U.S. Department of Energy (DOE)
Bioenergy Technologies Office (BETO)
2017 Project Peer Review

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

Date: March 9th, 2017

Technology Area Session: Biochemical Conversion

Principal Investigator: Kristi D. Snell, PhD

Organization: Yield10 Bioscience (Formerly Metabolix)

Goal Statement

Project Goal

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

Project Outcomes

- Camelina crop with significantly increased oil and 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: 9/30/2017
- Percent complete: 60%

Budget

	FY 16 Costs[1]	FY 17 Costs	Total Planned Funding (FY 17-Project End Date)
DOE Funded	$631,241	$1,365,355	1,996,596
Project Cost Share (Comp.)[2]	YTEN & MOI: $189,306 NCSU: $43,536	YTEN & MOI: $220,305 NCSU: $46,426	YTEN & MOI: $409,611 NCSU: $89,962

[1]DOE fiscal year 10/1/2015 to 9/30/2016.

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

Barriers Addressed

- Terrestrial Feedstock Genetics & Development *(Ft-C; improved Camelina feedstock productivity with new genome edited lines)*
- Terrestrial Feedstock Availability & Cost *(Ft-A; higher Camelina yields provide increased profit to farmers increasing production of oilseed feedstock)*
- Project helps to address terrestrial feedstock supply targets from the BETO Multi-Year Program Plan

Partners

- Yield10 Bioscience 45%*
- Metabolix Oilseeds** 35%
- NCSU 20%

**expressed as percentage of actual total project spending (DOE plus cost share) through FY2016 (Sept 30, 2016)*

***a wholly owned subsidiary of Yield10 Bioscience*

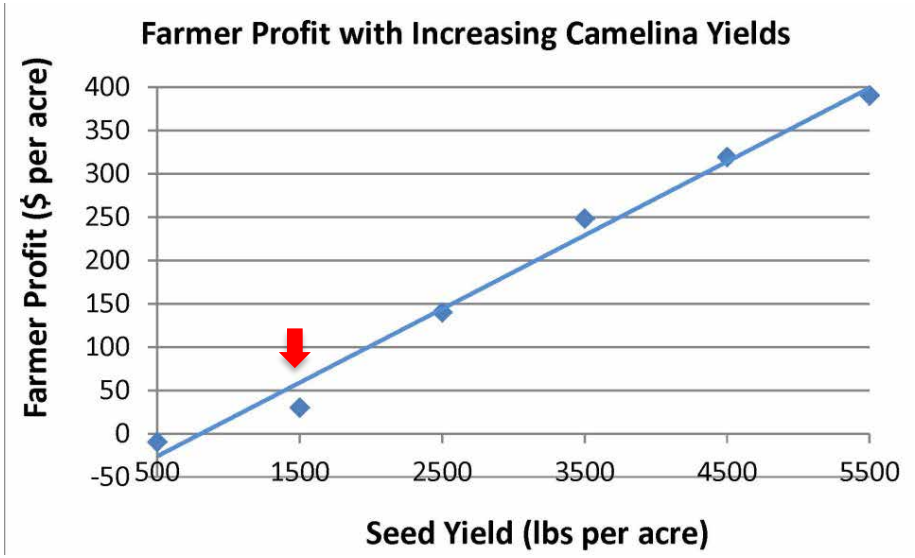
1 - Project Overview

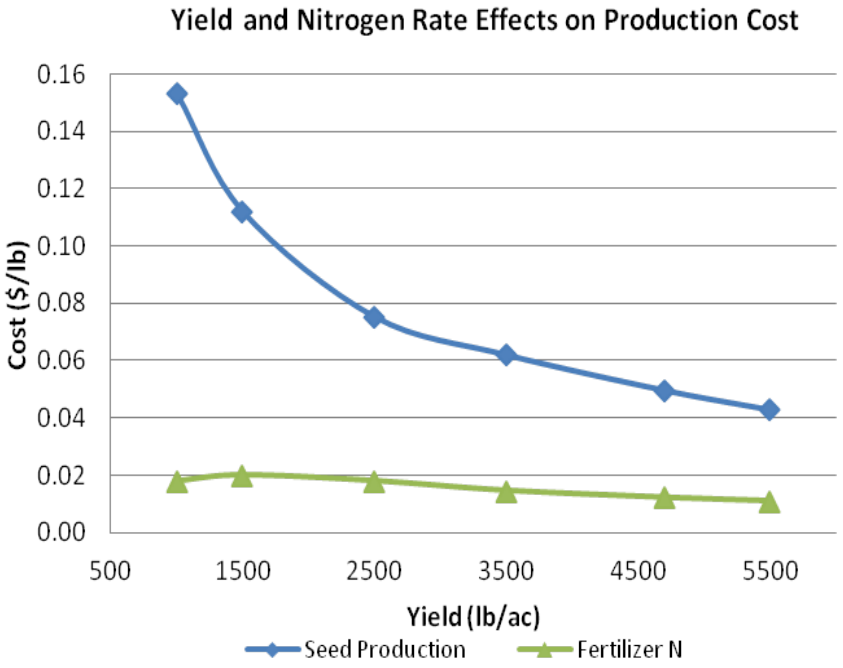
Camelina sativa is an oilseed that has received considerable interest as a crop for the domestic production of biodiesel and aviation fuels

- High oil content, low inputs for cultivation, primarily non-food

Why isn't Camelina currently a crop with significant acreage?

- Current Camelina seed yields of ~1,500 lbs/acre do not provide enough farmer profit





Average yield of Camelina at beginning of program 1500 lbs/acre

[1]*Calculations and estimates provided by Kelly Zering, NCSU, Agricultural and Resource Economics Department*

1 - Project Overview

Current camelina yields:

- 1,500 lbs seed/acre with 40% oil

Project Goal:

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

Program Deliverable Targets:

- **Intermediate** Camelina line delivering equivalent of 2,500 lbs seed/acre with 45% oil
- **End of Project** Camelina line delivering equivalent of 3,500 lbs seed/acre with 60% oil

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

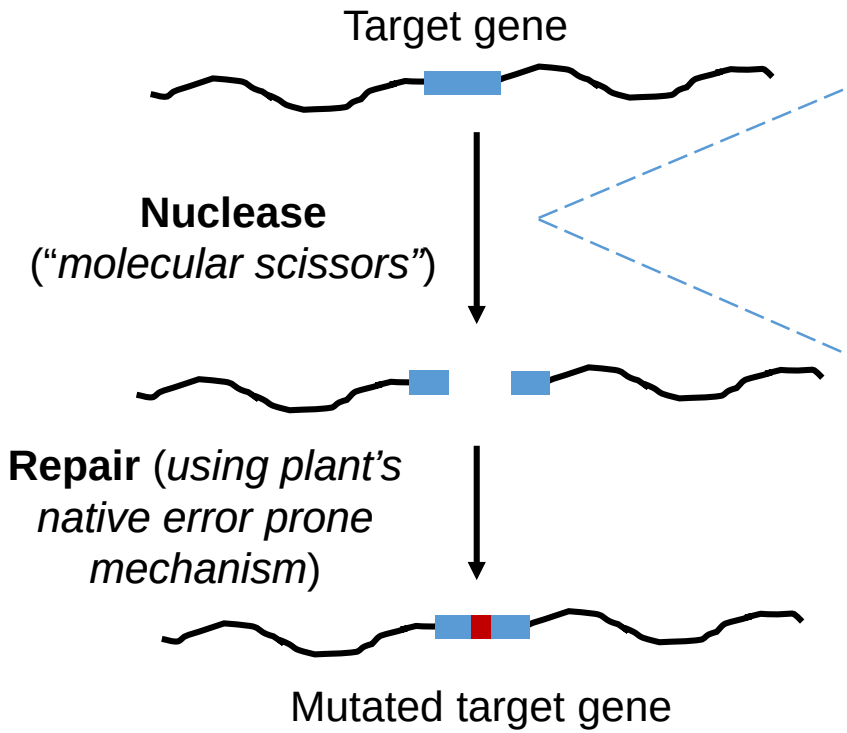
- Monthly conference calls to gauge and guide progress toward milestones and deliverables
- Subproject meetings at each site at least every two weeks
- Periodic face to face meetings (3 so far during project)

2 – Approach (Technical)

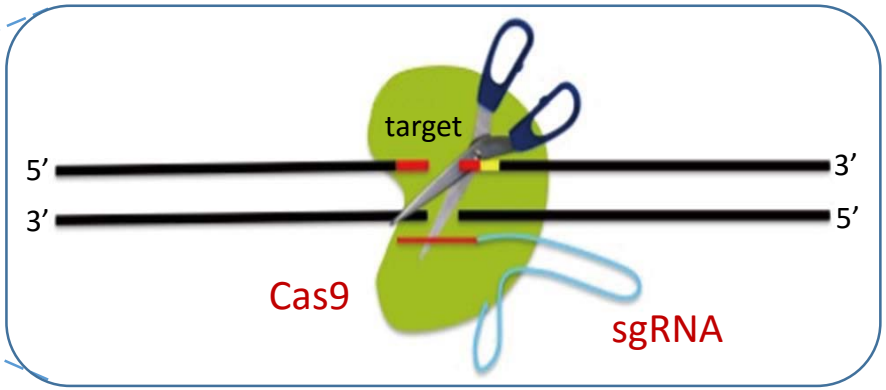
Genome Editing

- 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

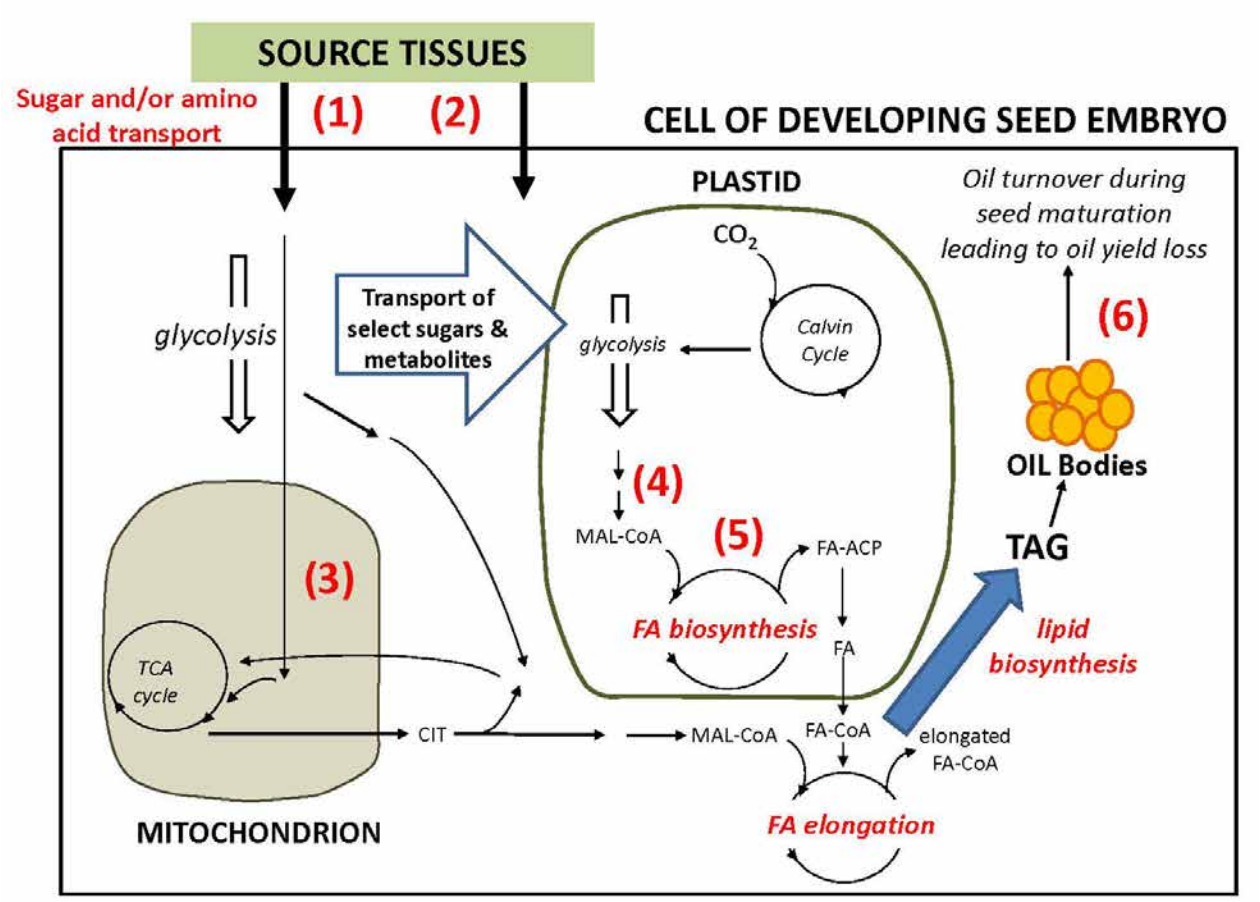


Cas9 efficiently generates double strand breaks (Cut) at sequences homologous to co-expressed gRNA

2 – Approach (Technical)

Genome Editing Targets

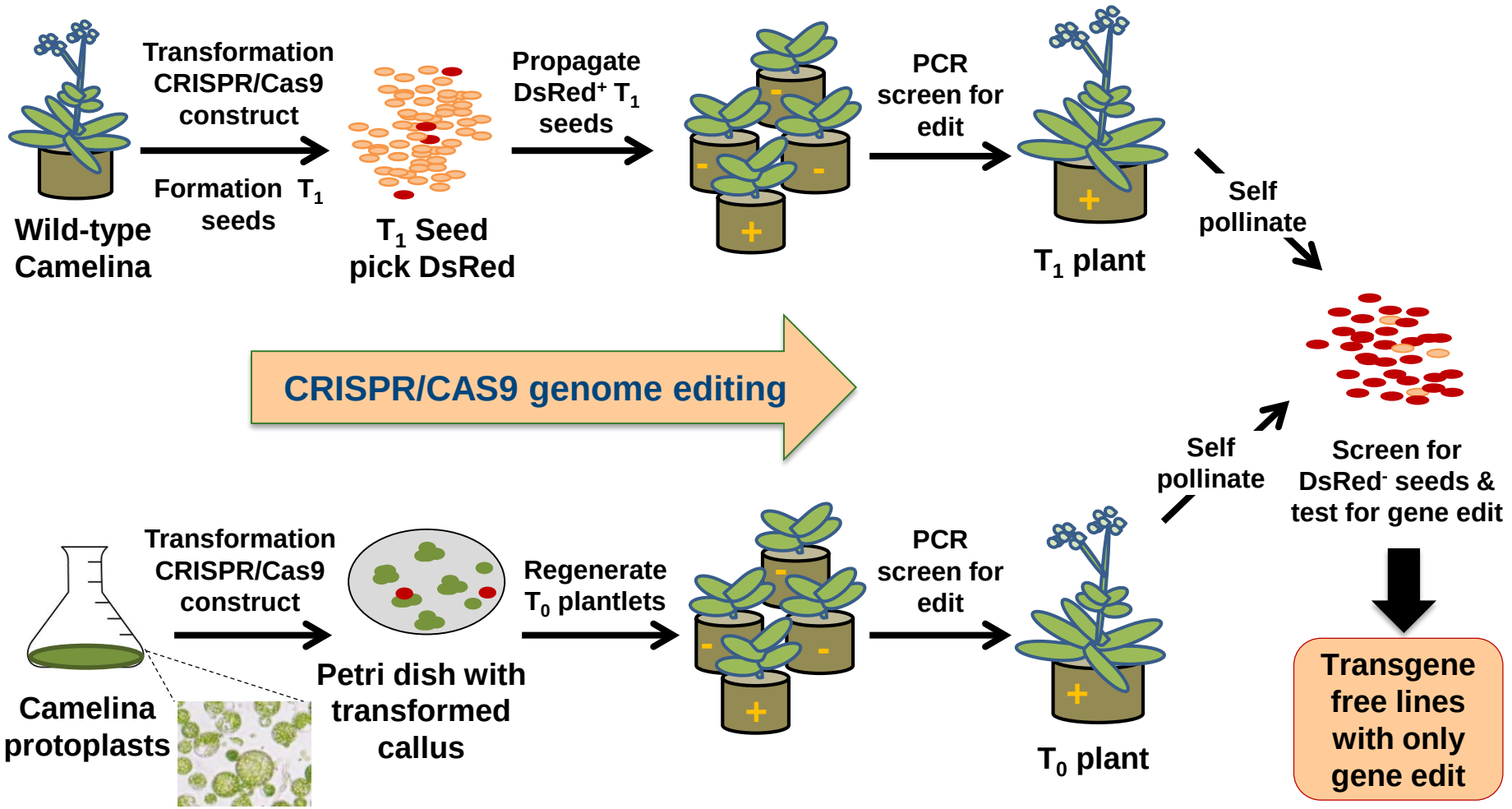
Multiple sites spanning different aspects of carbon transport into seed from source tissues, central metabolism, and fatty acid and oil synthesis



Red numbers indicate area of metabolism manipulated by genome editing. Number may designate multiple gene targets

2 – Approach (Technical)

1. Agrobacterium Transformation: for screening targets



2. Protoplast Transformation: for 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	▪ Currently screening for edits by isolating DNA and looking for change in a specific DNA sequence (restriction site) where edit occurred. ▪ DNA sequencing is then used to characterize the exact edit ▪ NC State is working to find alternative solutions that allow quicker screening of plants
Protoplast transformation for creating edits is a more challenging, labor intensive procedure than Agrobacterium transformation but has an easier regulatory path	▪ Screen multiple targets for increased yield using Agrobacterium transformation procedures ▪ Use protoplast transformation for a limited number of the best targets

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

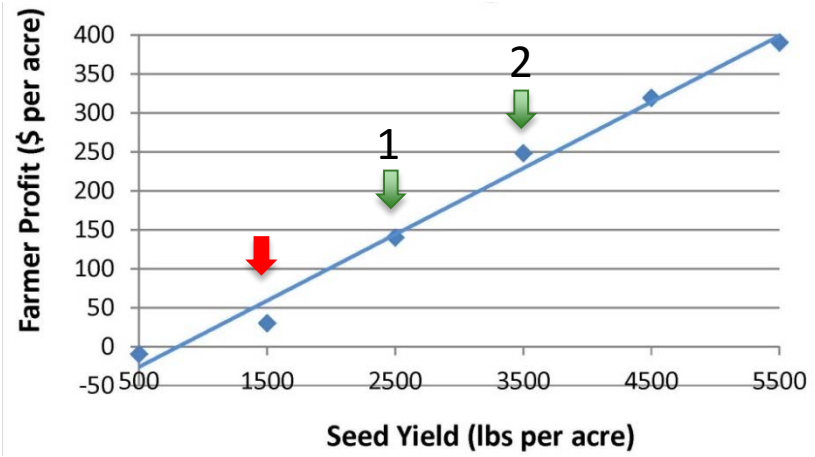
Project Technical Targets

Average yield of Camleina at beginning of program 1500 lbs/acre

1 Target goal 1: 2,500 lbs/acre

2 Target goal 2: 3,500 lbs/acre

Increased Crop Value Upon Achieving Technical Targets



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

Program divided into three tasks

Task A. Modification of individual gene targets for increased oil production

-Agrobacterium transformation to screen multiple targets

-Protoplast transformation on a limited number of targets, targets likely to enter commercial pipeline

Task B. Crossing of best lines from Task A to generate lines with multiple edits

Task C. Multiplex genome editing for increased oil and seed yield

-Simultaneously edit more than one gene target using Agrobacterium and protoplast transformation techniques

3 – Technical Accomplishments/Progress/Results

Progress with each task

Task A. Modification of individual gene targets for increased oil production

i. Protoplast transformation

- *Key Technical Accomplishment.* Transformation of protoplasts achieved and plantlets obtained achieving milestone. Screening of editing in progress
- *Next Key Milestone.* Successful genome edits for two separate target genes generated (due end of March 2017)

ii. Agrobacterium transformation

- *Key Technical Accomplishment.* Achieved and verified editing of 6 different gene targets achieving a program milestone
- *Fulfilled Program Go/No-Go Decision:* Decision point - If no successful deletions have been obtained, decide whether to re-evaluate strategy or continue program; Decision point originally due July 2016, extended to and met in Sept 2016
- *Next Key Milestone.* Oil content and seed yield determined for T3 transgene free seeds with targeted deletions (due end of March 2017)

3 – Technical Accomplishments/Progress/Results

Task B. Crossing of best lines from Task A to generate lines with multiple edits

Task scheduled to start March 2017

Task C. Multiplex genome editing for increased oil and seed yield

i. Protoplast transformation

- *Key Technical Accomplishment.* Completed and transformed genetic constructs achieving milestone
- *Next Key Milestone.* Successful multiple targeted deletions generated (due May 2017)

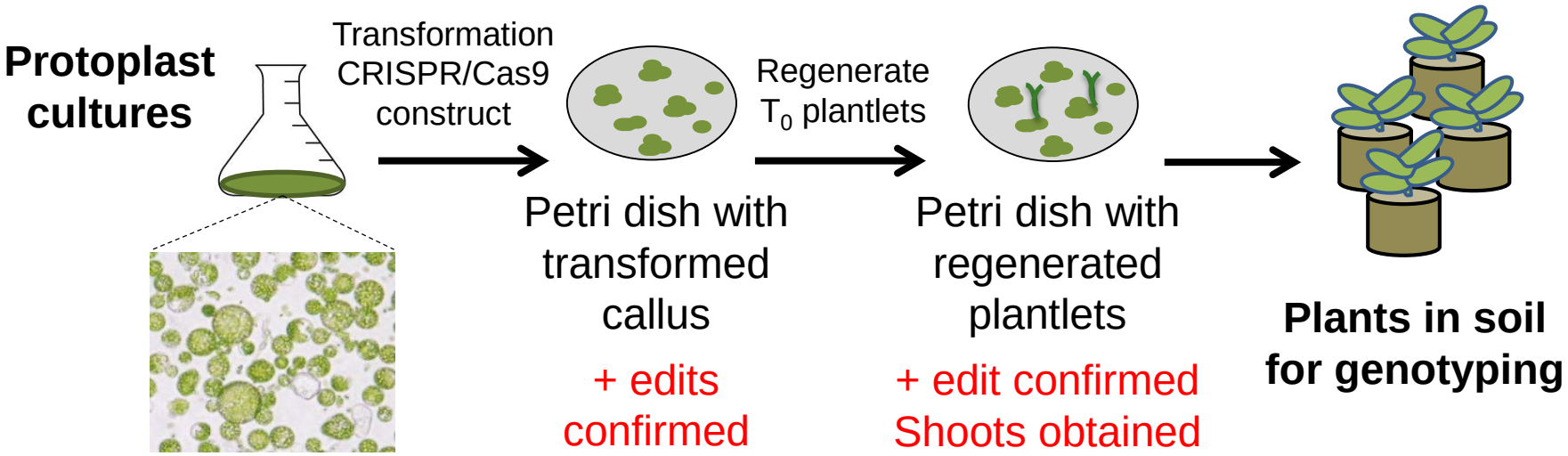
ii. Agrobacterium transformation for screening of multiple targets

- *Key Technical Accomplishment.* Completed and transformed genetic constructs achieving milestone
- *Next Key Milestone.* Successful multiple targeted deletions generated (due end of Jan 2017). Currently screening for lines with multiple edits

Improvements over past efforts

Developed modified protoplast transformation procedure where DNA encoding CRISPR/CAS9 machinery does not integrate into genome

- DNA encoding CRISPR/CAS9 machinery not integrated into genome thus does not need to be removed by segregation
- No selectable marker
- Significant result further easing regulatory path



4 – Relevance

Project Goal

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

Program Deliverables:

- **Intermediate** Camelina line delivering equivalent of 2,500 lbs seed/acre with 45% oil (1125 lbs oil/acre farmed)
- **End of Project** Camelina line delivering equivalent of 3,500 lbs seed/acre with 60% oil (2100 lbs oil/acre farmed)

Program Impacts:

- **Accelerated commercialization.** *Gene editing technology platform will create transgene-free plants with reduced regulatory hurdles*
- **Increased yields of seed & oil per acre.** *Will provide increased profit margins for farmers & processors stimulating interest in crop*
- **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

Key Future Work to Meet Program Goals:

1. Additional DNA sequencing of edited plants to select best lines to bring forward
2. Greenhouse growth of plants to measure seed oil content and seed yield

Future Work by Task:

Task A. Modification of individual gene targets for increased oil production

i. Protoplast transformation

- *Next Key Milestone.* Successful genome edits for two separate target genes generated (due end of March 2017)
- *Key Upcoming Milestone.* Oil content and seed yield determined for T3 transgene free seeds with targeted deletions (due Sept 2017)

ii. Agrobacterium transformation for screening of multiple targets

- *Key Upcoming Milestone.* Oil content and seed yield determined for T3 transgene free seeds with targeted deletions (due end of March 2017)

5 – Future Work

Task B. Crossing of best lines from Task A to generate lines with multiple edits

Task scheduled to start March 2017

- *Key Upcoming Milestone.* Oil content and seed yield determined for F2 lines with more than one gene edit (due end of Sept 2017)

Task C. Multiplex genome editing for increased oil and seed yield

i. Protoplast transformation

- *Key Upcoming Milestone.* Successful multiple targeted deletions generated (due May 2017)
- *Key Upcoming Milestone.* Oil content and seed yield determined for T2 transgene free seeds with multiple targeted deletions (due Sept 2017)

ii. Agrobacterium transformation for screening of multiple targets

- *Key Upcoming Milestone.* Successful multiple targeted deletions generated (due end of Jan 2017). Currently screening potential lines by DNA sequencing
- *Key Upcoming Milestone.* Oil content and seed yield determined for T3 transgene free seeds with targeted deletions (due end of Sept 2017)

5 – Future Work

Remaining budget

Funds remaining for program (January 1st through September 30th 2017) are $1,313,842 *($1,050,735 Federal; $263,107 cost share*)

This is sufficient to complete remaining work

Would like to obtain a no cost extension

- *would allow more complete analysis of lines*
- *would allow completion of screening of all targets initiated in program*
- *would increase the chance that some of the lines will meet the high oil and seed yield deliverable targets*

5 – Future Work

Future work after the end of BETO funding

Best lines from BETO program with highest oil and/or seed yield

Commercial Pipeline (*after BETO Incubator Program*)

Generate homozygous lines or perform backcrosses *(as needed)* → **Bulk up seed** → **Small scale field trials of best lines** → **Measure oil content & seed yield** → **Multi-site large scale field trials of lines with commercial potential**

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 oil content using a gene editing technology

Technical Accomplishments/Progress/Results:

- Created genome edited lines for 6 different gene targets; working to characterize seed yield and oil content

Relevance:

- Higher yielding Camelina lines will significantly 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:

- Complete characterization of genome edited lines.
- After program, lines with significant yield to go into field trials

Additional Slides

Publications, Patents, Presentations, Awards, and Commercialization

Patents

- No patents have been filed to date. We plan to file on novel targets that increase seed yield or oil content

Commercialization

- In July of 2016, the Metabolix board made the decision to exit the biobased products business, sell those assets, and focus the future of the company on developing crop yield enhancing technologies. In January of 2017, Metabolix changed its name to Yield10 Bioscience. The technology developed in this BETO funded program is relevant not only to increasing Camelina yield, but should also be useful for increasing yield in related crops such as canola.

- After completion of the BETO program, promising Camelina lines with high seed yield and/or oil content will be tested in field trials. Promising traits will enter Yield10's commercial pipeline.