DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

Production of High Oil, Transgene Free *Camelina sativa* plants

Date: March 4th-8th, 2019
Technology Session Area Review: Biochemical Conversion

Principal Investigator: Kristi D. Snell
Organization: Yield10 Bioscience, Inc. (formerly Metabolix, Inc.)

Goal Statement

Project Goal

- Develop a Camelina feedstock with significantly increased seed yield and/or seed oil content to maximize oil yields per acre using a genome editing technology

Project Outcomes

- Camelina crop with significantly increased oil and/or seed yields
- Camelina crop with an expedited path through regulatory approval

Project Relevance

- Current Camelina yields are not high enough to generate enough profit for farmers to choose to grow the crop
- Higher yielding Camelina lines will significantly increase grower profits resulting in more acreage dedicated to the crop
- This will result in increased production of Camelina feedstock stimulating its use in the bioenergy industry

Quad Chart Overview

Timeline

- Project start date: 10/1/2015
- Project end date: 6/30/2018
- Percent complete: 100%

Barriers addressed

- Ft-C. Feedstock Genetics and Variety Improvement *(Improved Camelina feedstock productivity with new genome edited lines)*

	Total Costs Pre FY17[1]	**FY 17 Costs[1]**	**FY 18 Costs[1]**	**Total Planned Funding (FY 19-Project End Date)**
DOE Funded	$631,240	$1,124,272	$241,086	$0
Project Cost Share	YTEN & MOI: $189,306 NCSU: $44,390	YTEN & MOI: $166,194 NCSU: $45,572	YTEN & MOI: $54,111 NCSU: $0	$0
Partners[2] **Funding FY 17-18 YTEN & MOI (87%); NCSU (13%)**				

Objective

- Develop a Camelina feedstock with significantly increased seed yield and/or oil content to maximize oil yields per acre

End of Project Goal

- Increase in seed yield of 67% (seed yield of 2500 lbs/acre[3]) or higher
- Increase in oil content of 13% (seed oil content of 45% seed weight[3]) or higher

[1]DOE fiscal year 10/1 to 9/30.

[2]Multiple cost-share partners: YTEN, Yield10 Bioscience; MOI, Metabolix Oilseeds, a wholly owned subsidiary of Yield10 Bioscience; NCSU, North Carolina State University

[3]Benchmark yields for Camelina at start of project were considered to be 1500 lbs/acre with an oil content of 40% of seed weight

1 - Project Overview

Project Goal:

- Create genome edited Camelina feedstock with increased seed oil content and/or seed yield to deliver increased oil yields per acre

Impact of increased Camelina seed yield and seed oil content on total oil produced

Line	Seed Yield *(lbs/acre)*	% Increase in Seed Yield	Seed Oil Content *(% seed weight)*	% Increase in Seed Oil Content	Oil Produced *(lbs/acre)*	% Increase in Oil Produced
Conventional Camelina Benchmark	1500[1]	-	40[1]	-	600	-
Improved Plants (Target Goal 1)	2500	67	45	13	1125	88
Improved Plants (Target Goal 2)	3500	133	60	50	2100	250

Project Advantage:

- Edited lines anticipated to have non-regulated status under USDA-APHIS rules significantly reducing time and expense for commercialization

[1]Yields vary depending on field conditions, soil type, and geography. These benchmark seed yields and oil content for conventional Camelina are an estimate of average yields obtained under field conditions.

2 – Approach (Management)

Project Structure

Kristi Snell – PI
Yield10 Bioscience

Yield10 Bioscience	Meghna Malik - coPI Metabolix Oilseeds	Heike Sederoff - coPI NCSU
• Genetic construct design – single & multiplex genome editing • Oil content & oil profile measurements • Regulatory analysis	• Large scale plant transformation for genome editing • Large scale greenhouse growth & agronomics of edited plants	• Genetic construct design – single genome editing • Small scale plant transformation for genome editing • Small scale greenhouse growth & agronomics of edited plants

Management Approach

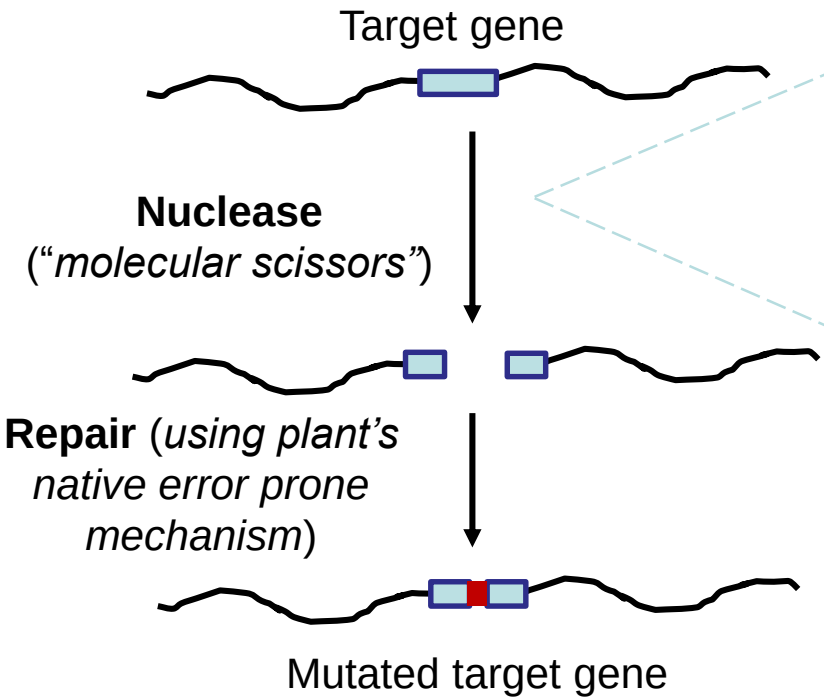
- Monthly conference calls to gauge and guide progress toward milestones and deliverables
- Subproject meetings at each site
- Periodic face to face meetings

2 – Approach (Technical)

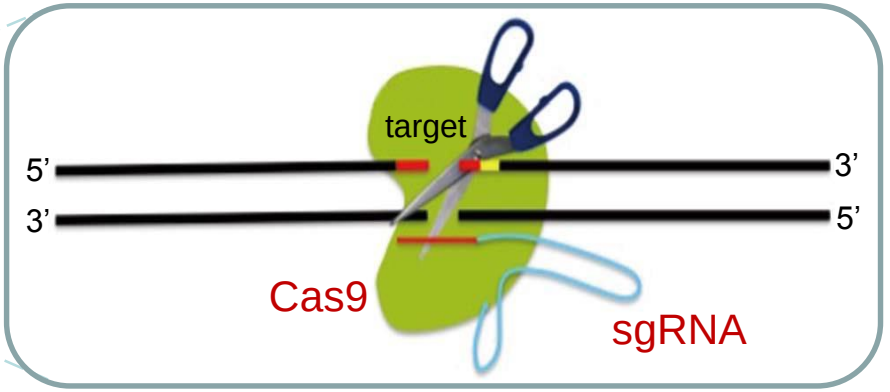
Genome Editing

- Sequence specific “molecular scissors” to cut DNA at desired sites to reduce or eliminate expression of gene. Multiple genome editing methods available
- CRISPR/Cas9 one of simpler methods to implement – requires Cas9 nuclease enzyme and a guide RNA

Genome editing



Genome editing with Cas9 nuclease

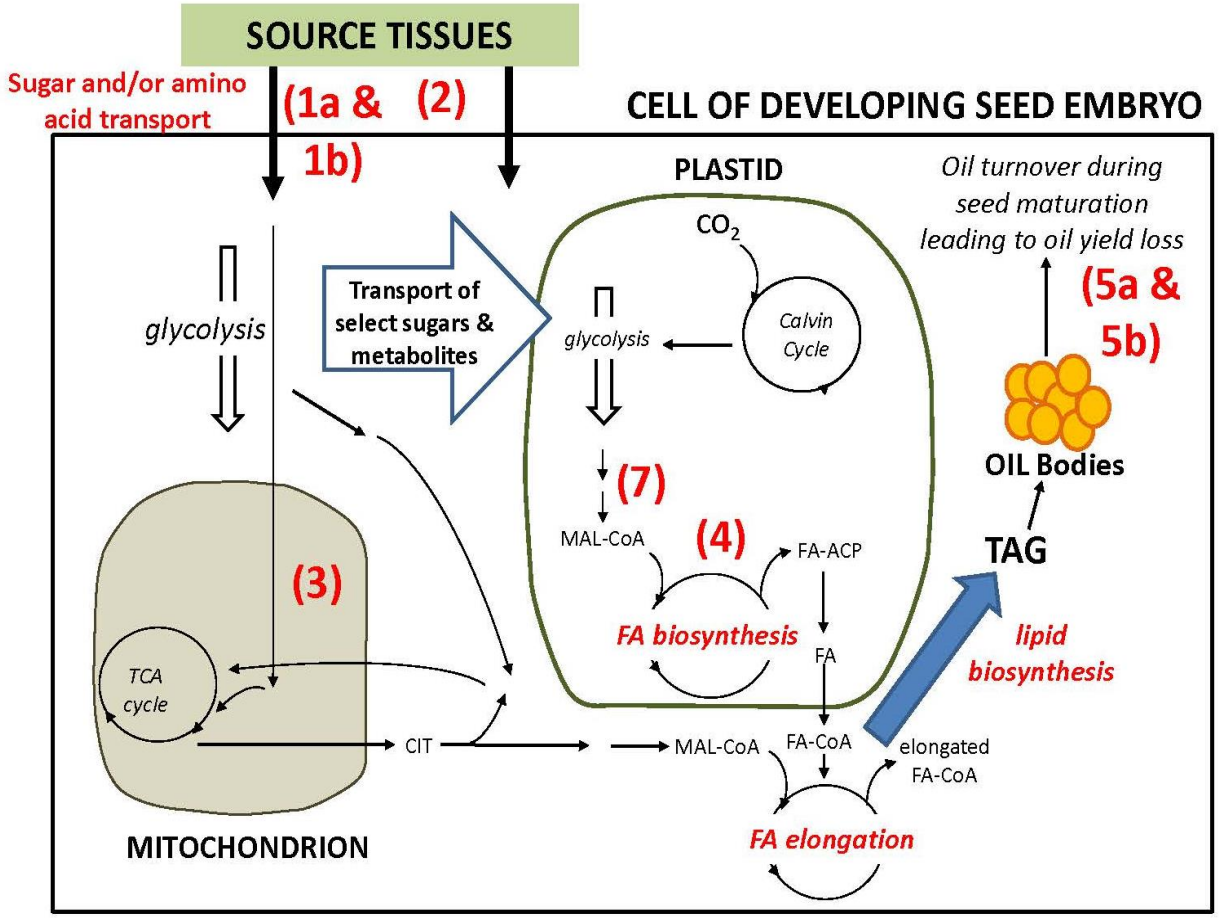


Cas9 efficiently generates double strand breaks (Cut) at sequences homologous to co-expressed gRNA

2 – Approach (Technical)

Genome Editing Targets

Multiple sites (genes) were chosen for genome editing that span different aspects of plant metabolism, including carbon transport into seed from source tissues, central carbon metabolism, and fatty acid and oil biosynthesis

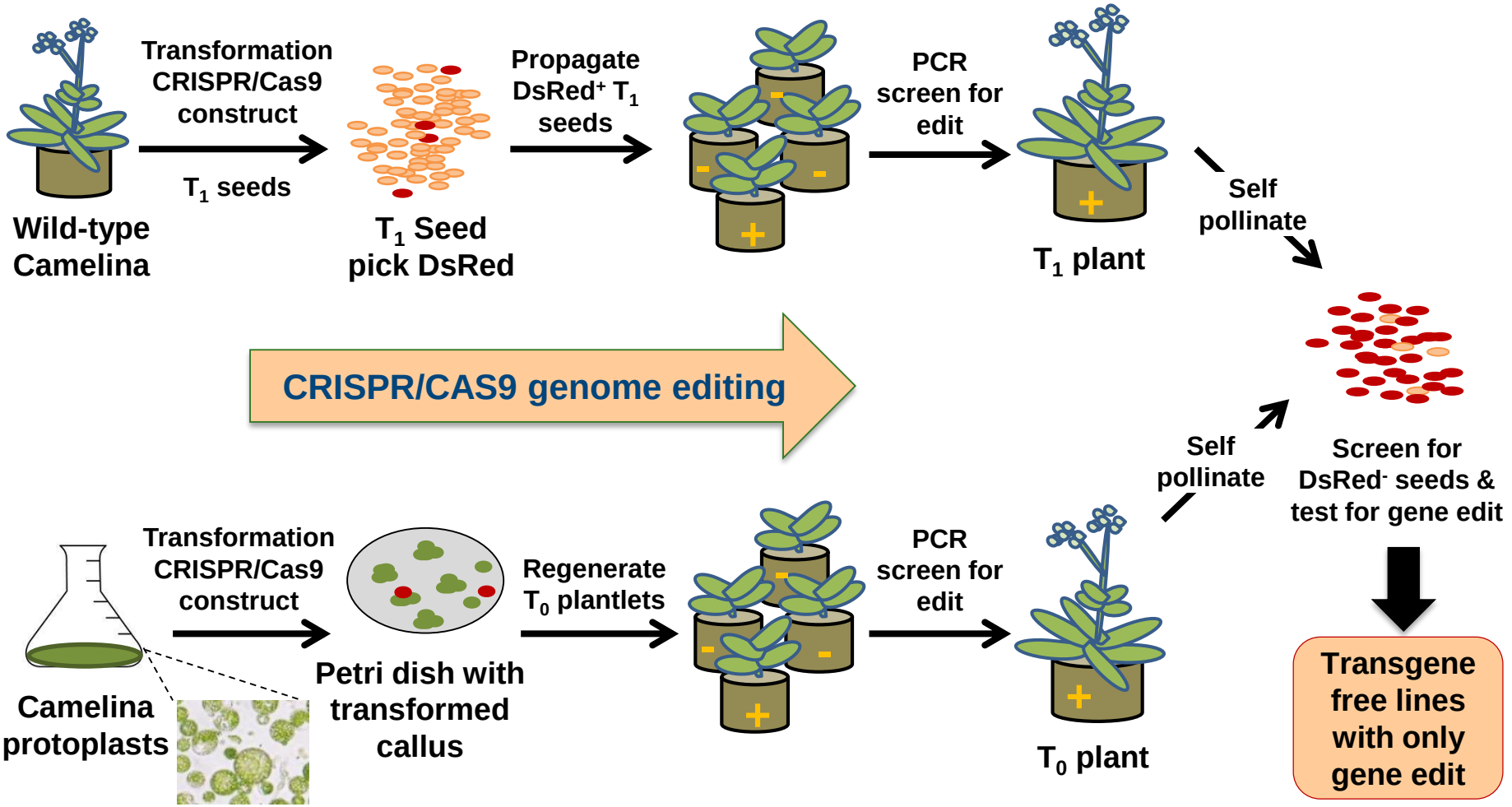


Due to IP issues, only general area of metabolism is indicated with a red target number. Number may designate multiple gene targets

Not shown, targets 6a and 6b involved in partitioning of imported sucrose between carbohydrate production and seed oil biosynthesis

2 – Approach (Technical)

1. Agrobacterium Transformation: for screening targets



2. Protoplast Transformation: generation of events for commercial pipeline

2 – Approach (Technical)

Technical Challenges

Challenge	Solution
Identification of a few genome edited plants in a large population of non-edited plants	▪ Pre-screening by restriction digest followed by amplicon sequencing enabled identification of edits
Protoplast transformation for creating edits is a much more challenging, labor intensive procedure than Agrobacterium transformation but has an easier regulatory path	▪ Determined that transgene-free edits generated by Agrobacterium transformation can be deemed non-regulated through USDA-APHIS “Am I Regulated?” process ▪ Discontinued protoplast transformation and focused on Agrobacterium methods
Obtaining homozygous edited lines in Camelina unexpectedly required multiple generations of breeding	▪ Obtained homozygous edited lines but had to reduce number of targets pursued

2 – Approach (Technical)

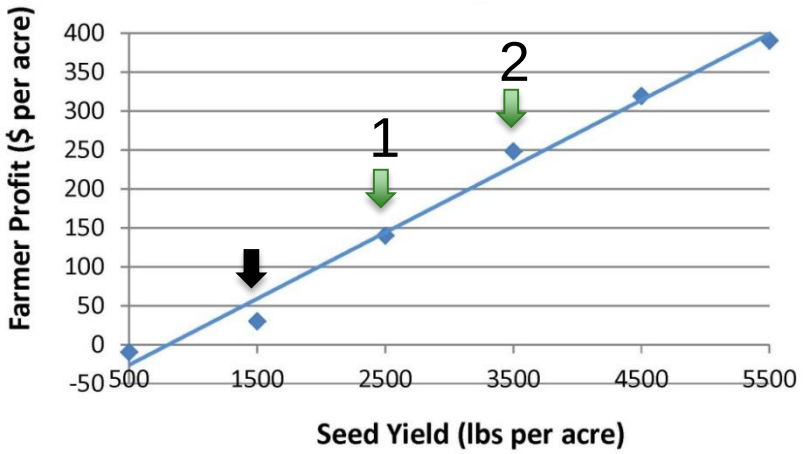
Critical Success Factors

- Yield per acre of Camelina needs to be increased for it to be a commercially viable crop for farmers to grow
- Supply of feedstock to bioenergy industry is dependent on farmers willing to grow crop

Project Technical Targets

- ⬇ Average yield of Camelina at beginning of program, 1500 lbs/acre
- 1 ⬇ Target goal 1: 2,500 lbs/acre *(~67% increase in seed yield)*
- 2 ⬇ Target goal 2: 3,500 lbs/acre *(~133% increase in seed yield)*

Increased Crop Value with Target Yield Increases



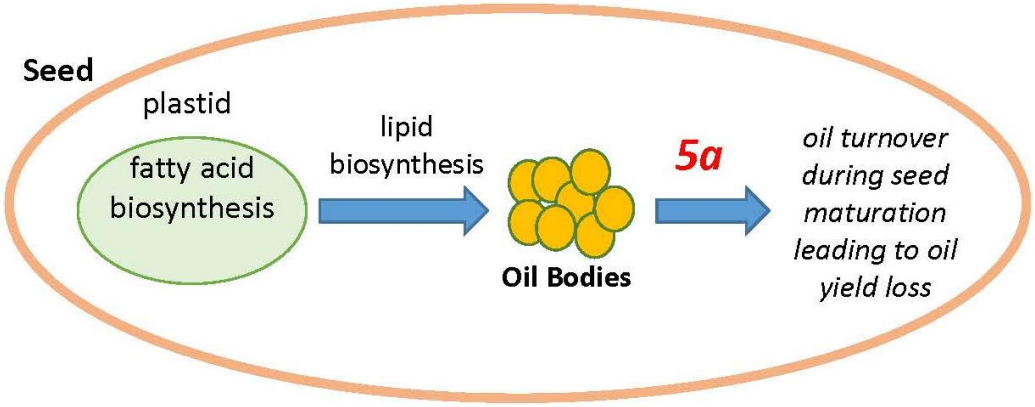
Calculations and estimates provided by Kelly Zering, NCSU, Agricultural and Resource Economics Department

3 – Technical Accomplishments/Progress/Results

- Since project research was performed in a contained greenhouse, yields per acre are difficult to calculate
- Project accomplishments were determined by measuring increases in seed yield per plant, percent oil content in seed, and total oil yield per plant compared to wild-type plants

Project Accomplishments: *Best lines from single gene edits*

Edited Line	% Increase in Seed Yield *(per plant)*		% Increase in Seed Oil Content *(% of seed weight)*		% Increase in Oil Produced *(per plant)*	
	Achieved:	**Goal:**	**Achieved:**	**Goal:**	**Achieved:**	**Goal:**
Line 1 with Target 5a edit	**+ 38**	+ 67 to 133	**+ 1.2**	+ 13 to 50	**+ 39**	+ 88 to 250
Line 2 with Target 5a edit	**+ 39**	+ 67 to 133	**+ 0.9**	+ 13 to 50	**+ 40**	+ 88 to 250

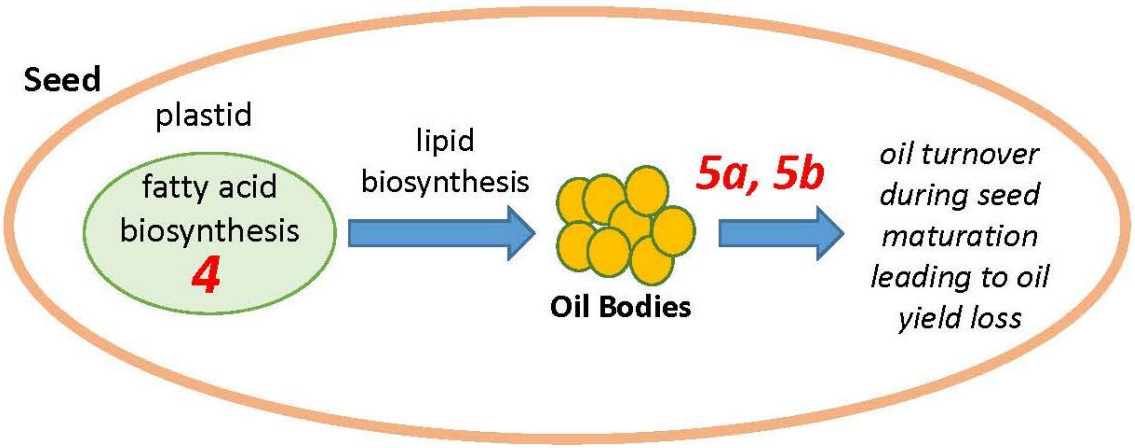


3 – Technical Accomplishments/Progress/Results

Project Accomplishments: *Best line from multiplex gene edits*

Edited Line Produced	% Increase in Seed Yield *(per plant)*		% Increase in Seed Oil Content *(% of seed weight)*		% Increase in Oil Produced *(per plant)*	
	Achieved:	**Goal:**	**Achieved:**	**Goal:**	**Achieved:**	**Goal:**
Line with Target 4, 5a, and 5b edits	- 4	+ 67 to 133	+ 9	+ 13 to 50	+ 5	+ 88 to 250

- Increased oil production reduced seed yield in lines with edits in Targets 4, 5a, and 5b
- Lines with higher amounts of oil produced per individual seed were isolated that were more compromised in seed production (next slide)



Multiplex Gene Edits

- Increases in mgs of oil per individual seed of 34 to 38% were observed in some lines
  - More significant decreases in seed number were observed in these lines
  - Demonstrates significant shift in carbon partitioning to oil
  - Observed yield drag suggests there is not enough carbon or reducing power to significantly increase oil content AND produce a normal number of seeds
  - Opportunity exists to further engineer lines to increase seed yield while maintaining oil content with other Yield10 gene targets

Edited Line	**Targets edited in plant[1]**			% Increase in oil per individual seed *(mgs)*	% Increase in individual seed weight *(mgs)*	% Increase in seed oil content *(% of seed weight)*	% Increase in number of seeds per plant	% Increase in oil produced per plant
	5a	**5b**	**4**					
A	XXX	XX_	XXX	+ 12	+ 1	+ 9	- 4	+ 5
B	XX_	XXX	XXX	+ 38	+ 17	+ 5	- 19	- 15
C	XXX	XXX	XXX	+ 34	+ 9	+ 6	- 29	- 26

[1]Since Camelina is an allohexaploid, expect 3 copies of each gene in the plant.
"X" designates fully edited copy of gene, "_" designates wild-type copy of gene as determined by Amplicon sequencing

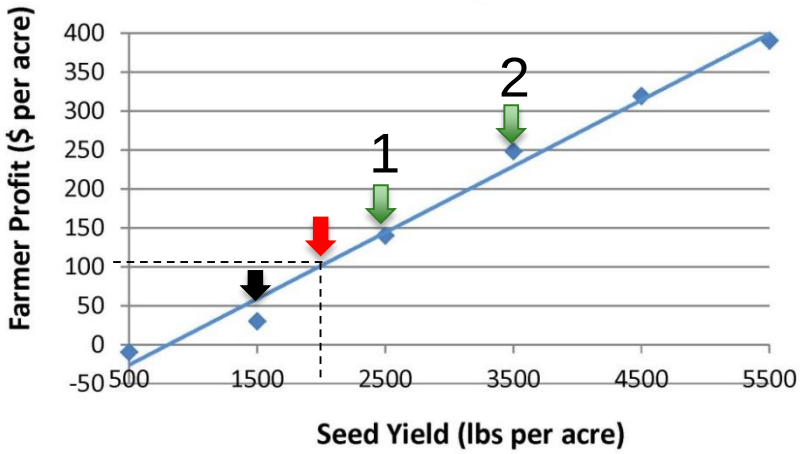
Critical Success Factors

- Best edited line showed 39% increase in yield in greenhouse
  - *If seed yield increase translates to the field, farmer profit is estimated to increase to ~$100 per acre*
- Represents a step forward in creating a Camelina crop that is commercially viable for farmers to grow

Increased Crop Value With Best Edit (⬇)

Key

- ⬇ Edited line with 39% increase in seed yield
- ⬇ Average yield of Camelina at beginning of program, 1500 lbs/acre
- 1 ⬇ Target goal 1: 2,500 lbs/acre *(~67% increase in seed yield)*
- 2 ⬇ Target goal 2: 3,500 lbs/acre *(~133% increase in seed yield)*



Calculations and estimates provided by Kelly Zering, NCSU, Agricultural and Resource Economics Department

Regulatory

- Demonstrated that edited lines generated in program can be designated as non-regulated through USDA-APHIS “Am I Regulated?” process
  - *Submitted letters and received responses of not regulated for one single edited and several multiplex edited lines*
- Lines were edited using Agrobacterium transformation methods with subsequent removal of DNA encoding CRISPR editing machinery
- Genome editing using time consuming, labor intensive protoplast work did not give regulatory advantage and was discontinued

4 – Relevance

Project Goal:

- Develop a Camelina feedstock with significantly increased seed yield and/or seed oil content to maximize oil yields per acre using genome editing

Program Achievements:

- Camelina lines with single gene edits delivering up to 39% increase in seed yield per plant in greenhouse growth
  - *If results are translatable to field, would be a major step forward towards improving Camelina as a crop*
- Camelina lines with multiple edits delivering up to 38% increase in oil produced per seed but with significantly reduced number of seeds produced per plant
  - *Demonstrates significant shift in carbon partitioning to oil*
  - *Opportunity to further engineer line to increase seed yield while maintaining oil content with other Yield10 gene targets*

4 – Relevance

Program Impacts:

- **Accelerated commercialization.** Demonstrated that gene editing technology platform will create transgene-free plants with reduced regulatory hurdles
    - *Two USDA-APHIS "Am I Regulated?" letters with project lines, agency designated lines as not regulated*
- **Increased yields of seed & oil per acre.** Will provide increased profit margins for farmers & processors stimulating interest in crop
    - *Lines generated in this program are a promising step forward*
- **Increased Camelina acreage.** Farmers will grow crops that are profitable.
- **Domestic Camelina renewable fuels/bioproducts industry.** Availability of feedstock will enable development of industry.
- **Project helps to address terrestrial feedstock supply targets from the BETO Multi-Year Program Plan**
- **Enhanced US energy and economic security**

5- Future Work (post-BETO program)

- Field trials of best lines
  - Single gene edited lines with up to 39% increase in yield in greenhouse growth
  - Multigene-edited lines with up to 38% increase in mgs per oil in individual seeds but with yield drag when grown in greenhouse
  - Submit additional “Am I Regulated?” letters to USDA-APHIS as needed for field work
- Engineer yield enhancing gene into multi-edited lines with up to 38% increase in mgs per oil in individual seeds but with yield drag
  - Attempt to maintain high oil achieved in edited seeds while increasing number of seeds
  - Yield10 Bioscience’s C3003 gene enhances carbon fixation and is a good candidate

Summary

Current Camelina yields are not high enough to generate enough profit for farmers to choose to grow the crop

Approach:

- Develop a Camelina feedstock with significantly increased seed yield and/or seed oil content using genome editing

Technical Accomplishments/Progress/Results:

- Best single edit lines had up to 39% increase in seed yield
- Best multi-edit lines had up to 38% increase in mgs of oil produced per individual seed. Significant shift in partitioning of carbon to oil resulted in reduced seed production in multi-edited lines
- Demonstrated editing technology will have an expedited regulatory path in US (2 USDA-APHIS “Am I Regulated?” letters submitted, lines are not regulated by agency)

Relevance:

- Higher yielding Camelina lines will increase grower profits resulting in more acreage dedicated to the crop; will result in increased production of Camelina feedstock stimulating its use in the bioenergy industry

Future work (post-BETO program):

- Field trials of best lines
- Engineer yield enhancing gene into lines with high oil containing seeds

Additional Slides

Response to Previous Reviewer's Comments

1. Has there been any policy development to indicate that editing will not be seen or evaluated in the same way as genetic engineering?

Information from USDA-APHIS BRS Stakeholder webinar (11/7/2018):

USDA-APHIS will continue regulating "plant breeding innovations" according to the categories laid out in Secretary Perdue's statement from March 2018 which includes NOT regulating plants with the following changes:

- **Deletions**—the change to the plant is solely a genetic deletion of any size.
- **Single base pair substitutions**—the change to the plant is a single base pair substitution.
- **Insertions from compatible plant relatives**—the change to the plant solely introduces nucleic acid sequences from a compatible relative that could otherwise cross with the recipient organism and produce viable progeny through traditional breeding.
- **Complete Null Segregants**.

2. Has the project considered the need for patent licenses to use CRISPR technology commercially in plants?

Yield10 has a research license to CRISPR from Corteva/Broad Institute and can negotiate a commercial license at the appropriate time

Go/No-Go Project Decision Point

- Go/No-Go Decision point due July 4th, 2016
  - A.GN.1, *If no successful gene deletions have been obtained, decide whether to re-evaluate strategy or continue program*
- First successful editing of Camelina was demonstrated before Go/No-Go Decision point for Target 4 during Q3 (FY 2016) of program
  - *Demonstrated that genome editing by Agrobacterium-mediated transformation is feasible in Camelina*
- DOE requested the demonstration of additional edits before signing off on project Go/No-Go Decision Point
  - *A 60 day extension was obtained to demonstrate editing of additional targets.*
  - *A Go/No-Go Decision memo was submitted September 4th, 2016 demonstrating the generation of additional edits in the Target 6a and 5a genes*
  - *Program was continued*

Publications, Patents, Presentations

Publications: Malik MR, Tang J, Sharma N, Burkitt C, Ji Y, Mykytyshyn M, Bohmert-Tatarev K, Peoples OP, Snell KD (2018) *Camelina sativa, an oilseed at the nexus between model system and commercial crop* Plant Cell Rep, **37**, 1367.

Patents: No patent applications to date, applications will be drafted

Presentations:

Kristi Snell (Yield10)

- Northeast American Society of Plant Biologist's (ASPB), The University of Massachusetts, Amherst, April 28-29th, 2018.
- CanolaWeek 2017, Saskatoon, Canada, December 7th, 2017.

Heike Sederoff (NCSU)

- Departmental seminar at NCSU (2016), Raleigh, NC
- Biolunch Seminar at NCSU (2016), Raleigh, NC
- International Conference on CRISPR technologies (2017). Raleigh, NC

Poster Presentations:

NCSU

- Postdoc Research symposium at NCSU, Raleigh, NC (2018)
- NCSU Plant Breeding Research symposium (2017)

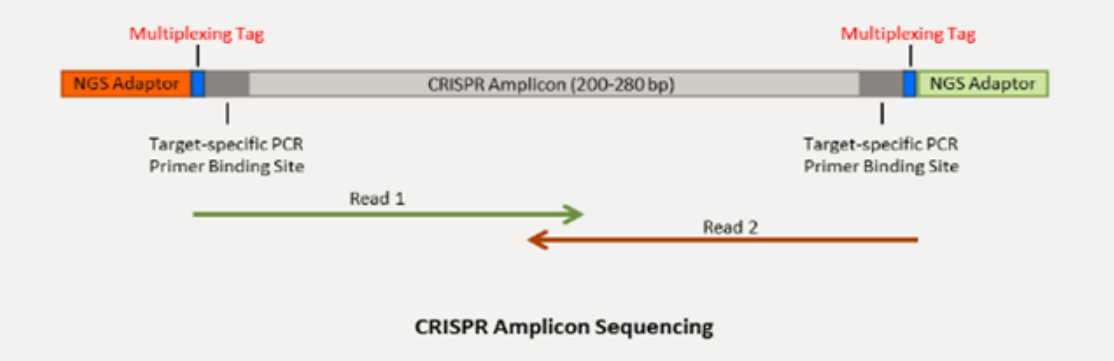
Commercialization:

Best lines from program will be evaluated by Yield10 in field tests

Appendix - Definitions and Terms

Amplicon sequencing

- CRISPR/Cas-induced mutations via deep sequencing of PCR amplicons



- 35K-50K reads per sample
  - Quantitative: Provides the count of edited reads
  - Qualitative: Identifies types of edits

Method for amplicon sequencing and reads obtained using a service provider. An amplicon of 200-280 bp is required for this method and is produced using PCR using target specific primers to generate a PCR product from the region where the CRISPR mutation is anticipated. The fee for service provider adds multiplex tags and Next Generation Sequencing (NGS) adaptors to the amplicon. Deep sequencing was then performed on the Illumina NGS platform.