



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

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# Safe Harbor Statement\*

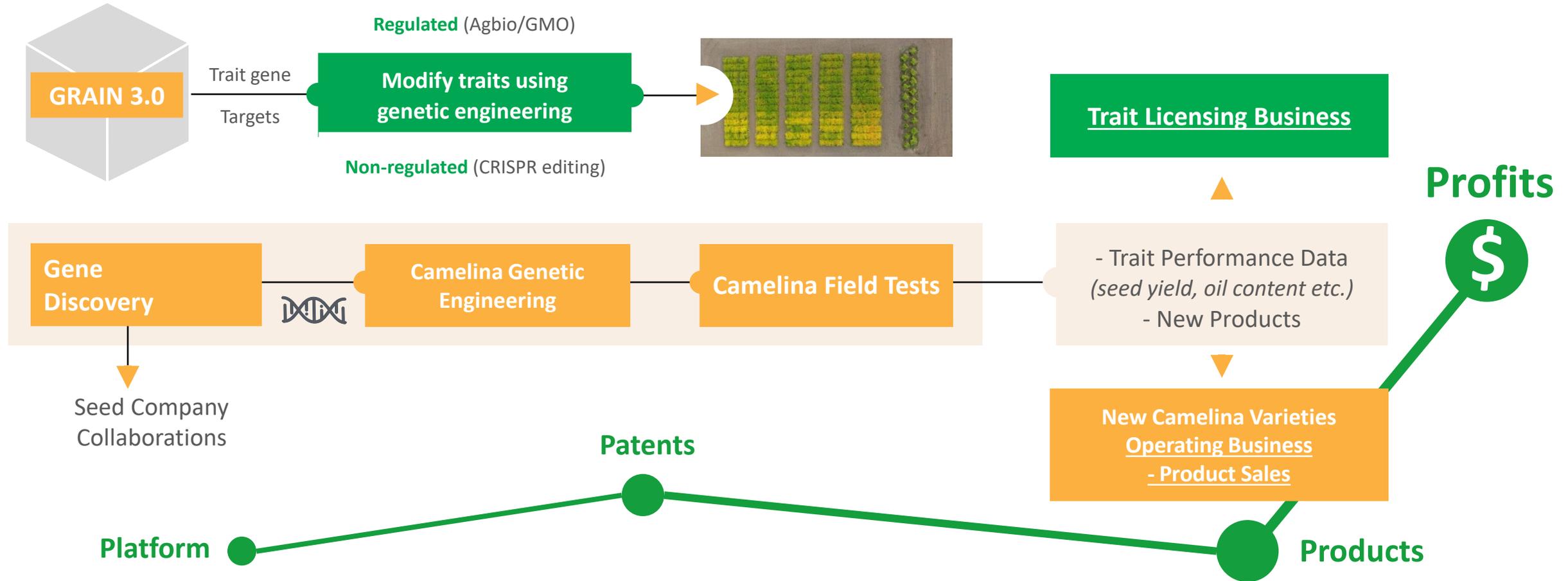
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**\*Under the Private Securities Litigation Reform Act of 1995**

# 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 oil/acre = seed yield/acre x 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

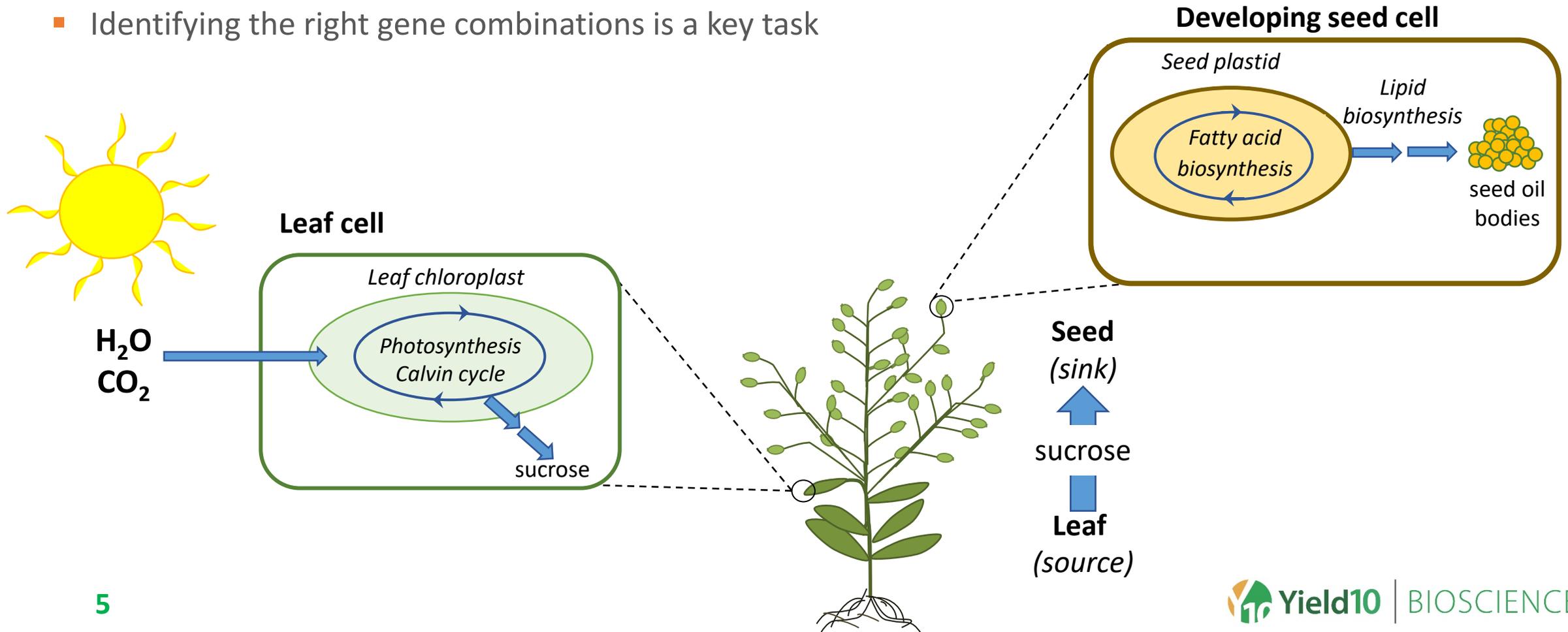
**Increases in seed size and/or seed oil content can impact overall seed yield**

- 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

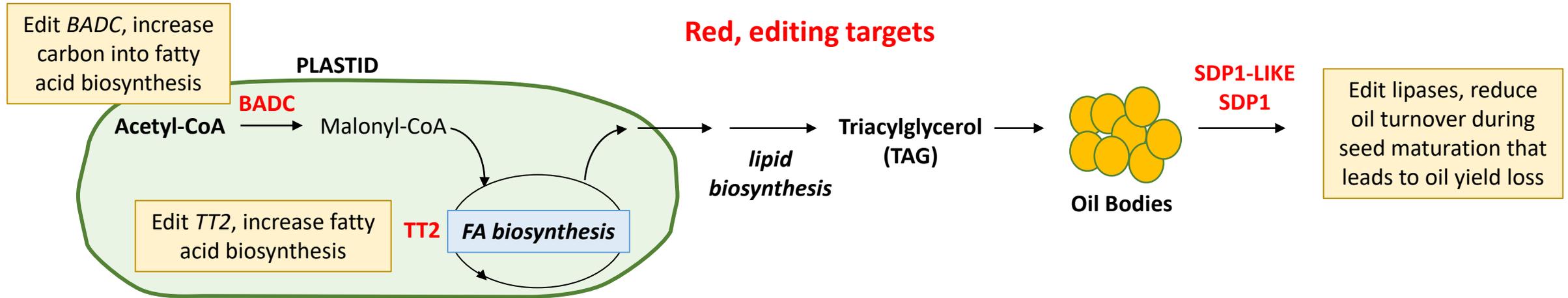
Yield10 is uniquely positioned to re-engineer the oil biosynthesis pathway in oilseed crops

## 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

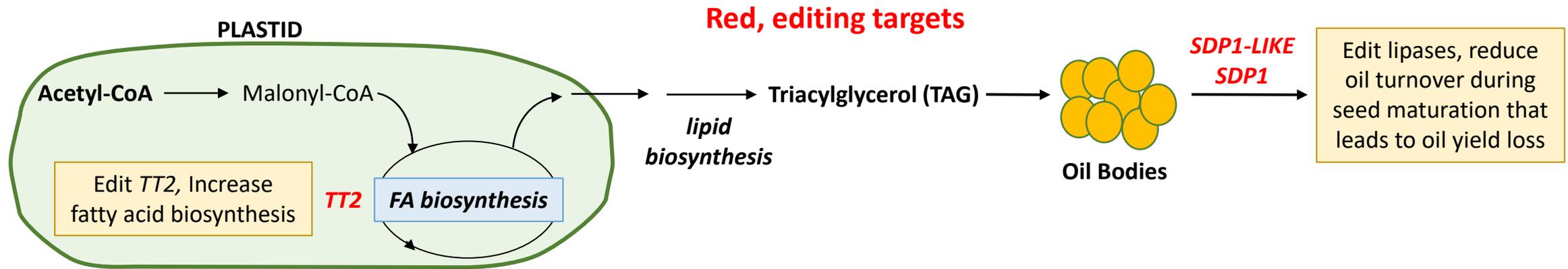
Editing work funded in part by US Department of Energy – BETO to Yield10 Bioscience



- **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.

## 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>

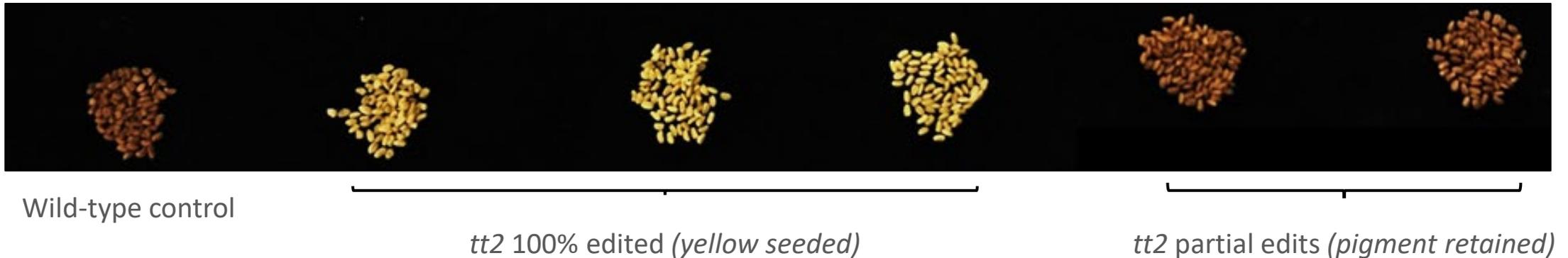
### 3. Multiplex Editing of *SDP1*, *SDP1-LIKE*, *TT2* at Yield10 and Metabolix Oilseeds

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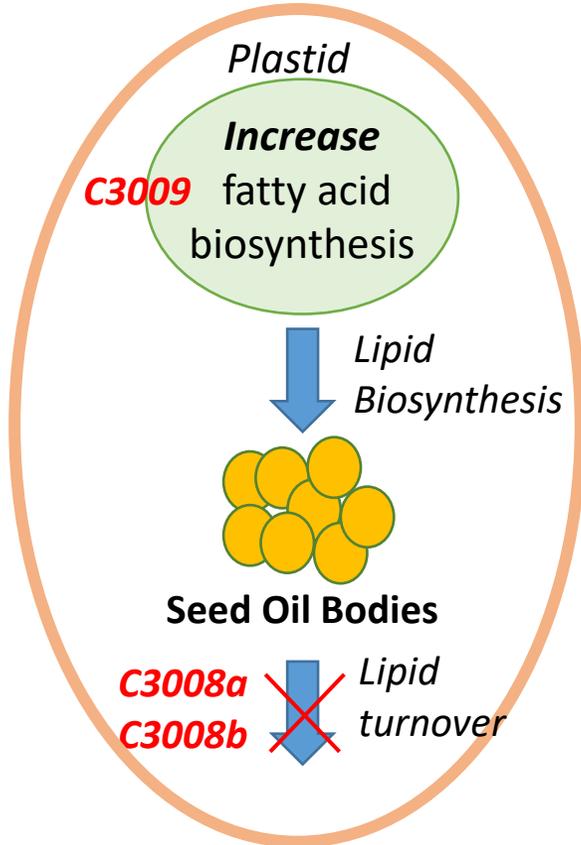
### Camelina with CRISPR triple edit: *sdp1*, *sdp1-like* and *tt2* (US)

- 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

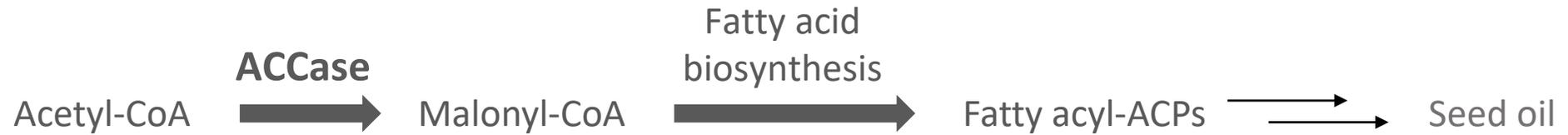
Site	% Increase, oil per individual seed (mgs)	% Increase, individual seed weight (mgs)	% Increase, seed yield (kg seed per hectare)	% Increase, seed oil content (% of seed weight)	% Increase, number of seeds harvested	% Increase, total oil produced per hectare
Field	<b>11.8</b>	<b>8.7</b>	9.7	<b>4.7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

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

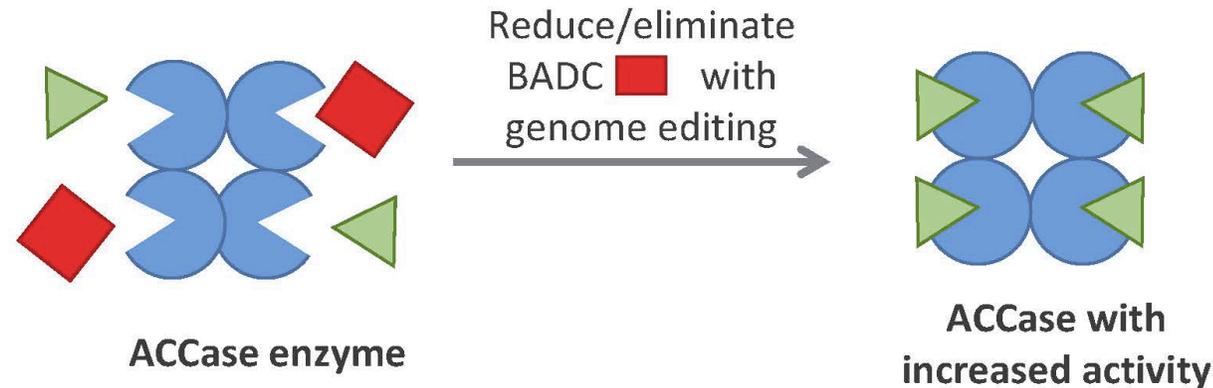
- Line 1 scaled up in 2020 field trials

# 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>Keereetawee 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



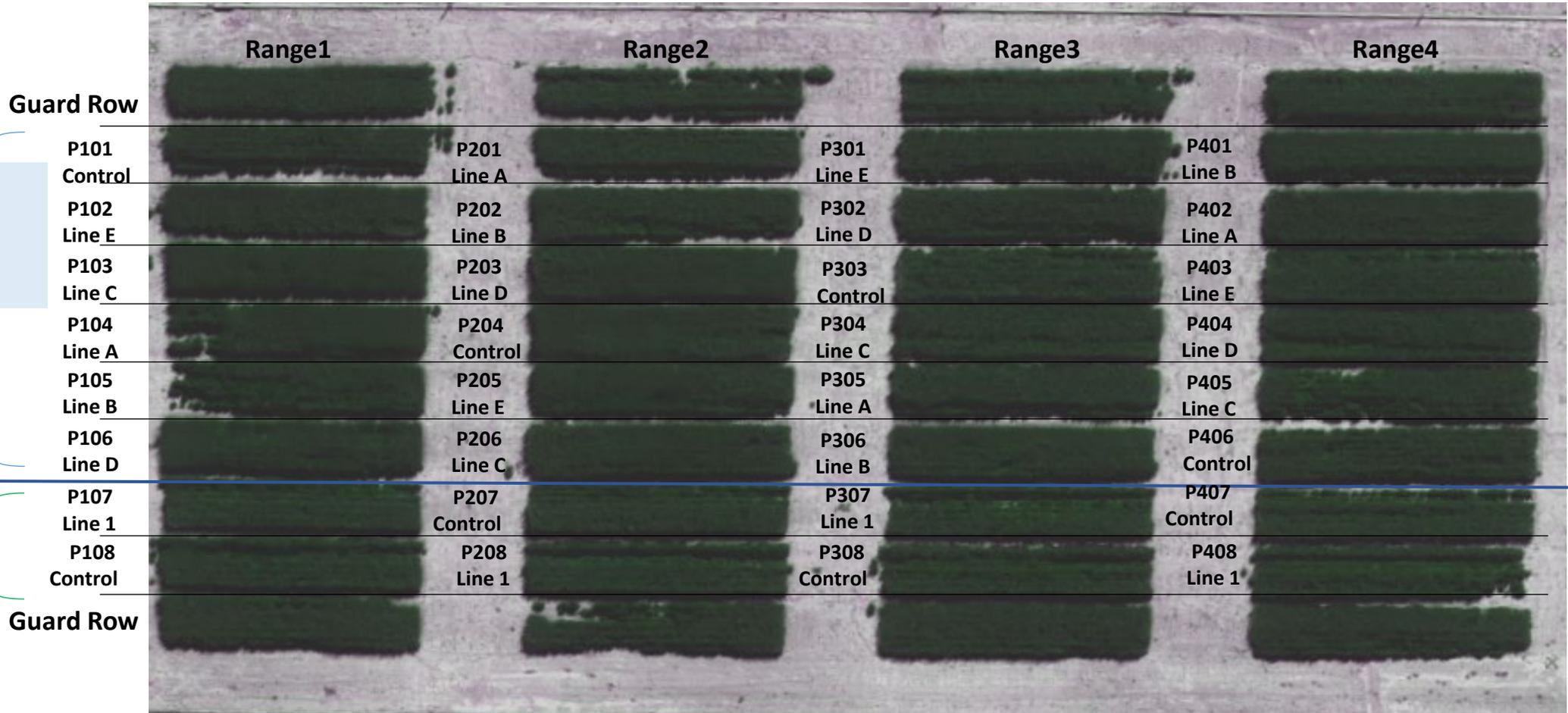
# 2020 Field Tests of Edited Lines

- 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)

Lines A-E,  
combinations of  
*badc* edits

Line 1  
*sdp1*, *sdp1-like*,  
*tt2*



## 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
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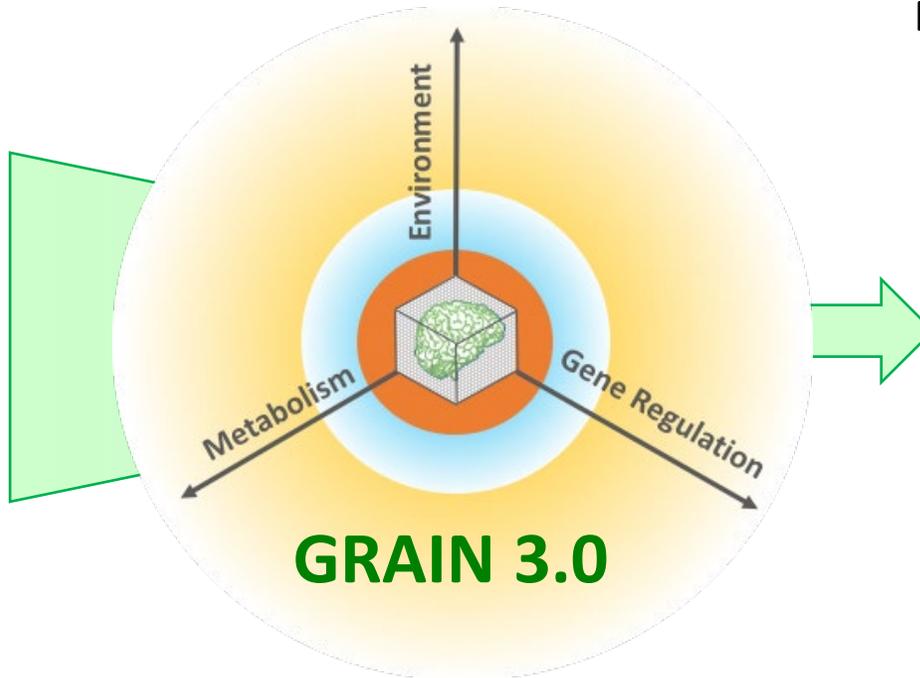
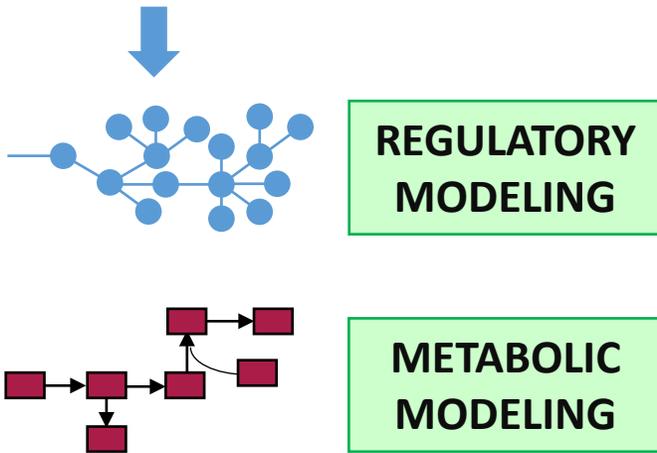
## Canola editing of C3007

- 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

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**Yield10**  
BIOSCIENCE

**QUESTIONS?**

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