



Yield10
BIOSCIENCE

Genome editing to increase seed yield and oil content

Kristi Snell
CSO and VP of Research
www.yield10bio.com

August 5th, 2019

PB19
PLANT BIOLOGY
AUGUST 3-7 | SAN JOSE, CA

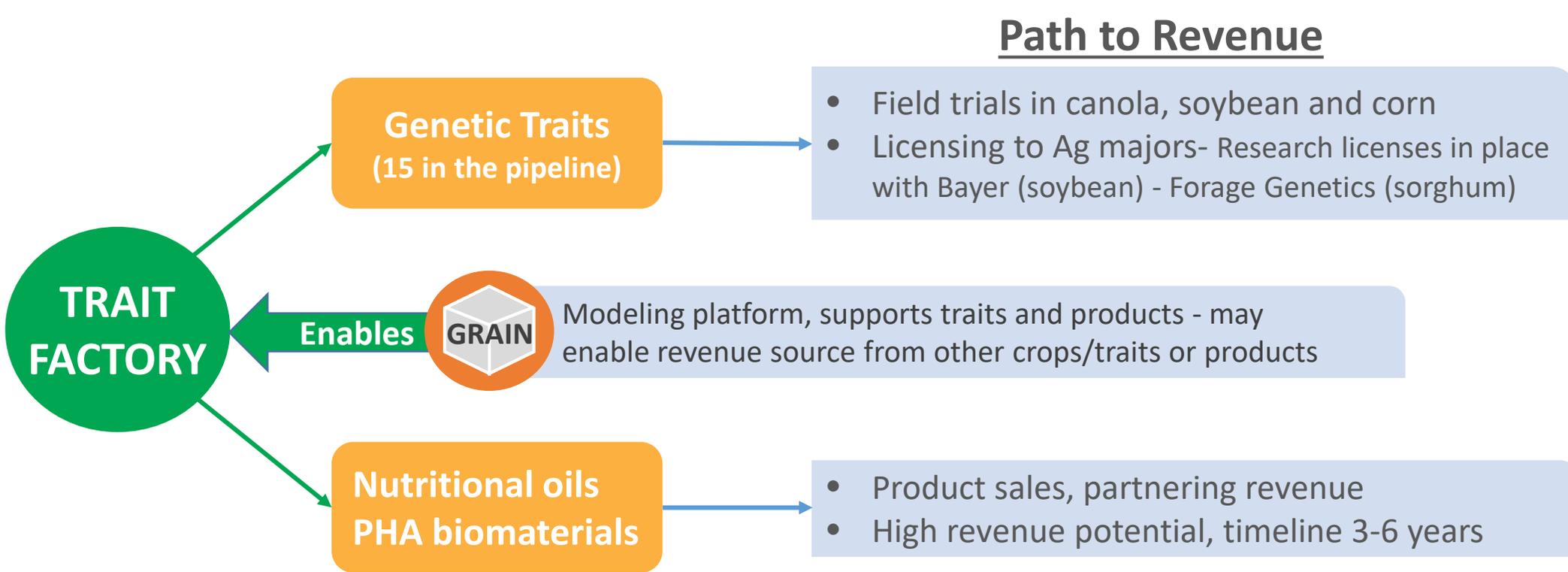


Safe Harbor Statement*

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, 2018 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.

Because forward-looking statements are inherently subject to risks and uncertainties, some of which cannot be predicted or quantified and may be beyond the Company’s control, you should not rely on these statements as predictions of future events. Actual results could differ materially from those projected due to our history of losses, lack of market acceptance of our products and technologies, the complexity of technology development and relevant regulatory processes, market competition, changes in the local and national economies, and various other factors. All forward-looking statements contained herein speak only as of the date hereof, and the Company undertakes no obligation to update any forward-looking statements, whether to reflect new information, events or circumstances after the date hereof or otherwise, except as may be required by law.

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



Multiple traits in development

Oilseed Crop Choice: Canola and *Camelina sativa*

Canola

- Commodity food oil crop, 22 million acres harvested (20 million tonnes) in Canada in 2018¹

Camelina

- Good platform for specialty/niche crops for high value products
 - specialty oils, biopolymers, products in food and animal feed
- Seed oil levels typically 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

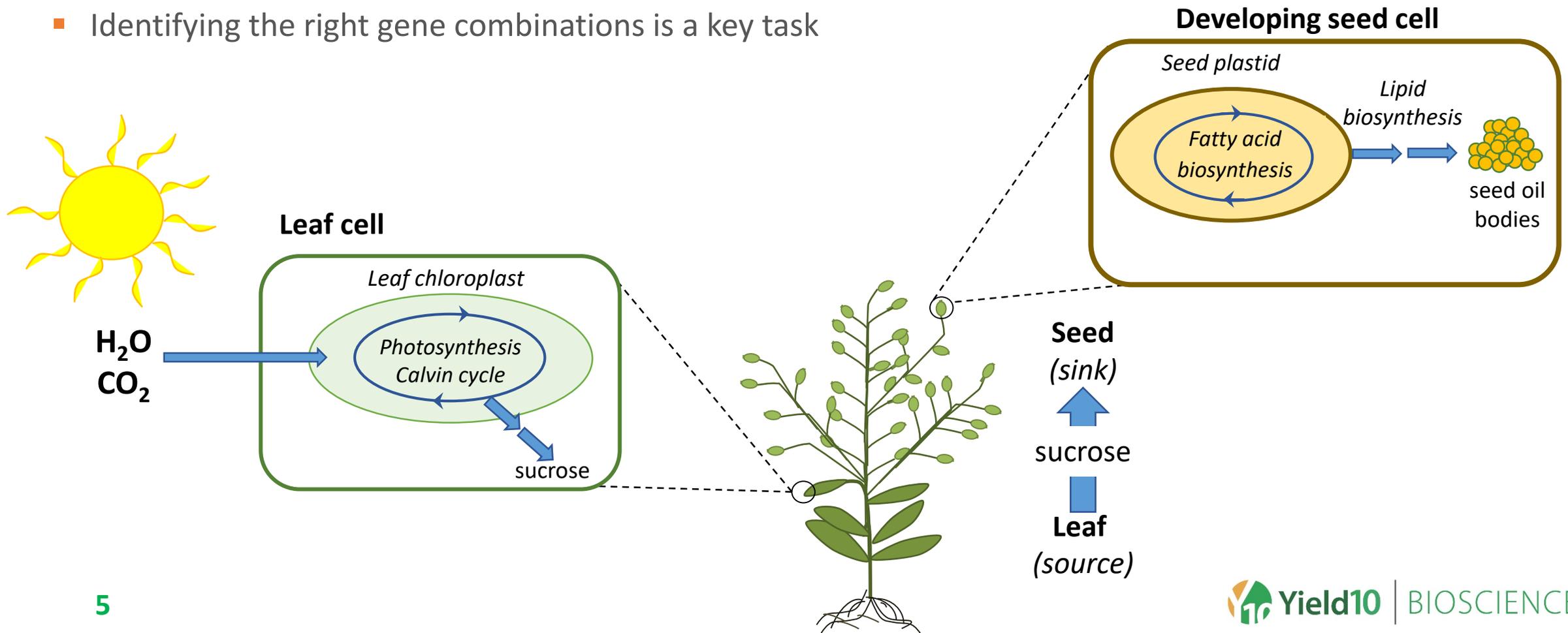
¹ <https://www.canolacouncil.org/markets-stats/statistics/harvest-acreage/>

² **Yield10 Camelina Review:** *Camelina sativa*, an oilseed at the nexus between model system and commercial crop.
Malik et al., Plant Cell Rep., 2018

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



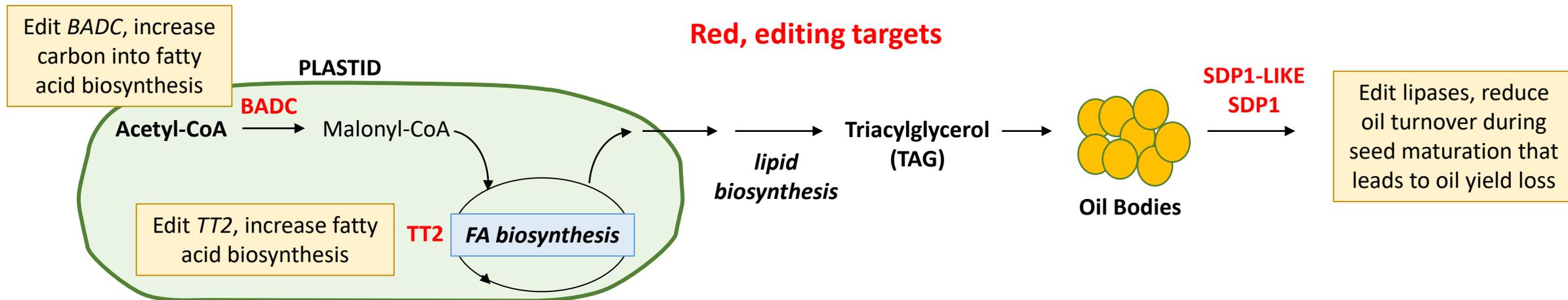
Traits for Boosting Seed Oil Content

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) and Canola (tetraploid)
- Obtain stable lines with homozygous edits
- Growth in greenhouse/growth chamber: preliminary snapshot of trait performance
- Submit “Am I Regulated Letter?” to USDA-APHIS to determine regulatory status of trait
- Field test to determine trait performance

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



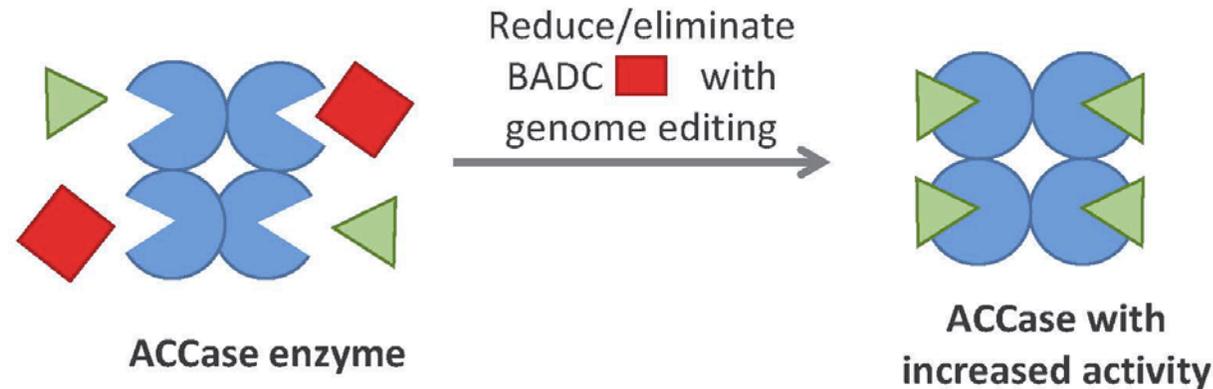
- **BADC** = biotin/lipoyl attachment domain containing protein - a negative regulator of acetyl-CoA carboxylase
- **TT2** = transparent testa2 - transcription factor that reduces expression of fatty acid biosynthetic genes
- **SDP1** (sugar-dependent 1 protein) and **SDP1-LIKE** (sugar-dependent 1-like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

Genome Editing Targets for Increasing Oil Content: BADC

- 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
- **Yield10 has an exclusive license to BADC from University of Missouri**



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

Genome Editing Targets for Increasing Oil Content: BADC

Previous work with BADC

- Jay Thelen Lab, University of Missouri: 3 *BADC* genes in Arabidopsis, seed specific gene silencing of *BADC1* increased oil per individual seed^{1,2}
- 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 obtained³
 - Best line *badc1 badc3* mutant, up to 30% more seed total fatty acids (% dry weight)

Progress at Yield10 and Metabolix Oilseeds

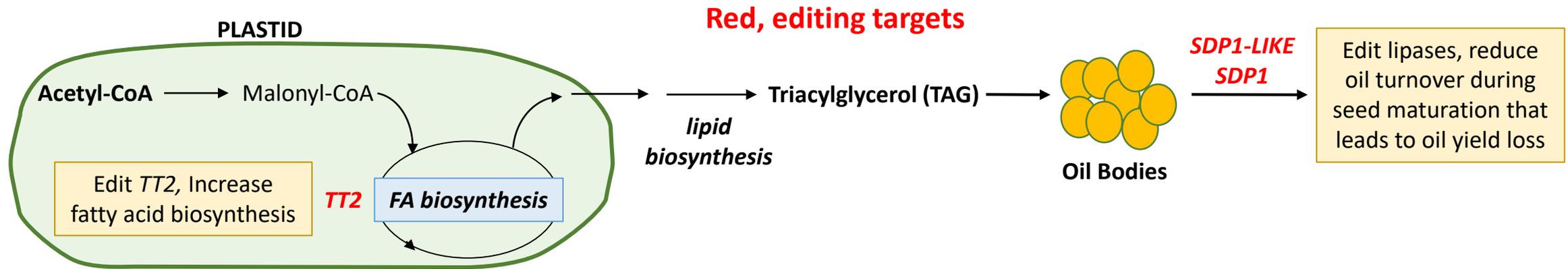
- Identified multiple *BADC* genes in Camelina and canola
 - Camelina 3 *BADC* genes (9 alleles total)
 - Canola 3 *BADC* genes (6 alleles total)
- Obtained stable edits for select *badc* genes/gene combinations in Camelina and canola
 - **Complete editing of all alleles was not obtained, possibly lethal**
- Edited camelina and canola lines are being characterized
- Next step, “Am I Regulated?” letter to USDA-APHIS prior to field trials

¹Salie et al., 2016, Plant Cell, 28, 2312; ²PCT/US2016/041386;

³Keereetawee et al., Plant Physiology, 2018, 177, 208

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^{1,2}
 - 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 size³
 - impaired oil breakdown during postgerminative growth, normal growth after photosynthesis established²
- Arabidopsis *sdp1-like* mutant, no growth phenotype²

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

³Kim 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 seeds¹

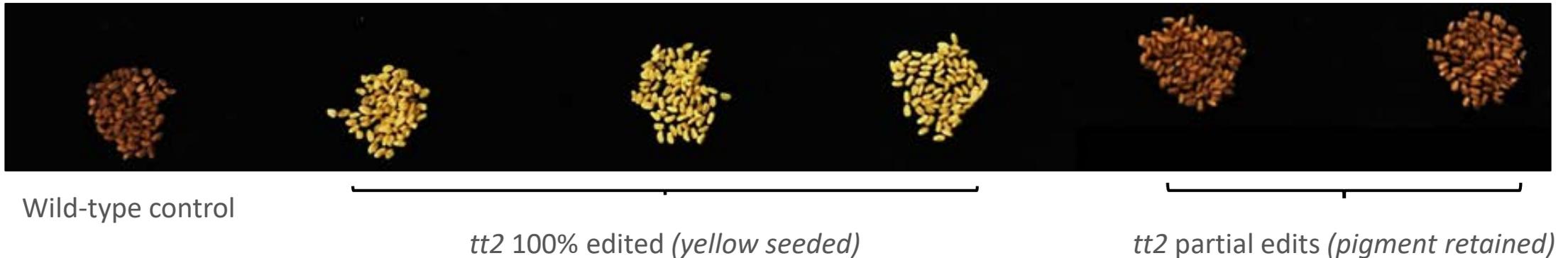
3. Multiplex Editing of *SDP1*, *SDP1-LIKE*, *TT2* at Yield10 and Metabolix Oilseeds

- 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, only 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
 - 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
 - Partial editing of *sdp1* (editing of 2 of 3 alleles, line type 1) provides 5% increase in total oil produced per plant

	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	- 4	+ 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*
- US field tests in progress

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



June 25, 2019



July 30, 2019

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

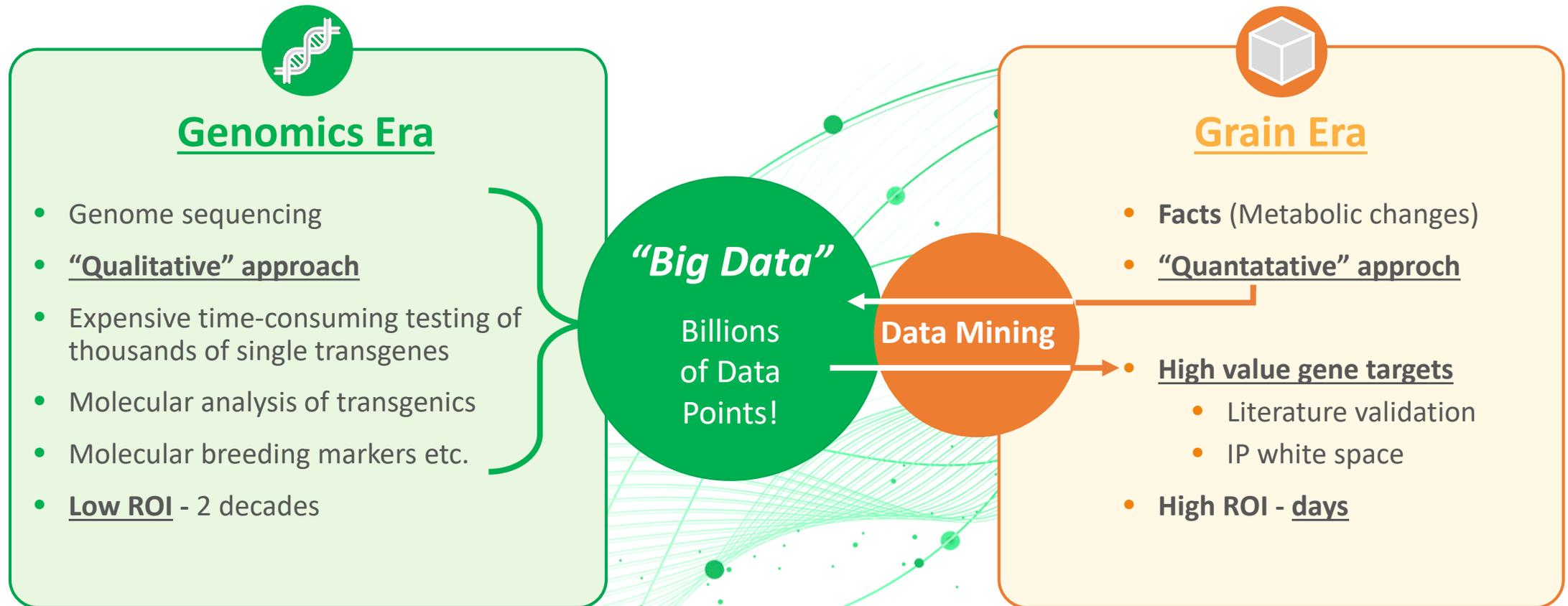
Line 3 = *sdp1* (100%), *sdp1-like* (100%), *tt2* (100%) edits

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

Line 4 = 100% *sdp1-like* edits

GRAIN 3.0 (Gene Ranking Artificial Intelligence Network)

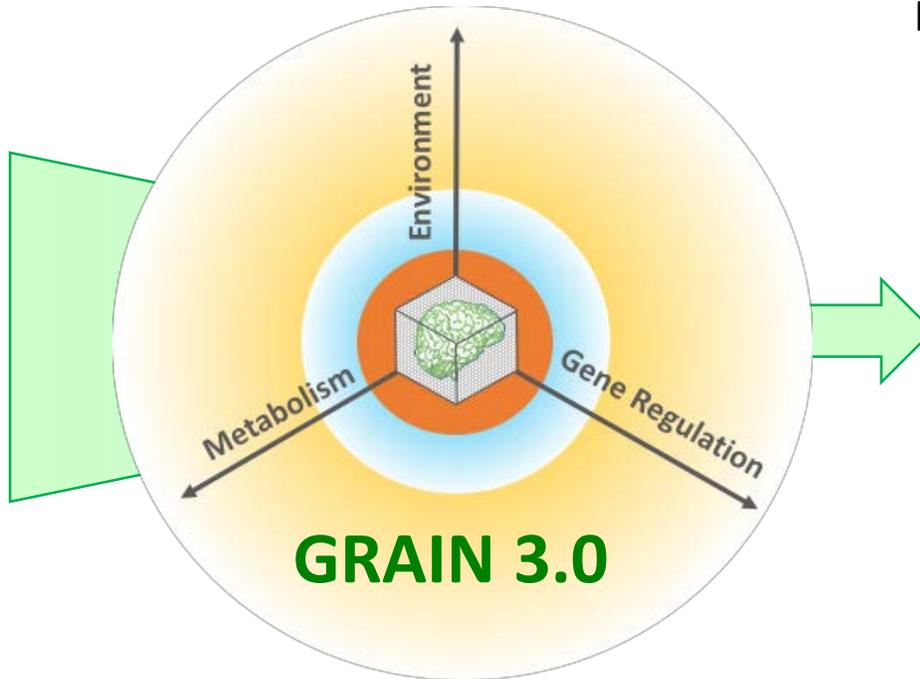
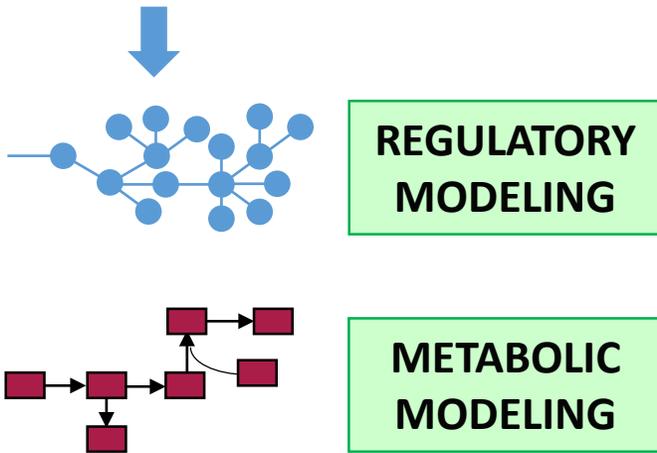
- On some level a plant phenotype or trait reflects altered metabolism due to genetic variation
- GRAIN uses metabolism to rationally mine genomics data



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

Identification of WRI1 and MYB56 Validates Approach

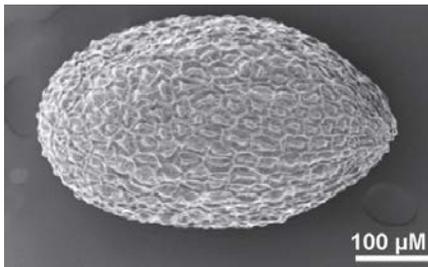


WRINKLED1, A Ubiquitous Regulator in Oil Accumulating Tissues from *Arabidopsis* Embryos to Oil Palm Mesocarp

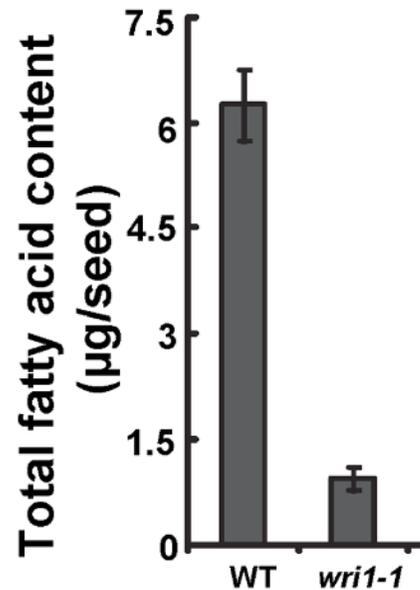
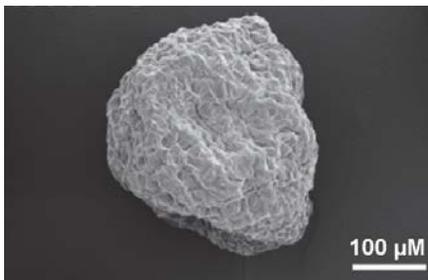
Wei Ma , Que Kong, Vincent Arondel, Aruna Kilaru, Philip D. Bates, Nicholas A. Thrower, Christoph Benning, John B. Ohlrogge

Published: July 26, 2013 • <https://doi.org/10.1371/journal.pone.0068887>

Wild-type seed



Wri1 mutant seed



Research Article

MYB56 Encoding a R2R3 MYB Transcription Factor Regulates Seed Size in *Arabidopsis thaliana*

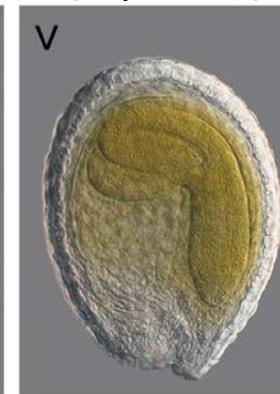
Yanjie Zhang, Wanqi Liang, Jianxin Shi, Jie Xu, Dabing Zhang 

First published: 03 August 2013 | <https://doi.org/10.1111/jipb.12094> |

Wild-type seed



Mutant (myb56-1)



Mutant (myb56-2)



Scale bars = 100 μM

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

Methods are needed to increase seed yield and/or seed oil content to maximize oil yields per acre

- Yield10 is creating 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)

Significant increases in seed size and/or seed oil content can impact overall seed yield

- Edited *sdp1*, *sdp1-like*, and *tt2* lines significantly shifted carbon partitioning to oil at expense of total seed
 - *Tradeoff suggests not enough carbon or reducing power to significantly increase oil content AND produce normal number of seeds*
- Opportunity exists to further engineer lines 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



Yield10
BIOSCIENCE

QUESTIONS?

A large photograph of a soybean field with rows of green plants under a blue sky with clouds. A large white '10' is overlaid on the bottom right corner of the image.

10