANDARDS OF SUCCESS

Measurable Deliverables

Objective 1 and 2: Seed expansion 4Q23 through 4Q24 with field trials starting with spring planting in 2025. APHIS application for field trials and for transferring of seed into U.S. from Finland will commence in 3Q24. Field trials in spring 2025 and data analysis following harvest assessing both physical and biochemical phenotype. Field trials repeated in spring 2026 and assessment of physical and biochemical phenotype. If data is sufficient, in collaboration with APHIS and other federal regulatory entities, submit an application for commercial use.

Objective 3: Transformation of two varieties of camelina with class-2 herbicide tolerance with a construct containing purple acidic phosphatase-2 (PAP2). Determine successfully transformed plants and plant T1 generation seeds. Assess seed size and seed yield of T1 plants, selecting plants to move forward T2 generation and repeat assessment and selection for T3-T5 generations. Expand T5 plants and begin application for transferring seed to the U.S. and field trials. This will occur 4Q23-4Q25. Field trials are anticipated in spring 2026.

Value to North Dakota

A transformed camelina for use by producers to enhance field performance and production of camelina for biofuel feedstock provides options to add another suitable crop for use as a rotation crop in the more arid locations in North Dakota. Further, the initial success of CamBioGene will help raise capital for projects in the pharmaceutical space, with much higher returns for our most successful producers. Our long-term vision is to use camelina to product AGR-131, which is an etanercept (Enbrel) biosimilar in a drug family that currently has about a $45B market cap. Using our patented protein expression system, we anticipate being able to substantially lower the cost for this drug commonly used to treat rheumatoid arthritis and psoriatic arthritis. This represents a strong potential reduction in drug costs to insurers and to the State visa vie Medicaid. Additional pharmaceuticals are in the pipeline.

Public and Private Sector Use

The public sector is not the primary beneficiary of this project beyond what is highlighted above. Private sector use will be planting seed for producers and crushing options for our emerging oil seed crushing facilities. Oil will be used by our state's biorefineries, and the meal can be used in state for livestock feed. A letter of no objection has been issued by the FDA for use of camelina meal in poultry and cattle rations.

Commercial Use of Deliverables

Commercial use is camelina oil as a feedstock in the production of biofuels and camelina meal for use as a livestock ration. The plants expressing PAP2 we anticipate will have enhanced seed yield and bigger seed size, which will increase harvest yield.

Enhance Research, Development, and Marketing for North Dakota's Renewable Energy Resources

Funding of this application will help establish a plant sciences company in North Dakota. Providing funding helps move two projects through commercialization and initiate a project that will enhance key agronomic properties of camelina. It is critical to diversify the economy of North Dakota and adding a plant biosciences company links two elements we do well agriculture and downstream processing including biofuel production. But unlike many efforts in agriculture our long-term goal is to have all facets of CamBioGene located in Grand Forks, North Dakota. By leading the way in the use of camelina-derived oil for biofuels, we uncouple biofuel production from using food grade oils, leading the way on the fuel vs food debate.

Satisfying Mission of Grant Program

This project promotes a sustainable feedstock for traditional biodiesel and feed stock for deoxygenated biofuel production. One important element is a feedstock must compete with petroleum-based oil in the absence of subsidies, and camelina does that because of its low cost of production, whereas soybean oil is much less competitive in the $70-85 per barrel price range. That ensures sustainability as a renewable energy feedstock and long-term market stability. This project is focused on improving a crop that can be introduced in North Dakota, providing producers with a cropping option that uncouples them from the traditional commodity market driven pricing structure associated with other crops. This adds wealth for landowners and our agriculture producers, aiding in maintaining a robust rural economy.

But most importantly, as noted previously, it is critically important to have at-scale feedstocks to feed biofuel needs without using human food grade oils. Soybean oil is traditionally used, but in the long-term this oil should be targeted for human consumption. This project will position North Dakota to be a leader in alternative seed oil-derived feedstocks, a positive position in the food vs fuel debate.

BACKGROUND/QUALIFICIATIONS

CamBioGene is a new plant biotechnology company based in Grand Forks, ND that is leveraging over twenty (20) years of expertise working with Camelina sativa (camelina). It has exclusively licensed an extensive, world-leading intellectual property (IP) portfolio giving it freedom to operate (FTO) as a fully operational plant science company. Using this technology and existing intellectual infrastructure in Helsinki, FI, CamBioGene will have the capability to significantly improve key traits in camelina using proven techniques for genetic engineering. This project is designed to take two existing projects to field trials in collaboration with North Dakota State University Research Extension Centers' APHIS qualified field trial locations.

The first project is a high lauric/myristic acid camelina that putatively overcomes suitability problems for using camelina oil as a feedstock for traditional biofuels, e.g. biodiesel. Camelina has been used extensively in the past as a biojet feedstock. It was successfully used by KLM in a daily flight from Amsterdam to Frankfurt using 100% camelina derived biojet in a continuous manner as well as by the U.S. Navy to fly an F-18 fighter jet on 100% camelina oil derived biojet. However, when used in traditional biodiesel, camelina underperforms other seed oils, in part due to its high iodine number (high degree of unsaturation in the fatty acid chains) and due to a relatively high gel point, often just above standards. By increasing the medium chain saturated fatty acids coupled with a reduction in the total amount of unsaturation, the goal is to produce a camelina oil that could be used to make traditional biodiesel as well as deoxygenated fuels, including biojet.

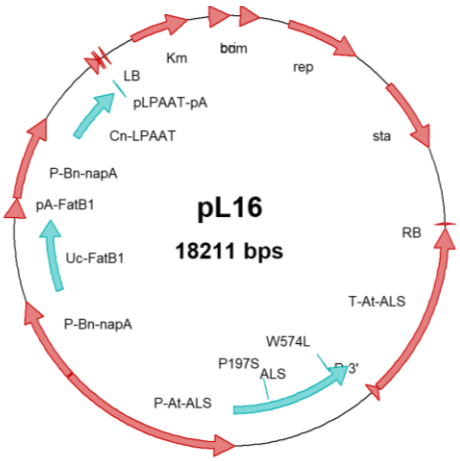
The second project is a class-2 herbicide tolerant camelina that overcomes the normal sensitivity of camelina to residual class-2 herbicides in the soil. This plant is bioengineered to replicate successful mutations in the active site of acetolactate synthase that impart tolerance to class-2 herbicides in weeds. By using a combinatorial approach, we elucidated which amino acids in the active site had to be modified to gain tolerance to chlorsulfuron and imidazolinone.

Projects 1 and 2 are ready for seed expansion, final analysis for an application for field trials, and then field trials. This is essential for commercialization.

The third project is designed to overexpress purple acidic phosphatase-2 (PAP-2) in camelina to increase yield and seed size, both of which impact yield off the field. Work presented in the literature indicates that both traits are increased by PAP-2 expression in camelina. Seed size is an important consideration as seed is lost during harvesting and just a small increase in camelina seed size is advantageous for greater off field yield. Thus, increasing seed yield per plant as well as yield during harvest would add to the profitability of camelina.

PROJECT 1.

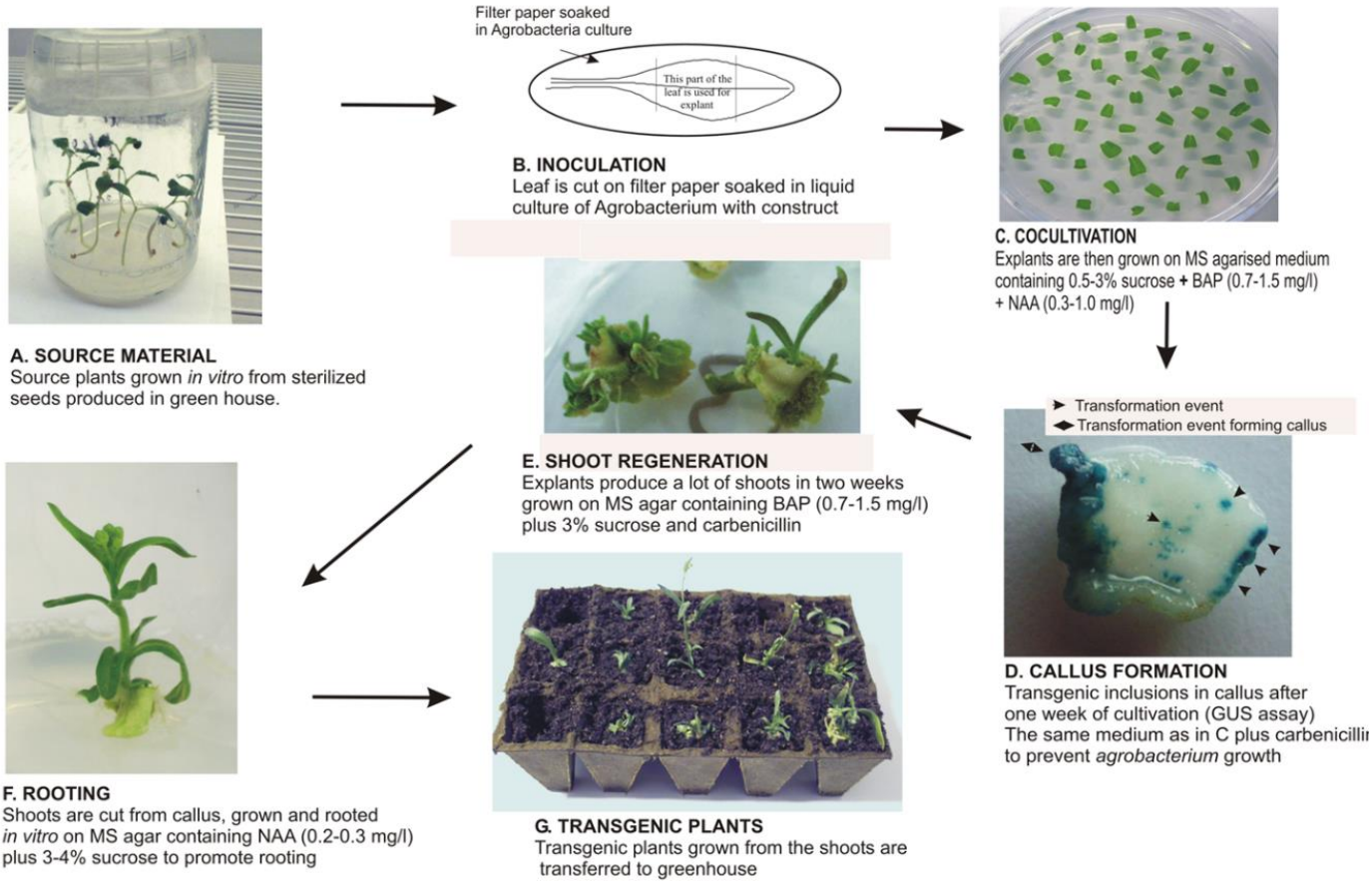
Figure 1. Construct for transforming camelina.



The construct used to transform camelina to produce lauric (12:0) and myristic (14:0) acid is shown in **Figure 1**. This construct contains the genes from *Umbellularia californica* (California bay) thioesterase (Uc-FatB1) under control of *Brassica napus* NapA-promoter and *U.c.* thioesterase terminator. *Cocos nucifera* lysophosphatidic acid acyltransferase (Cn-LPAT) under control of *Brassica napus* NapA-promoter and Cn-LPAT terminator is also included. This construct also contains the gene for ALS with the P197S and W574L mutations to impart optimal tolerance to class-2 herbicides and provide a non-antibiotic selection marker. This construct is placed in agrobacterium that is then used to infect camelina leaflets and inserts this construct randomly into the camelina genome. The process is demonstrated in **Figure 2** below, where sterile leaflets are grown, the leaflets harvested and inoculated with agrobacterium containing the construct in Figure 1. These leaflets are cultivated on medium and callus formation occurs and these calluses are

removed and put on medium to induce shoot generation. Successful shoots are transferred to rooting medium and following root development, put into soil to grow T0 plants. These plants produce less seeds than normal camelina. Plant tissue is assessed for mRNA levels of constructs and plants with the highest expression level have their seeds planted (T1 plants) and those seeds harvested. This process is then repeated for successive generations and transformation stability is assessed.

Figure 2. Transformation Protocol for Camelina



Camelina normally does not synthesize a fatty acid with a chain length shorter than 16:0 carbons (**Table 1.**). Thus, to produce a shorter chain fatty acid, we transformed camelina to express the thioesterase from the California bay plant. This cleaves the fatty acids from fatty acid synthetase when the chain length is 12 and 14 carbons, thus producing 12:0 and 14:0. Using the lysophosphatidic acid acyl transferase from coconut, the incorporation of these fatty acids into the plant oil in the seed is facilitated. Currently have plants in the T7+ generation and the level of 12:0 and 14:0 is slightly less than

in Table 1, but still very commercially viable. A similar strategy was employed to make a canola with high lauric acid by Calgene and grown in the Park River, ND region a few years ago.

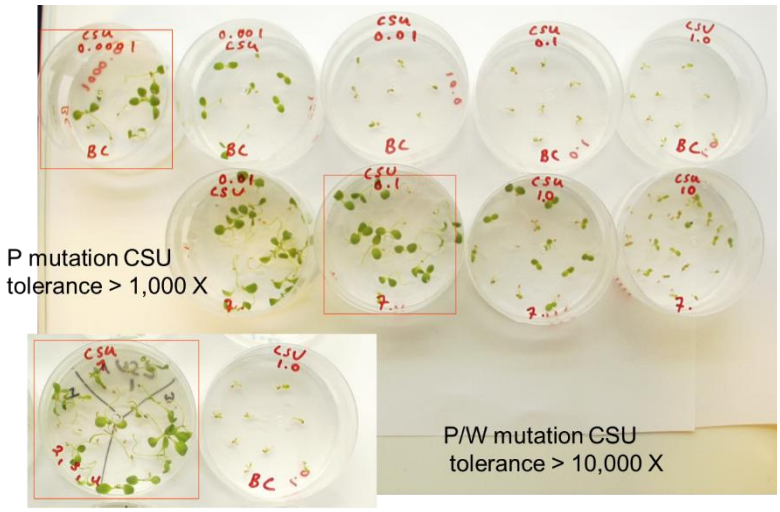
Table 1. Fatty acid composition of camelina producing 12:0 and 14:0

Fatty acid	1	2	4	5	6	7	8	9	10	Control	Vector
Lauric 12:0	3.4	6.4	23.1	19.8	0.2	14.5	13.5	10.7	4.4	0.0	0.0
Myristic 14:0	0.7	1.1	4.2	3.8	0.1	2.5	3.0	2.4	0.8	0.0	0.0
Palmitic 16:0	6.0	6.0	7.0	4.3	4.8	6.2	5.6	5.6	5.6	5.8	6.3
Stearic 18:0	3.4	2.8	2.1	2.3	2.9	2.8	2.5	2.5	3.2	4.0	4.4
Oleic 18:1n-9	14.8	14.0	9.9	8.8	16.9	10.9	10.6	11.1	11.8	17.2	14.7
Linoleic 18:2n-6	16.4	17.1	11.7	12.4	17.3	16.6	13.8	14.3	13.8	16.1	14.6
Linoleic 18:3n-3	31.7	29.7	28.9	30.8	32.3	27.2	30.3	33.5	36.8	31.5	34.1
Arachidic 20:0	1.9	2.0	1.8	2.0	1.9	2.5	2.3	2.2	2.7	2.0	2.4
Eicosenoic 20:1n-9	12.6	12.9	8.1	8.7	13.8	10.2	9.7	9.4	12.4	13.6	12.8
Eicosadienoic 20:2n-6	1.8	1.6	0.9	1.1	1.7	1.3	2.1	1.5	1.6	2.4	1.8
Eicosatrienoic 20:3n-3	1.4	1.2	0.8	1.0	1.4	0.9	1.2	1.3	1.6	1.5	1.7
Behenic 22:0	0.7	0.6	0.4	0.5	0.5	0.4	0.7	0.5	0.5	0.6	0.9
Erucic 22:1n-9	3.5	3.4	2.7	3.0	3.2	3.2	3.5	3.5	3.0	3.1	4.3
Lignoseric 24:0	0.4	0.3	0.3	0.3	0.6	0.5	0.2	0.3	0.3	0.3	0.3
Nervonic 24:1n-9	1.1	0.9	0.7	0.8	1.1	0.9	1.0	1.2	1.1	1.3	0.9
Camelina parent line	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC*

As highlighted in red are lauric (12:0) and myristic (14:0) levels in transformed line of the Blaine Creek varietal, which is a good performing camelina in fields. Note that there is normally no 12:0 or 14:0 in camelina. There is a reduction in 18:0 (stearic acid) and in 18:1 (oleic acid), although the essential fatty acids remain at a level similar to seeds from control and vector control plants. There is a modest reduction in 18:2, which when combined with the reduction in 18:1, would depress the iodine number. Similar changes in the fatty acids is maintained in successive generations (proprietary date, not presented).

Hence, we have plants that are stably transformed to produce 12:0 and 14:0. The seed stock of the best lines are being expanded for field trials as proposed in this application.

PROJECT 2.



A similar construct was made to transform camelina to express acetolactate synthase with up to three of the following mutations: A122T, P197S, and W574L. We found that while single P197S mutation increased tolerance 1,000-fold, but a combination of the P197S and W574L mutation provided the greatest increase in tolerance to chlorsulfuron >10,000-fold.

Figure 3. Enhanced tolerance to chlorsulfuron by P197S mutation and stacked P197S and W574L

Table 2. Single and combined mutations of ALS active sit on metsulfuron, chlorsulfuron, and imi tolerance in transformed camelina.

	IMI	MSU	CSU
C.microcarpa wt	< 0.001	< 0.001	<< 0.001
C.microcarpa W-mutation	> 0.1 100 X	> 0.1 10 -100 X	0.001- 0.01 100 X
Camelina wt (Blaine Creek)	0.01	0.0001	0.0001
Camelina W -mutation		0.0001	0.01 10 -100 X
Camelina P/W -mutation	10 1000 X	1 10 000 X	1 10 000 X
Camelina P/W -mutation/ *C.microcarpa* W-mutation	10 X	100 X	100 X

In **Table 2.**,increased tolerance of mutated camelina was assessed and compared wildtype camelina and to wildtype camelina macrocarpa and to mutated camelina macrocarpa (W574L). The red values represent the of herbicide where tolerance is lost. The W574L mutation demonstrated less tolerance to metsulfuron than chlorsulfuron but had no effect on imi tolerance. Combining the W574L mutation with the P197S mutation resulted in a 1,000-fold increase in tolerance to imi and a 10,000-fold increase in tolerance to mutsulfuron and chlorsulfuron. Additional stacked mutations did not add any additional tolerance to these class-2 herbicides. Hence, we moved forward with the plants with the combined W574L and P197S mutations.

These seed stocks for the best transformants is being expanded and will be used in field trials as indicated in Objective 2.

PROJECT 3.

In this project, purple acidic phosphatase-2 (PAP2) will be overexpressed in camelina. PAP2 is involved in regulation of plant carbon metabolism and when camelina is transformed with PAP2, seed weight per 100 seeds is increased 2-fold, indicating a heavier seed and a bigger seed (Zhang et al, 2012). In addition, seed yield increased 1.6-fold in one trial and 2-fold in another trial, indicating that seed yield is significantly increased. The plants also grow taller and have more flowers, consistent with increased seed formation. These data are very promising and suggest that this is a good target to enhance yield of camelina via two mechanisms, more seed and a seed that is 2X heavier, making harvesting easier.

Camelina will be transformed with PAP2 using our transformation strategy. We anticipate it will take 1.5 years to achieve stable transformed lines and an additional 0.5 years to expand the seed stock.

In **Figure 4.** camelina expressing PAP2 is considerably taller than the null transformed control camelina, which is about the same size as the wildtype control. Note the presence of more flower buds on the PAP2 overexpressing camelina.

Figure 4. OE lines expressing PAP2 and null transformed controls and wildtype control.

MANAGEMENT

The CamBioGene team has over 17+ years of work experience with senior management located in Grand Forks and laboratory operations based in Helsinki. Helsinki laboratory operations have been led by Dr. Viktor Kuvshinov for over 10 years. While our previous efforts were for Agaragen, OY, we will continue under CamBioGene to finish what we started several years ago.

We have substantial experience navigating this distance to keep projects on time and productive. This is done via remote management using a combination of:

- Weekly calls with Dr. Kuvshinov
- Data transmission via email and simultaneous analysis is Grand Forks and Helsinki followed by a discussion
- Zoom calls as needed
- Quarterly visits for face-to-face meetings in Helsinki
- Adjusting strategy based upon data obtained
- This recognizes that science is plastic, and we must adjust as needed to overcome obstacles to progress

Evaluations points are based upon phenotype analysis of each plant, including biochemical analysis as needed. Best performing lines are continued, with poorer performing lines stored. For Objectives 1 and 2, this is planting seed, analysis of phenotype, and continuation of selected lines to expand seed.

For Objective 3, this involves several additional evaluation points. This includes evaluation of transformation success, which may necessitate evaluation of construct organization, e.g. the sequence of genes and the polarization, e.g. 5' to 3' versus 3' to 5' insertion into the construct. The second key evaluation point is to assess transgene mRNA levels to determine transgene expression. Because this is a random insertion into the camelina genome, at times the plant will silence an insertion that it biologically cannot tolerate. Clearly of the 1000's of transformation events, not all are successful. The third key evaluation point is selecting T0 plants that produce seeds for the T1 generation. The T1 generation is planted and then evaluated for target phenotype, which is repeated for successive generations through minimally T5 generation, at which point the phenotype is considered stable. Hence, a key evaluation point in Objective 3 is the performance of each generation in the growth room and a selection process in which only the best transformed lines are moved forward to the next generation.

Overall, the CamBioGene team has a long history working together and honed a management strategy that is highly effective despite the distance.

TIMETABLE

Objectives 1 and 2

Seed Expansion and phenotype analysis	4Q23 through 4Q24
Final phenotype analysis and APHIS application	3Q24 through 1Q25 INTERIM REPORT in 1Q25

Planning field trial #1	3Q24 through 1Q25
Field trial #1	2Q25 through 3Q25
Data analysis field trial #1	3Q25 through 4Q25 INTERIM REPORT in 4Q25
Planning field trial #2	1Q25
Field trial #2	2Q26 through 4Q26
Data analysis field trial #2	3Q25 through 4Q25
Seed expansion	2Q26 through 3Q26
FINAL REPORT	1Q26

Anticipated submission of application for approval of specific lines of ALS mutated camelina and high lauric/myristic acid camelina for commercialization to APHIS.

Objective 3

Construct design and synthesis	4Q23 through 1Q24
Transformation of camelina	1Q24 through 2Q24
Phenotype assessment and data analysis	2Q24
Plant T1 seeds	2Q24 through 3Q24 INTERIM REPORT in 3Q24
Phenotype assessment and data analysis	3Q24
Plant T2 seeds	3Q24 through 4Q24
Phenotype analysis and data analysis	4Q24
Plant 4 seeds	4Q24 through 1Q25
Phenotype analysis and data analysis	1Q25
Plant T4	1Q25 through 2Q25
Phenotype analysis and data analysis	2Q25
Plant T5	3Q25 through 4Q25 INTERIM REPORT in 4Q25
Application to APHIS for field trials	4Q25
Plan field trials	1Q26
Field trial #1	2Q26 through 3Q26
Data analysis field trial #1	3Q26
FINAL REPORT	4Q26

BUDGET

Project Associated Expense	NDIC's Share	Applicant's Share (Cash)	Applicant's Share (In-Kind)	Other Project Sponsor's Share
Salaries + Fringe	350,000	$300,250	$108,000	$
Equipment	$0	$140,000	$	$
Supplies	70,000	$5,000		
Field Trials	$0	$40,000		
Travel	$0	$48,000		$
Indirect	$80,000	$10,000		
Total	$500,000	$543,250	$108,000	

Salaries
Viktor Kuvshinov $80,000 Svetlana Kuvshinova $50,000 Fringe $35,000 Total/year $165,000 x 3 = $495,000 TBA scientist (MS or PhD) $75,000 for 1.5 years $155,250,
IN KIND Eric J. Murphy $36,000 x 3 = $108,000
CBG- $300,250 NDIC-$350,000 Total cash $650,250

Equipment
Growth chamber, laminar flow hood, PCR machine, spectrophotometer, freezer, misc glassware and plasticware CBG $140,000 NDIC $0 Total $140,000

Supplies
Soil and growth room supplies $10,000 x 2.5 yrs = $25,000, DNA construct services $10,000, transformation supplies $15,000, PCR, mRNA, Southerns, phenotype testing $13,000, Oil and protein testing $12,000, other misc supplies $15,000 CBG $5,000 NDIC $70,000 Total $75,000

Field Trials and Seed Expansion
NDSU estimated $40,000 CBG$40,000 NDIC $0 Total $40,000

Travel
12 trips to Helsinki x $4,000 =$48,000 CBG $48,000 NDIC $0 Total $48,000

Indirect
Rent including utilities $90,000 CBG- $10,000 NDIC $80,000 Total $90,000

It is anticipated that this is an integrated budget for all three Projects. However, if other this application is partially funded, then the scope of the work would have to be adjusted to the altered budget, including the match by CamBioGene (CBG)

CONFIDENTIAL INFORMATION

A person or entity may file a request with the Commission to have material(s) designated as confidential. By law, the request is confidential. The request for confidentiality should be strictly limited to information that meets the criteria to be identified as trade secrets or commercial, financial, or proprietary information. The Commission shall examine the request and determine whether the information meets the criteria. Until such time as the Commission meets and reviews the request for confidentiality, the portions of the application for which confidentiality is being requested shall be held, on a provisional basis, as confidential.

If the confidentiality request is denied, the Commission shall notify the requester and the requester may ask for the return of the information and the request within 10 days of the notice. If no return is sought, the information and request are public record.

*Note: Information wished to be considered as confidential should be placed in separate appendices along with the confidentiality request. The appendices must be clearly labeled as confidential. If you plan to request confidentiality for **reports** if the proposal is successful, a request must still be provided.*

To request confidentiality, please use the template available at https://www.ndic.nd.gov/renewable-energy-program/rep-applicant-council-information.

If you are not requesting confidentiality, please note that below.

No confidential information has been disclosed and all information contained herein has been previously publicly disclosed.

PATENTS/RIGHTS TO TECHNICAL DATA

Again, there is no disclosure herein of proprietary information. Intellectual property will be protected via plant variety protection mechanisms.

STATE PROGRAMS AND INCENTIVES

Bioscience Innovation Grant Request $960,000 Funding $80,000, which requires a $40,000 match.