



# Yield10 Trait Development: Using CRISPR to Increase Seed Yield and Oil Content in Camelina

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# Safe Harbor Statement\*

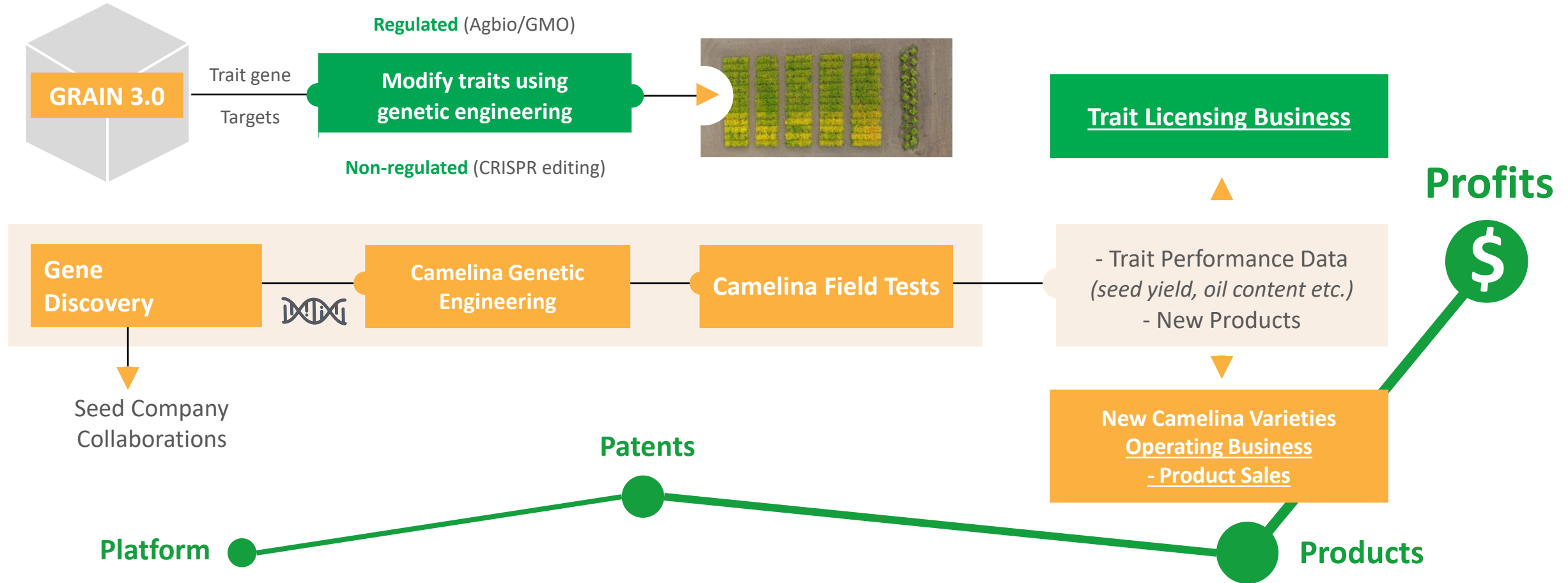
The statements made by Yield10 Bioscience, Inc. (the “Company,” “we,” “our” or “us”) herein regarding the Company and its business may be forward-looking in nature and are made pursuant to the safe harbor provisions of the Private Securities Litigation Reform Act of 1995. Forward-looking statements describe the Company’s future plans, projections, strategies and expectations, including statements regarding future results of operations and financial position, business strategy, prospective products and technologies, timing for receiving and reporting results of field tests and likelihood of success, and objectives of the Company for the future, and are based on certain assumptions and involve a number of risks and uncertainties, many of which are beyond the control of the Company, including, but not limited to, the risks detailed in the Company’s Annual Report on Form 10-k for the year ended December 31, 2019 and other reports filed by the Company with the Securities and Exchange Commission (the “SEC”). Forward-looking statements include all statements which are not historical facts, and can generally be identified by terms such as anticipates, believes, could, estimates, intends, may, plans, projects, should, will, would, or the negative of those terms and similar expressions.

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**\*Under the Private Securities Litigation Reform Act of 1995**

# The Yield10 - Trait Factory and Business Model

**“Trait Factory”:** Accelerated Trait Gene Discovery and Development Platform



# Genome Editing to Increase Seed Oil Content

**For oilseed crops : harvest value is driven by  $\text{oil/acre} = \text{seed yield/acre} \times \text{seed oil content}$**

**Increasing seed yield and/or seed oil content can maximize oil yields per acre**

- Yield10 is developing Camelina lines with multiple genome edits to increase oil yields
  - Editing combinations of *BADC*, *SDP1*, *SDP1-LIKE*, and *TT2* gene targets, additional targets to follow
- Yield10 is editing *BADC* in canola, additional targets to follow
- Potential to combine (stack) these edits with oil composition traits (e.g. high oleic, omega fatty acids)
  - Yield10 recently announced a collaboration with Rothamsted Research to develop advanced technology for producing omega-3 nutritional oils in Camelina

**Increases in seed size and/or seed oil content can impact overall seed yield**

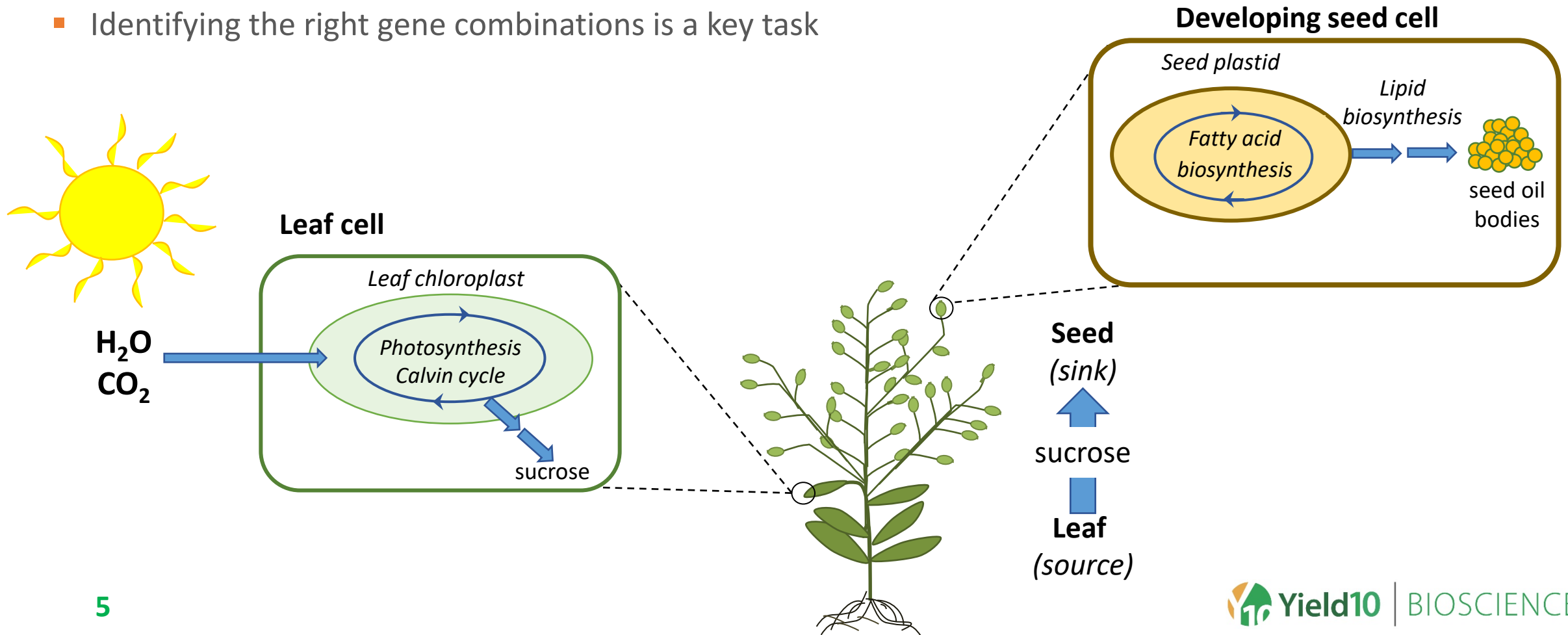
- Edited *sdp1*, *sdp1-like*, and *tt2* Line 1 significantly increased oil content in greenhouse and field tests
- Opportunity exists to further engineer Lines 1, 2 and 3 to increase seed yield while maintaining oil content
- Yield10 is using GRAIN 3.0 to identify traits to increase seed yield
- GRAIN uses metabolism to rationally mine genomics data to identify unique genes



# Target: Increased Seed Yield and Oil Content

For niche oils: cost of goods is driven by harvested oil/acre (= seed yield/acre x seed oil content)

- Step-change increases in seed yield and oil content will likely require increased photosynthetic carbon fixation and delivery of the increased fixed carbon to the seed
- Identifying the right gene combinations is a key task



# Oilseed Crop Choice: Canola and *Camelina sativa*

## Canola

- Commodity food oil crop, 20 million acres harvested (19 million tonnes) in Canada in 2020<sup>1</sup>

## Camelina

- Good platform for specialty/niche crops for high value products
  - specialty oils, biopolymers, products in food and animal feed
- Seed oil levels typically 35-40% of seed weight
- Does not outcross with *Brassica napus*
- Both spring and winter varieties available
  - winter varieties possible cover crop for excess N from corn and soybean
- Camelina is currently planted on a very small acreage

<sup>1</sup> <https://www.canolacouncil.org/markets-stats/statistics/harvest-acreage/>

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

# Traits for Boosting Seed Oil Content

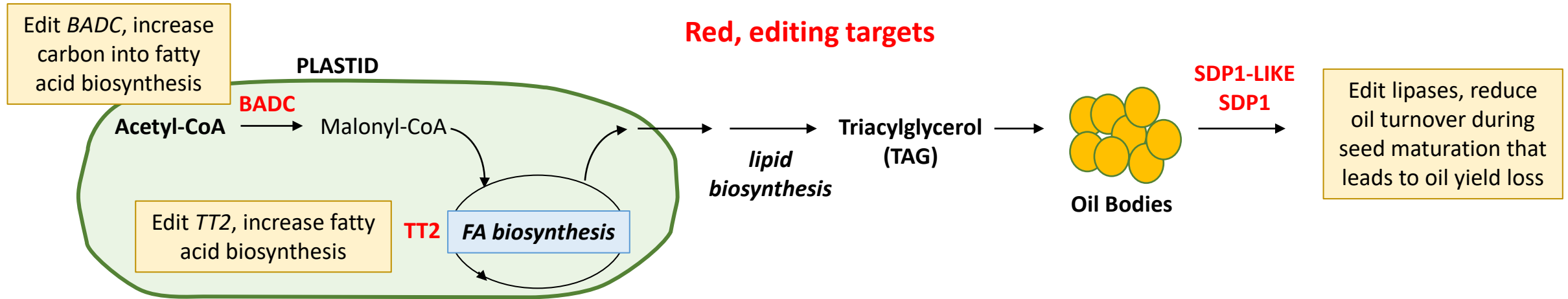
Yield10 is uniquely positioned to re-engineer the oil biosynthesis pathway in oilseed crops

## Objective: Maximize oil production per acre

- CRISPR editing gene combinations in Camelina (allohexaploid)
- Obtain stable lines with homozygous edits
- Growth in greenhouse/growth chamber: preliminary snapshot of trait performance
- Submit a request to USDA-APHIS for confirmation of exemption from regulation under 7 CFR part 340, (formerly “Am I Regulated Letter?” process) to clarify regulatory status of trait
- Field test to determine trait performance

# Gene Editing Targets

Editing work funded in part by US Department of Energy – BETO to Yield10 Bioscience

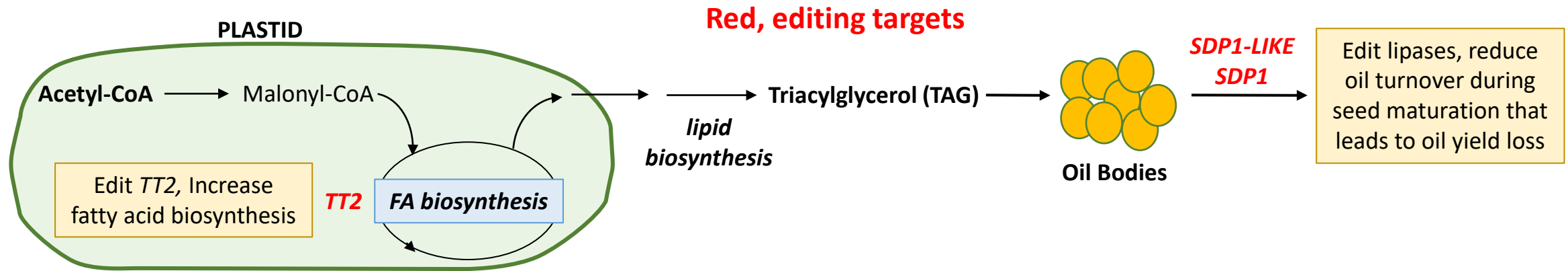


- **BADC** = biotin/lipoyl attachment domain containing protein - a negative regulator of acetyl-CoA carboxylase
- **TT2** = transparent testa2 - transcription factor that reduces expression of fatty acid biosynthetic genes
- **SDP1** (sugar-dependent 1 protein) and **SDP1-LIKE** (sugar-dependent 1-like protein) - oil body lipases



# Camelina: Multiplex Genome Editing of Three Genes (9 alleles)

## Editing of lipase genes (*SDP1*, *SDP1-LIKE*) and transparent testa2 (*TT2*) gene



### 1. Lipase gene targets to reduce oil turnover during seed maturation

- *SDP1* (sugar-dependent 1 protein) and *SDP1-like* (sugar-dependent 1-like protein) are oil body associated lipases<sup>1,2</sup>
  - account for 95% of TAG lipase activity during seed germination in Arabidopsis
  - expressed during seed maturation and desiccation in Arabidopsis, possible involvement in oil loss in mature seed
- Arabidopsis *sdp1* mutant
  - increased seed lipid content and seed size
  - impaired oil breakdown during postgerminative growth, normal growth after photosynthesis established<sup>2</sup>
- Arabidopsis *sdp1-like* mutant, no growth phenotype<sup>2</sup>

<sup>1</sup>Eastmond et al., 2006, Plant Cell, 18, 665; <sup>2</sup>Kelly et al., Plant Physiology, 2011, 157, 866

<sup>3</sup>Kim et al., 2014, Biotechnology for Biofuels, 7, 36.

# Multiplex Genome Editing of Camelina

## Editing of lipase genes (*SDP1*, *SDP1-LIKE*) and transparent testa2 (*TT2*) gene

### 2. Transparent testa2 transcription factor target to upregulate fatty acid biosynthesis

- Negative regulation of embryo fatty acid biosynthetic enzymes
- Positive regulation of proanthocyanidin enzymes in seed coat
- Arabidopsis *tt2* mutant:
  - Increased seed fatty acid content and fatty acid elongation, decreased seed size and seed weight, colorless seeds<sup>1</sup>

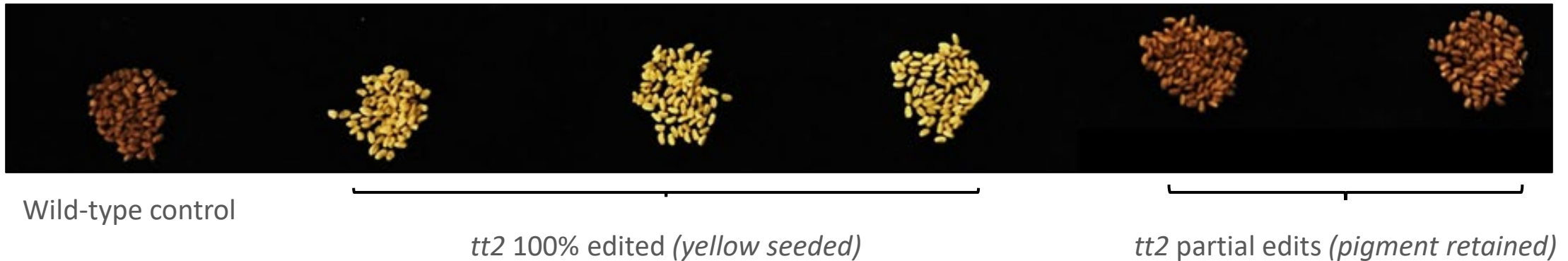
### 3. Multiplex Editing of *SDP1*, *SDP1-LIKE*, *TT2* at Yield10 and Metabolix Oilseeds

- 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, one line obtained with all 9 genes edited

# Multiplex Genome Editing of Camelina

## Multiplex editing of lipase genes (*SDP1*, *SDP1-LIKE*) and transparent testa2 (*TT2*)

- Fully edited *tt2* gene, loss of pigmentation in seed coat due to reduced expression of enzymes for proanthocyanidins
  - Unique distinction to track edited seed



# Multiplex Genome Editing of Camelina

## Multiplex editing of lipase genes (*SDP1*, *SDP1-LIKE*) and transparent testa2 (*TT2*)

- Preliminary snapshot of trait performance from greenhouse growth
  - Partial editing of *sdp1* (editing of 2 of 3 alleles, line type 1) provides 5% increase in total oil produced per plant
  - Increases in oil produced per individual seed and individual seed weight resulted in decrease in seed number
  - Demonstrates significant shift in carbon partitioning to oil

	Gene Targets			% Increase, oil per individual seed (mgs)	% Increase, individual seed weight (mgs)	% Increase, seed oil content (% of seed weight)	% Increase, number of seeds per plant	% Increase, total oil produced per plant
	<i>sdp1</i>	<i>sdp1-like</i>	<i>tt2</i>					
Line type 1	X X _	X X X	X X X	+ 12	+ 1	+ 9	- 5.2	+ 5
Line type 2	X X X	X X _	X X X	+ 38	+ 17	+ 5	- 19	- 15
Line type 3	X X X	X X X	X X X	+ 34	+ 9	+ 6	- 29	- 26

*Patent pending*

- Observed tradeoff between seed oil content with seed number
  - *Suggests not enough carbon/reducing power to significantly increase oil content AND produce normal number of seeds*
- Opportunity exists to further engineer edited lines to increase seed yield

# 2019 Field Tests of Edited Lines

## Editing of lipase genes (*SDP1*, *SDP1-LIKE*) and transparent testa2 (*TT2*)

- Received confirmation in 2018 that USDA-APHIS does not consider lines to be regulated pursuant to 7 CFR part 340\*

2019 field test of edited lines at site in US  
(randomized complete block design, 4 edited lines  
replicated 6 times)



July 30, 2019

**Line 1** = *sdp1* (66%), *sdp1-like* (100%), *tt2* (100%) edits

**Line 2** = *sdp1* (100%), *sdp1-like* (66%), *tt2* (100%) edits

**Line 3** = *sdp1* (100%), *sdp1-like* (100%), *tt2* (100%) edits

**Line 4** = 100% *sdp1-like* edits

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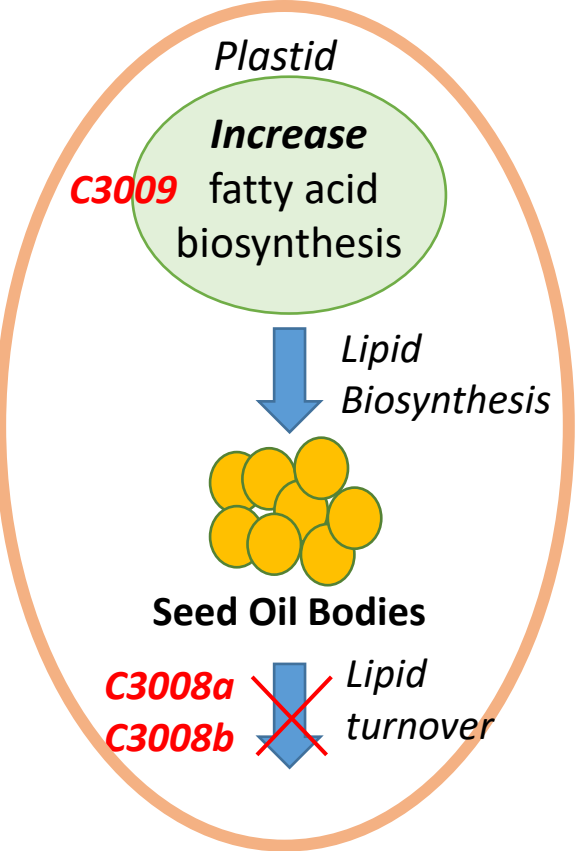
### Camelina with CRISPR triple edit: *sdp1*, *sdp1-like* and *tt2* (US)

- Best line (Line 1: E3902) produced 11.8% increase in oil/individual seed, 8.7% increase in individual seed weight, and 4.7% increase in seed oil content as percentage of bulk seed weight. No significant change in oil composition

\* May still be subject to regulation by FDA and EPA

# Spring Camelina Line 1 (E3902)

## Multiplex editing to increase oil content



Line 1 (E3902) Gene Targets		
<i>sdp1</i>	<i>sdp1-like</i>	<i>tt2</i>
X X _	X X X	X X X

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### Greenhouse Data

Site	% Increase, oil per individual seed (mgs)	% Increase, individual seed weight (mgs)	% Increase, seed yield (seed weight/plant )	% Increase, seed oil content (% of seed weight)	% Increase, number of seeds per plant	% Increase, total oil produced per plant
Greenhouse	+ 12	+ 1	- 4	+ 9	- 5	+ 5

### Field Data

Site	% 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
Field	11.8	8.7	9.7	4.7	-3.7	15.0

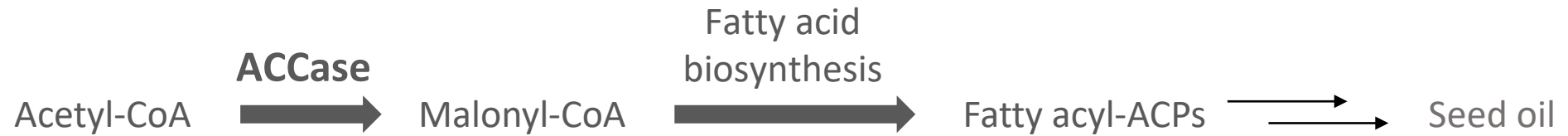
Yellow shaded boxes are statistically significant (t-test)  
Patent pending

- Line 1 scaled up in 2020 field trials

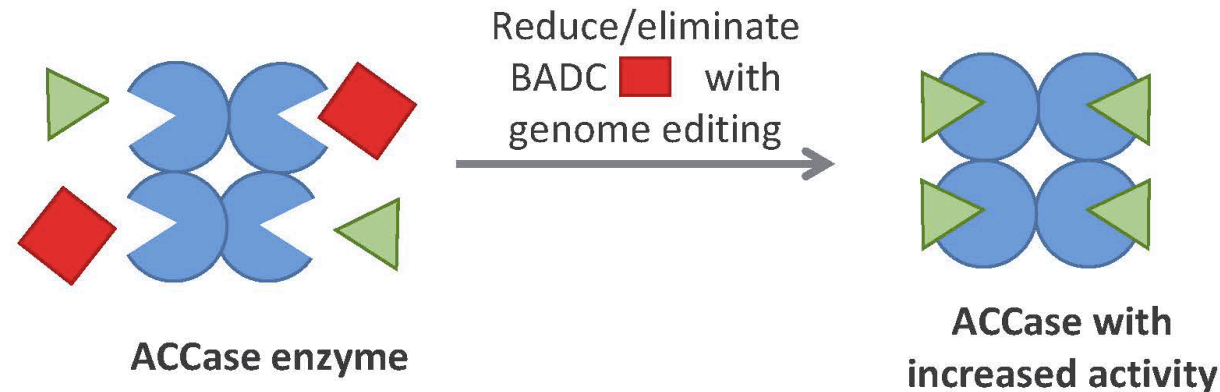




# C3007 (BADC) – Targets for Increasing Oil Content:

- BADC is a novel negative regulator of the heteromeric acetyl-CoA carboxylase (ACCase), a key enzyme in fatty acid biosynthesis



- BADC proteins displace biotin carboxyl carrier proteins (BCCP) required for ACCase activity
- C3007 (BADC) trait in-licensed from University of Missouri** (Prof. J. Thelen)



 BADC = biotin/lipoyl attachment domain containing proteins  
 BCCP = biotin carboxyl carrier protein

## Academic work with BADC

- Jay Thelen Lab, University of Missouri:
  - 3 *badc* genes identified in Arabidopsis, seed specific gene silencing of BADC1 increased oil per individual seed<sup>1</sup>
- John Shanklin Lab, Brookhaven National Laboratory
  - Arabidopsis T-DNA insertion lines used to analyze single and double mutants
  - Not all double mutant combinations could be obtained<sup>2</sup>
    - Best line *badc1 badc3* mutant, up to 32% more total triacylglycerols in seeds (% dry weight)<sup>2</sup>
- More recent work by Thelen Lab suggests that BADC plays a role in inhibiting ACCase in the dark or low light conditions<sup>3</sup>
  - ***BADC mutants would enable more fatty acid synthesis in the dark***

## Work at Yield10 and Metabolix Oilseeds in Camelina

- Identified multiple *badc* genes
  - Camelina 3 *badc* genes (9 alleles total)
- Obtained stable edits for select *badc* genes/gene combinations
  - **Complete editing of all alleles was not obtained, possibly lethal**

<sup>1</sup>Salie et al., 2016, Plant Cell, 28, 2312; <sup>2</sup>Keereetaweep et al., Plant Physiology, 2018, 177, 208;

<sup>3</sup>Ye et al., Journal of Biological Chemistry, 2020, 295, 99901

# C3007: A Genome Editing Target for Increasing Oil

## Camelina editing of C3007

- Nine C3007 gene targets, subset of targets edited, increase in oil produced per plant obtained (greenhouse)
- USDA-APHIS “Am I Regulated?” letter approved April 2020
- Lines in 2020 field trial

### 2020 Camelina C3007 Field Trial, flowering stage

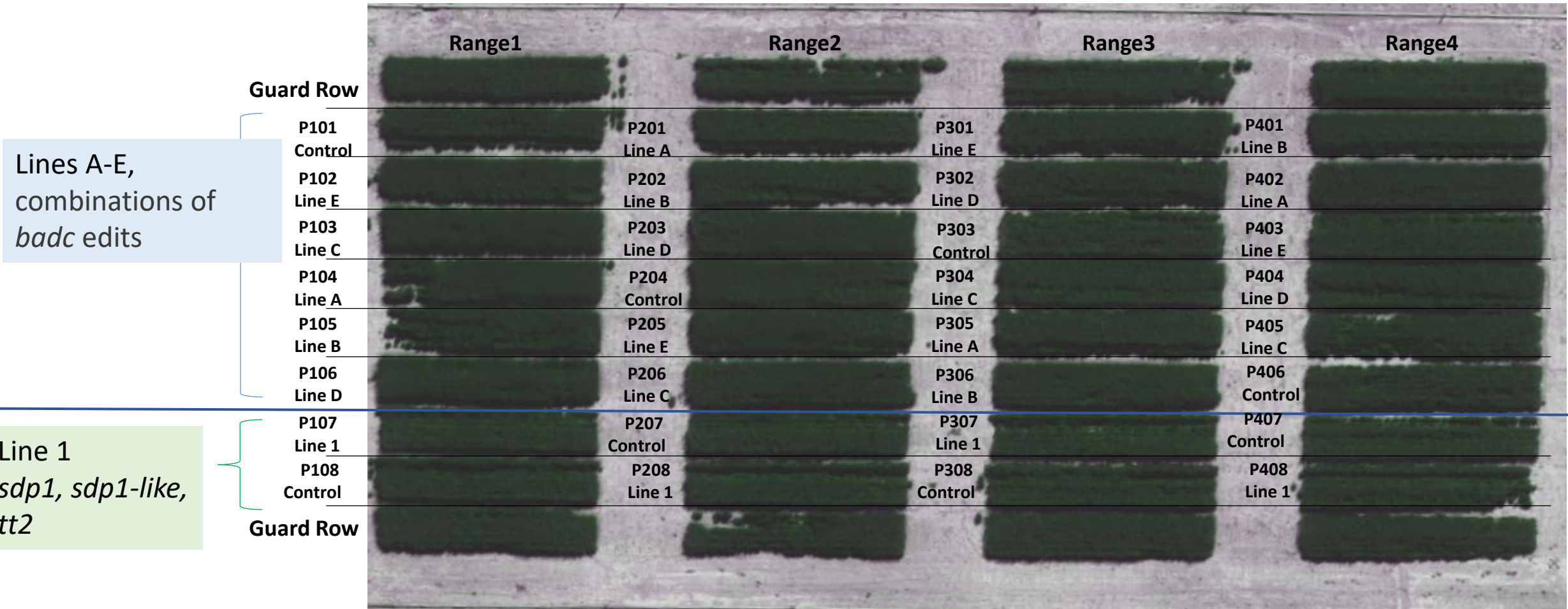




# 2020 Field Tests of Edited Lines

- First field test of *BADC* edited lines in the US
- Field tests to validate the Line 1 *sdp1*, *sdp1-like* and *tt2* 2019 data in US

(randomized complete block design, 6 edited lines replicated 4 times)



## Work at Yield10 and Metabolix Oilseeds in Canola

- Identified multiple *badc* genes
  - Canola 6 *badc* genes (12 alleles total)
- Obtained stable edits for select *badc* genes/gene combinations
  - **Complete editing of all alleles was not obtained, possibly lethal**

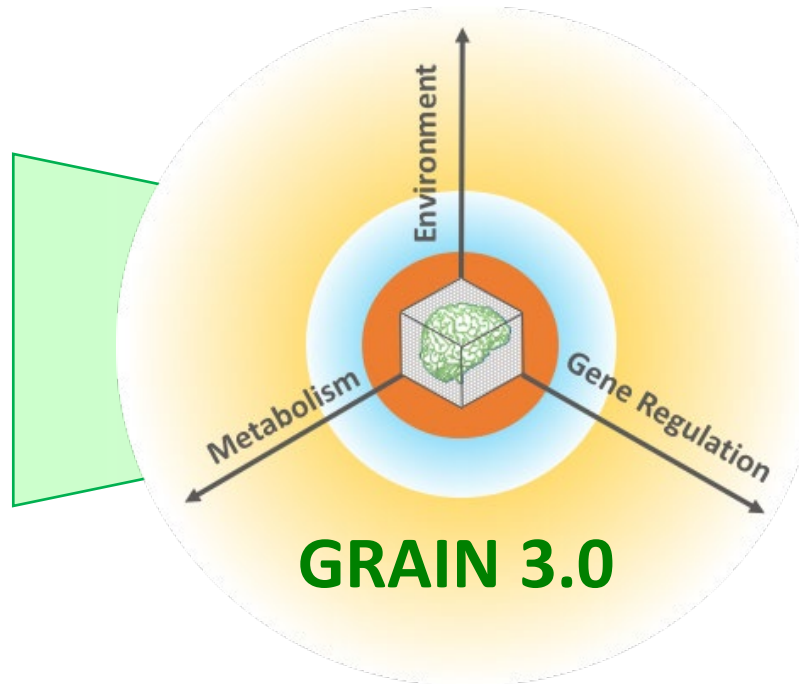
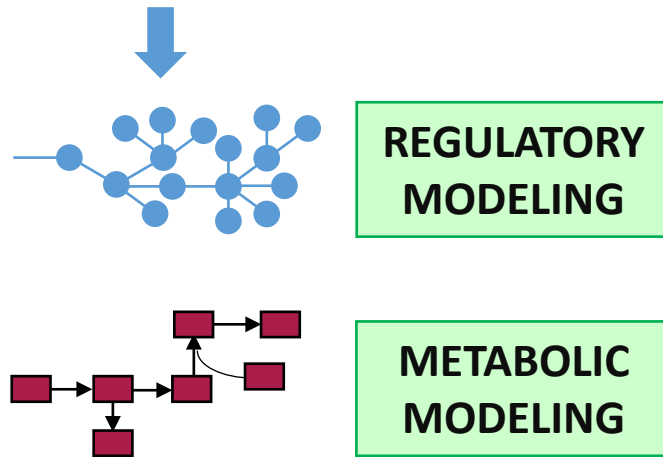
## Canola editing of C3007

- Six C3007 gene targets, subset of targets edited, increase in oil produced per plant obtained (greenhouse)
- “Am I Regulated?” letter approved in August 2020
- Field Trials planned for 2021 in the US
  - Seed increase in progress

# GRAIN 3.0: Using Metabolism to Rationally Mine Genomics Data

Combined regulatory and metabolic modeling components to identify transcription factors (TFs) to increase seed and oil yield in *Camelina*

## Camelina Genomics Data



## Prioritized List of Gene Targets

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

- Ranked list of TFs obtained
- Known TFs that impact oil and/or seed yield observed in top spots in list validating approach (WRI1, MYB56)
- Many uncharacterized genes identified, IP white space
- Currently pursuing multiple uncharacterized TFs



# Genome Editing to Increase Seed Oil Content

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# QUESTIONS?

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