



Yield10 Trait Development: Towards a value-added Camelina crop

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**10TH PLANT GENOMICS &
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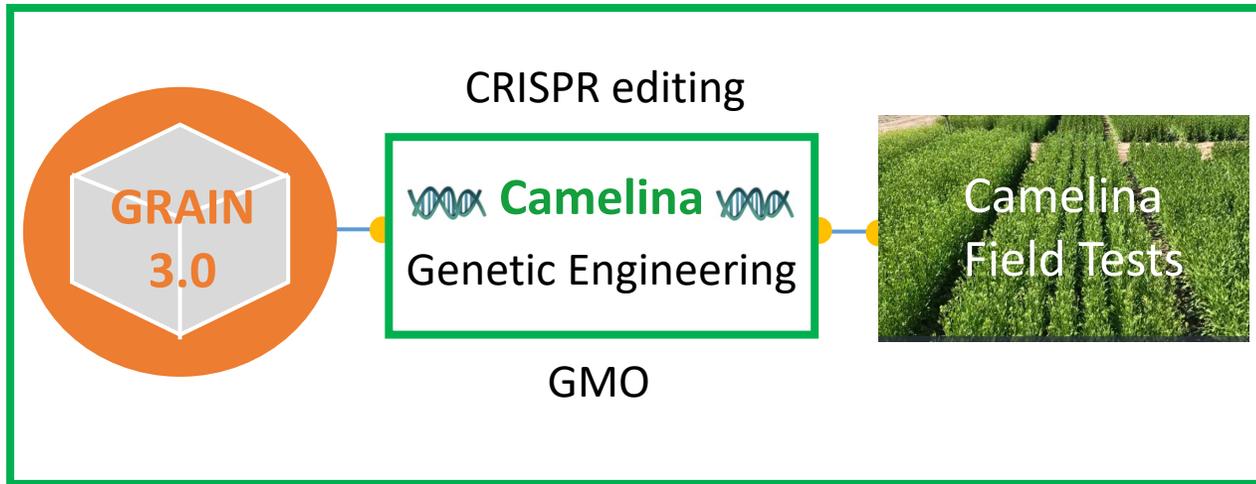
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From Crop Science to Market

Technology Platform - "Trait Factory"¹



Camelina Seed Products

1. Feedstock Oil (Biofuel)



2. Omega-3 Oil (EPA+DHA)



3. PHA Bioplastics



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
- 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

Camelina Based Biofuel Feedstocks

The Potential of the Camelina Crop for Biofuel Feedstocks is Driven by:

Grower adoption – Weed control and seamless integration into crop rotations

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

- Camelina grain (seed) yield per acre, oil as a percent of seed weight (oil/acre)
- Carbon intensity (CI) score of the oil (carbon score as a trait target?)
- Improved protein meal value

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

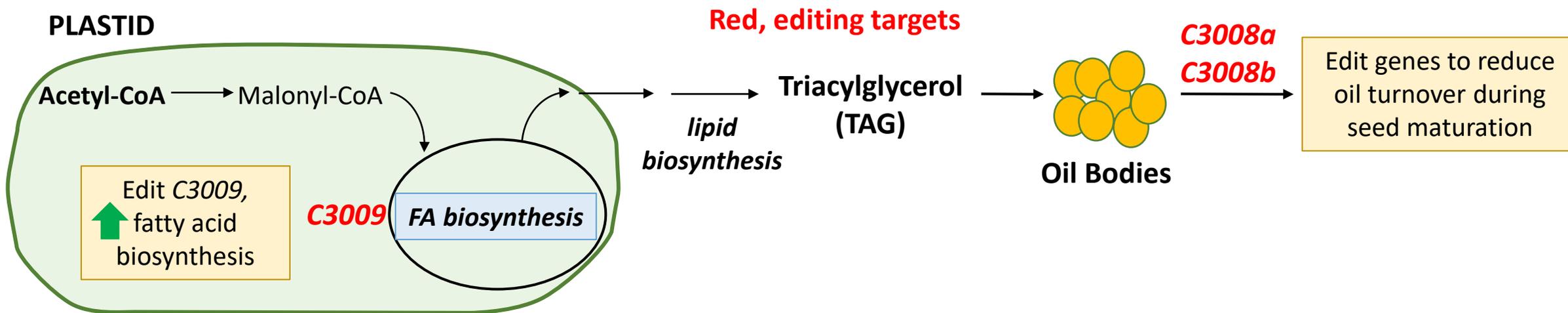
Yield10 has pipeline of Camelina lines and proprietary gene traits to increase value of Camelina

Priority 1: Herbicide tolerant Camelina to enable seamless integration into grower crop rotations

Priority 2: Seed yield and seed oil content to increase the harvest value for biofuels

Priority 3: Meal quality traits to improve meal value

Gene combinations to increase oil biosynthesis and prevent oil turnover¹



1. C3009 - transcription factor target to upregulate fatty acid biosynthesis

— 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

¹Editing work supported in part by U.S. Department of Energy – BETO, Grant No. DE-EE0007003

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

- Identified best Camelina orthologs to Arabidopsis genes based on homology
- 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*)

Received 2018 confirmation that USDA-APHIS does not consider lines to be regulated¹

2019 field test of edited lines at site in US

(randomized complete block design, lines replicated 6 times)



- E3902 oil trait stable in 2019, 2020, 2021, 2022 field trials. Demonstrated increased total oil produced per acre

2019 Field Data for E3902

% Increase, oil per individual seed (mgs)	% Increase, individual seed weight (mgs)	% Increase, seed yield (kg seed per hectare)	% Increase, seed oil content (% of seed weight)	% Increase, number of seeds harvested	% Increase, total oil produced per hectare
11.8*	8.7*	9.7	4.7*	-3.7	15.0

*statistically significant (t-test)

Patent pending

¹pursuant to 7 CFR part 340*

E3902 is Yield10's first spring variety in pre-commercial stage

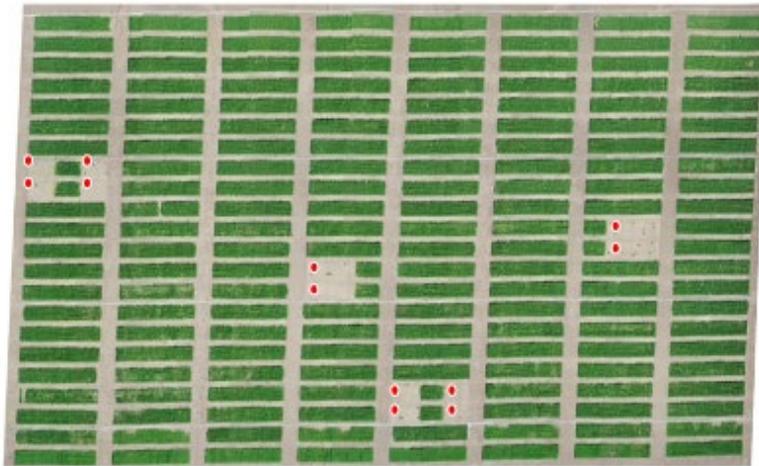
- US: Am I Regulated Process, line not considered to be regulated article under 7 CFR part 340
- Chile: Ministry of Agriculture on Agricultural Protection- line would not be subject to regulation
- Argentina: Ministry of Agriculture, Livestock and Fisheries in Argentina - line would not be subject to regulation
- Documentation to determine regulatory status in Canada is being compiled

Trait stacking in E3902

Herbicide Tolerance Traits: E3902 is germplasm background for our herbicide tolerant lines

- Over the top spray weed control line
- Group 2 residue tolerance, over the top spray weed control line

Omega 3 oil: Develop lines producing EPA and DHA



Herbicide Tolerant event selection trials in the U.S. in spring 2022.

• Red dots in photos, control plots where plants died with herbicide application

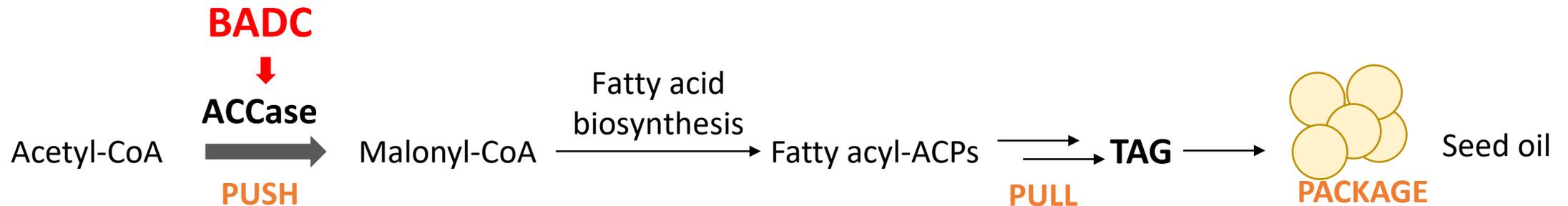


Camelina containing the omega-3 EPA trait growing at acre-scale in the U.S. in spring 2023.

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

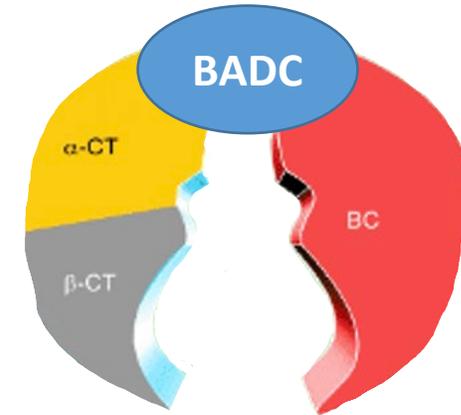
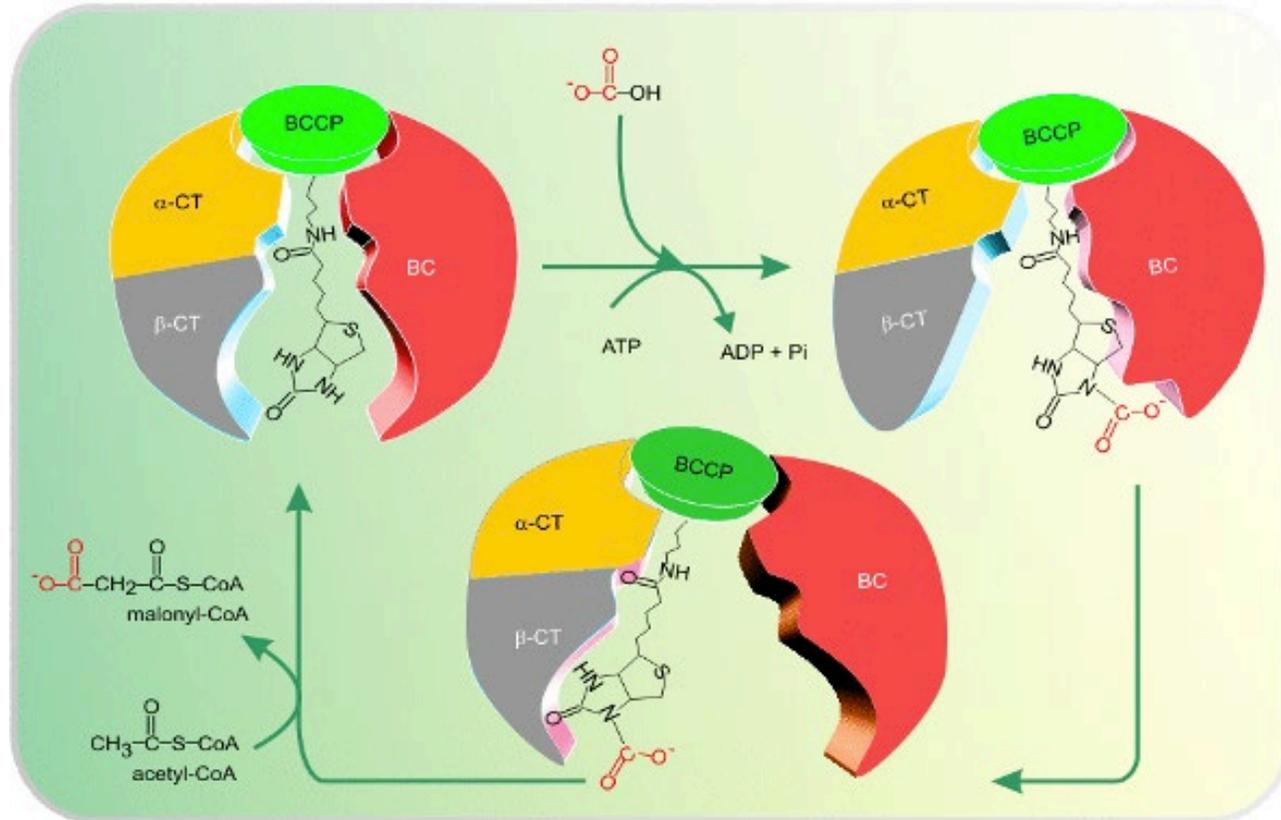


C3007 (BADC) trait in-licensed from University of Missouri

BADC can displace BCCP, but lacks active site motif

Active heteromeric ACCase with **BCCP** and biotinylation motif in active site

Inactive heteromeric ACCase, **BCCP** displaced by **BADC**



No biotinylation motif essential for ACCase enzyme activity

Li-Beisson Y et al., (2013) Acyl-lipid metabolism. Arabidopsis Book 11: e0161

Reduce/eliminate BADC with genome editing to increase ACCase activity

Genome Editing of *badc* Genes

Species	Ploidy/genome	# of BADC homologs
<i>Arabidopsis</i>	2n=2x, diploid	3 (<i>badc1</i> , <i>badc2</i> , <i>badc3</i> ; <i>Salie et al.</i>) ¹
<i>Camelina</i>	2n=6x, hexaploid (Cs-G1, -G2, -G3)	9 (3 <i>badc1</i> , 3 <i>badc2</i> , 3 <i>badc3</i>) (1 copy on each subgenome; <i>Yield10</i>) ²

Work at Yield10 to edit *badc* in Camelina

- Identified 3 Camelina *badc* genes (9 alleles total)
- Obtained stable edits for select *badc* genes/gene combinations
 - **Complete editing of all alleles was not obtained, possibly lethal**
- Crossed lines edited in all 3 copies of *badc1* with lines containing combinations of *badc2* or *badc3* edits
- Field trials completed in 2022, 2023

¹Salie et al., 2016, Plant Cell, 28, 2312. ²Camelina genes identified at Yield10

Field Trial of Edited *badc* Lines

Lines tested in field trial

Entry	<i>badc1</i>	<i>badc2</i>	<i>badc3</i>
E4757	X X X		
E4724-C	X X X		
E4806-B	X X X		
E4816-A	X X X		
WT43-F	---	---	---
E6146	X X X	X X _	
E6154	X X X	X X _	
E6119	X X X		X X _
WT43-G	---	---	---

“X” denotes gene is edited; “_” denotes wild-type gene; Control line.

US field site (July 14, 2022)



plots (63 days old)

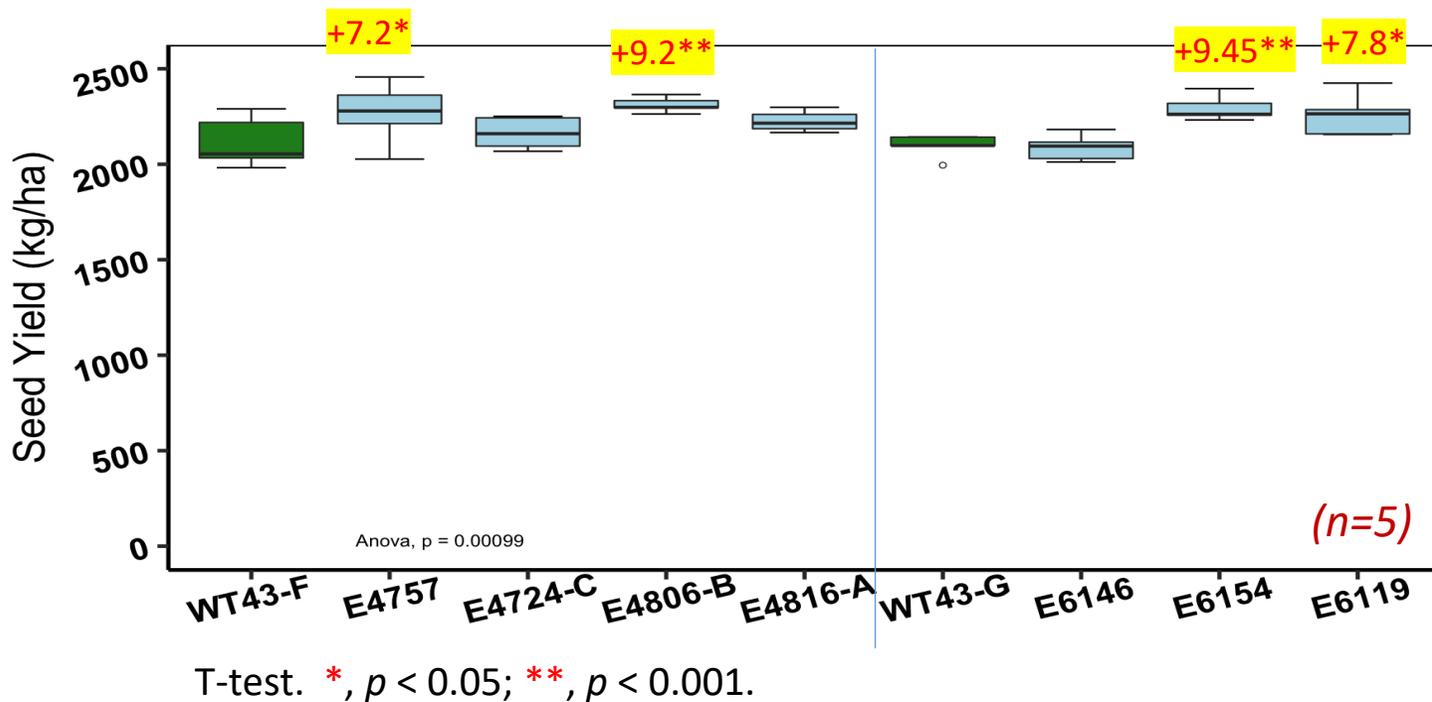
Field trial drone photo



Plan to submit lines with all 3 *badc1* genes edited through USDA-APHIS Regulatory Status Review (RSR)

Spring 2022 Camelina *badc* Field Trial – Seed Yield

Seed Yield of C3007 Edited Lines, Measured in Replicated Plots



Lines tested in field trial

Entry	<i>badc1</i>	<i>badc2</i>	<i>badc3</i>
E4757	X X X		
E4724-C	X X X		
E4806-B	X X X		
E4816-A	X X X		
WT43-F	---	---	---
E6146	X X X	X X _	
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WT43-G	---	---	---

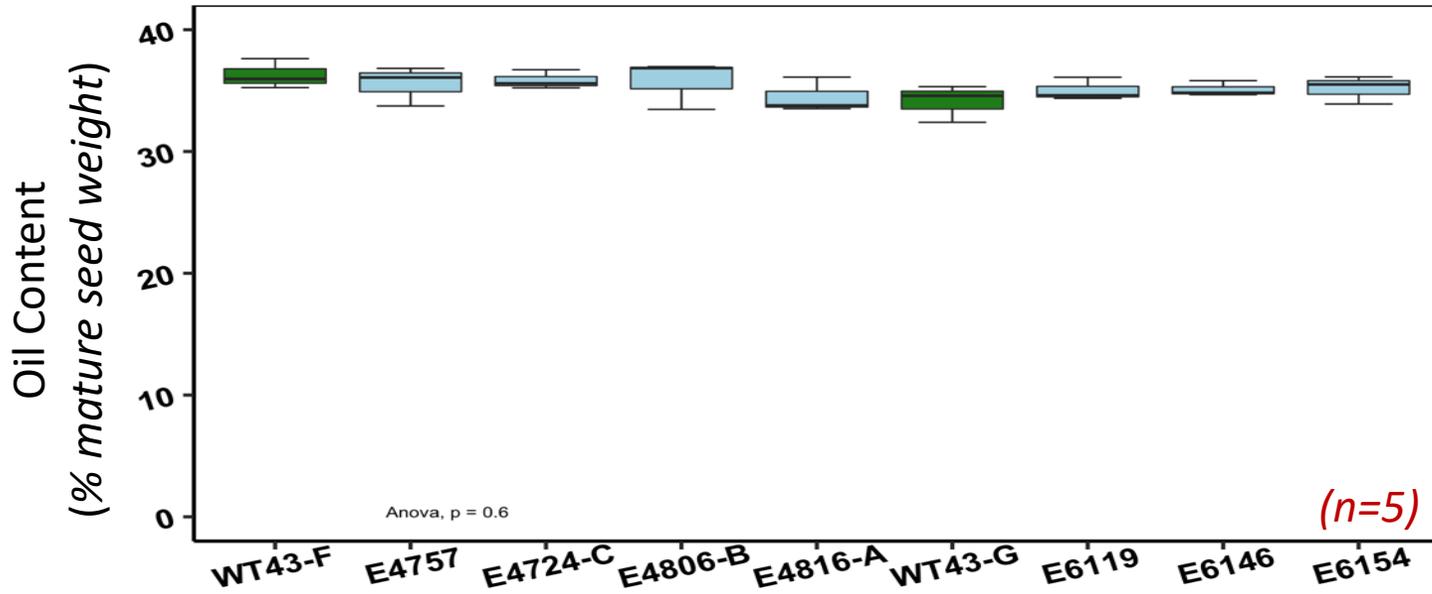
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Significant yield increase observed for four C3007 (*badc*) edited lines

Plan to submit lines with all 3 *badc1* genes edited through USDA-APHIS Regulatory Status Review (RSR)

Spring 2022 Camelina *badc* Field Trial – Seed Oil Content

Seed Oil Content of C3007 Edited Lines, Measured from Replicated Plots



T-test. *, $p < 0.05$; **, $p < 0.001$.

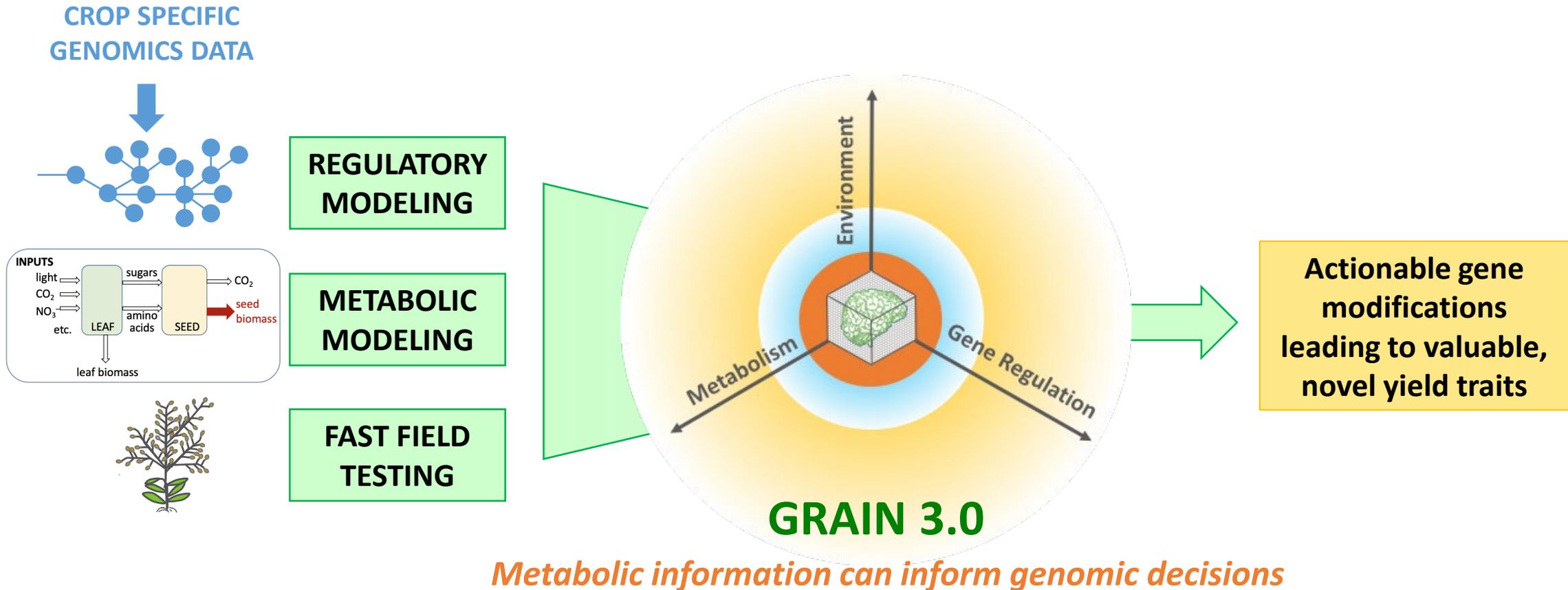
Lines tested in field trial

Entry	<i>badc1</i>	<i>badc2</i>	<i>badc3</i>
E4757	X X X		
E4724-C	X X X		
E4806-B	X X X		
E4816-A	X X X		
WT43-F	---	---	---
E6146	X X X	X X _	
E6154	X X X	X X _	
E6119	X X X		X X _
WT43-G	---	---	---

“X” denotes gene is edited; “_” denotes wild-type gene; Control line.

- No significant change in oil content observed in C3007 (*badc*) edited lines
- *badc* edits analyzed increase seed yield but not seed oil content
- Field trials repeated in spring 2023

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

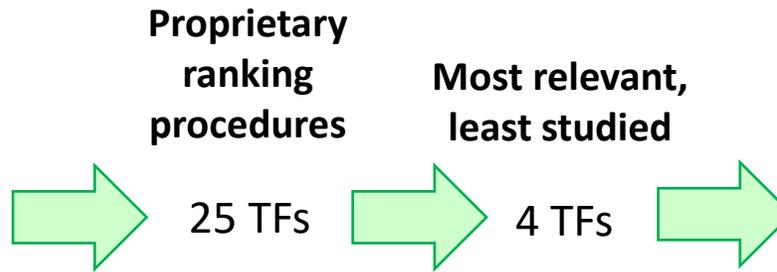


GRAIN Identifies New Gene Targets: C3019 – C3022

GRAIN - Searches for transcription factors (TFs) to increase seed oil content in Camelina

Ranked List of Gene Targets

211 Identified TFs	
TF1 = WRI1	TF9
TF2 = WRI1	TF10
TF3 = WRI1	TF11
TF4 = MYB56	TF12
TF5	TF13
TF6	TF14
TF7	TF15 = MYB56
TF8	TF16



Overexpression of TFs

Trait	Effect
C3020	+10% oil
C3019	Negative
C3021	Negative
C3022	Negative

- Ranked list of TFs obtained - Includes known genes in top spots validating approach (WRI1, MYB56)
- Many uncharacterized genes identified, IP white space
- Overexpression of C3020 increases seed oil content by up to 10%, validated in 2021 and 2022 field seasons.
- Genes overexpressed giving a negative phenotype are good editing targets C3019, C3021 and C3022
- Editing of C3021 resulted in higher number of tertiary branches, more orthologs may need editing
- Yield10 now has ~10 Camelina gene targets for combinatorial editing to achieve step change increases in oil

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*
- *Completed field testing 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

- *Edited E3902 line has ~5% increase in oil in multiple years of field trials*
- *Additional testing of badc edited lines that have shown increased seed yield in the field - in progress*
- *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

- *Discussions with potential partners in progress*



www.yield10bio.com

NASDAQ: YTEN

Thank you

Sustainable Growth Starts with a Seed

