

Update on increasing seed oil content through gene editing

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Sustainable Growth Starts with a Seed



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Yield10's Trait Factory

From Crop Science to Market

Technology Platform - "Trait Factory" CRISPR editing GRAIN Mr. Camelina Mr. 3.0 Genetic Engineering GMO GMO

Portfolio of Camelina Seed Products

1. Feedstock Oil (Biofuel)



- 2. Omega-3 Oil (EPA+DHA)
- **3.** PHA Bioplastics



Near Term Focus: Establish & demonstrate value chain

Value	Seed	Grower	Grain/Oil	Crush/
Chain	Production	Contracts	Offtake	refining
Status	¥	¥	¥	underway



Camelina grain harvest & delivery to customer - *Alberta, 7/2023*

Delivered Camelina grain - *Alberta, 7/2023*



Why Camelina?

- Promising oilseed crop
 - Seed oil levels ~ 40% of seed weight
- Both spring and winter varieties
 - Winter varieties, potential use as cover crop for corn and soybean acres
- Doesn't outcross with canola
- Proven Biofuel Feedstock
- Low CI (Carbon Intensity) score
- Excellent platform crop for novel high value seed products value proposition for farmer



Greenhouse grown Camelina



Camelina field plots at flowering



Large scale winter Camelina growth

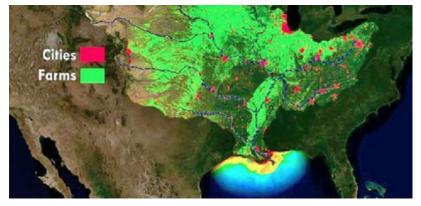


Yield10 Camelina Review: *Camelina sativa*, an oilseed at the nexus between model system and commercial crop. Malik et al., Plant Cell Rep., 2018

Benefits of Cover Cropping

1. Reduce or prevent nutrient runoff

- fertilizers contaminate ground water or end up in rivers/streams creating "deadzones"
- dead zone in Gulf of Mexico linked to nutrient inputs from cities and farms in Mississippi River Basin¹



*Gulf of Mexico dead zone 3,058 square mile in 2023 (NOAA)*²

Algal blooms Alg

2. Protect and improve farmer's soil

- Boost soil quality, prevent erosion, increase organic matter in root zone
- Retain moisture in field, snow retained instead of lost to wind

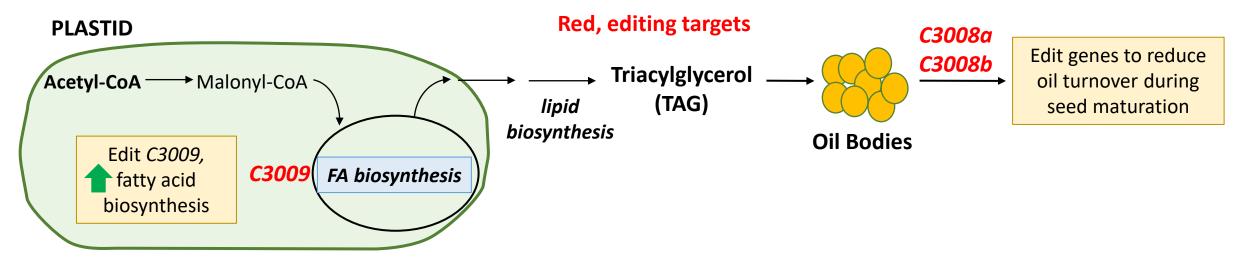
Farmers reluctant to plant cover crops due to negative return on investment
 Yield10 winter Camelina seed products can provide income to farmer

^{1. &}lt;u>https://mississippiriverdelta.org/learning/explaining-the-gulf-of-mexico-dead-zone/</u>

https://coastalscience.noaa.gov/news/below-average-summer-2023-dead-zone-measured-in-gulf-of-mexico/#:~:text=In%20June%202023%2C%20NOAA%20forecasted,miles%20was%20set%20in%202017

Editing Combinations of Known Genes to Increase Oil Content

Gene combinations to increase oil biosynthesis and prevent oil turnover¹



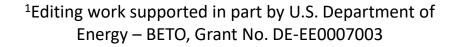
<u>1. C3009 - transcription factor target to upregulate fatty acid biosynthesis</u></u>

- regulation of embryo fatty acid biosynthetic genes, + regulation of genes responsible for pigment in seed coat

2. C3008a and C3008b - gene targets to reduce oil turnover during seed maturation

C3008a and C3008b, oil body associated lipases

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Multiplex Genome Editing of Three Genes in Camelina

Strategy 1: Editing of lipase genes (C3008a, C3008b) and transcription factor gene (C3009)

- Simultaneous editing of 9 genes (3 target genes present in 3 copies each) using CRISPR
- Lines with different combinations of edits obtained and characterized
 - Very difficult to get all 9 gene copies edited in same line, only one line obtained with all 9 genes edited
- Fully edited *C3009* gene, loss of pigmentation in seed coat
 - Unique distinction to track edited seed



Wild-type control

C3009 100% edited (yellow seeded)

Greenhouse harvested seeds showed increase in seed oil content



E3902 Status and Stacking of Herbicide Tolerance Traits

E3902 Performance

- E3902 oil trait stable in 2019, 2020, 2021, 2022 field trials
- Average increase in seed oil content ~5%

E3902 Regulatory

- US: Am I Regulated Process, growth of line not considered to be regulated¹
- Canada: Document compilation to determine regulatory status in progress

E3902 is germplasm background for Yield10 herbicide tolerant (HT) lines

E3902 Trait Stacking of Herbicide Tolerance (HT)

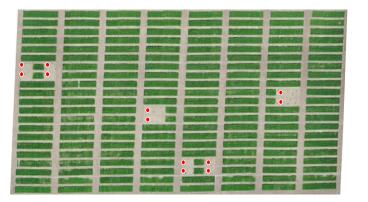
- Tolerance to glufosinate over the top spray
- Tolerance to glufosinate over the top spray <u>&</u> Group 2 soil residues

E3902 Herbicide Stack Regulatory

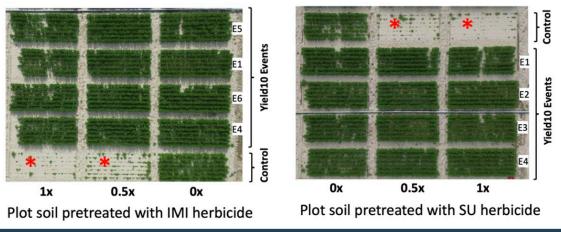
- US: Regulatory Status Review, growth of lines not considered to be regulated¹
- Canada: Document compilation to determine regulatory status in progress

Field photos of Yield10 herbicide tolerant lines

E3902 glufosinate tolerant events Spring 2022 field trial, Drone image, lead events identified



E3902 glufosinate & group 2 tolerant events Spring 2023 field trial, plots pretreated with IMI or SU herbicide prior to planting, young plants sprayed with glufosinate



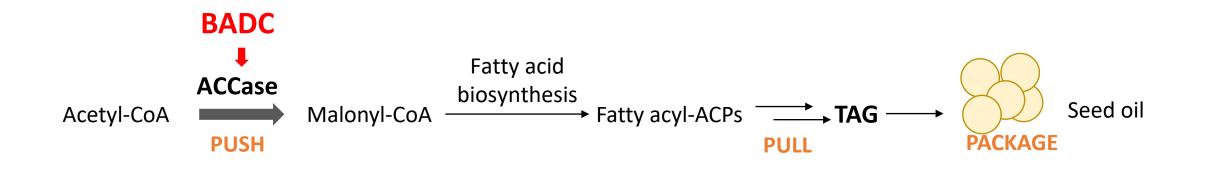
¹pursuant to 7 CFR part 340

c or ***** in photos, control plots where plants died with herbicide application

Gene Combinations to Increase Oil Content

Strategy 2: Edit a negative regulator of acetyl-CoA carboxylase (ACCase)

- ACCase considered to be rate-limiting step in fatty acid biosynthesis
- Jay Thelen (University of Missouri) identified role for BADC as a novel negative regulator of the heteromeric ACCase



C3007 (BADC) trait in-licensed from University of Missouri



C3007: A Genome Editing Target for Increasing Oil

Work at Yield10 in Camelina

- Identified 3 Camelina badc genes (9 alleles total)
 - Complete editing of all alleles was not obtained, possibly lethal
- Line edited in all 3 copies of badc1 genes and containing combinations of badc2 or badc3 edits
- <u>Field trials</u>: significant increase in seed yield of up to 10%

Work at Yield10 in Canola

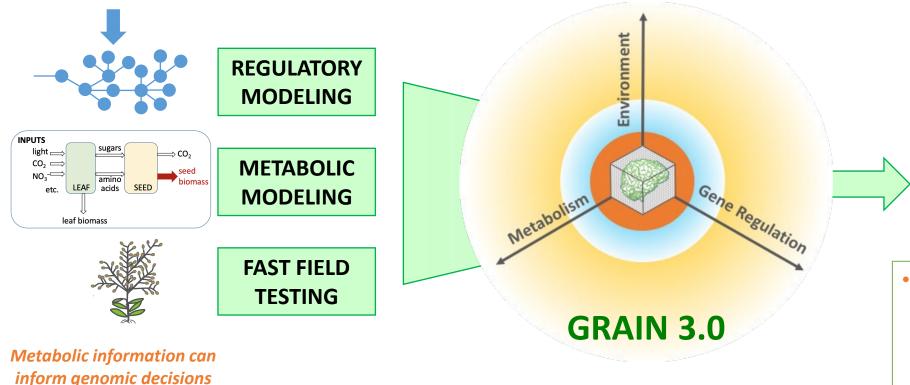
- Identified 6 badc genes
- Obtained stable edits for select badc genes/gene combinations
 - Complete editing of all alleles was not obtained, possibly lethal
- <u>Greenhouse trials</u>: increases in oil content (2.6 to 4.8%) with seed yield increases up to 17%
- <u>Field trials:</u> increases in oil content (2.1-4.3%), drought conditions prevented seed yield measurements
- <u>US Regulatory</u>: lines not considered to be regulated article under 7 CFR part 340¹



GRAIN 3.0: Identify Unique Gene Combinations for a Trait

How do you move beyond known genes and identify new combinations?

CROP SPECIFIC GENOMICS DATA



Actionable gene modifications leading to valuable, novel yield traits

- Identified C3020: Increases seed oil content by up to 10% in Camelina (2021 & 2022 field trials).
- Multiple other potential targets identified for testing



Yield10 Review Paper on Metabolic Engineering in Plants: Skraly et al., Metabolic engineering to increase crop yield: From concept to execution, 2018, Plant Science

Yield10 is Harnessing The Potential of Camelina for Biofuel Feedstocks

Grower adoption – Weed control

- Developed and field-tested herbicide tolerant lines for over-the-top spray weed control
- → Field trials of next generation stacked herbicide tolerance lines (over-top-spray & soil residue tolerance) in spring 2023

Grower adoption & business success – Revenue – increased harvest value for biofuel feedstocks

- ightarrow Edited E3902 line has ~5% increase in oil in multiple years of field trials
- ightarrow Testing of badc edited lines that have shown increased seed yield in the field
- ightarrow GRAIN modeling has identified additional genes to increase oil content
- → Improved protein meal value: Gene editing targets have been identified

Grower adoption & business success – Partnerships across the biofuel value chain

 \rightarrow Discussions with potential partners in progress





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Thank you



