



Genome editing to increase seed yield and oil content

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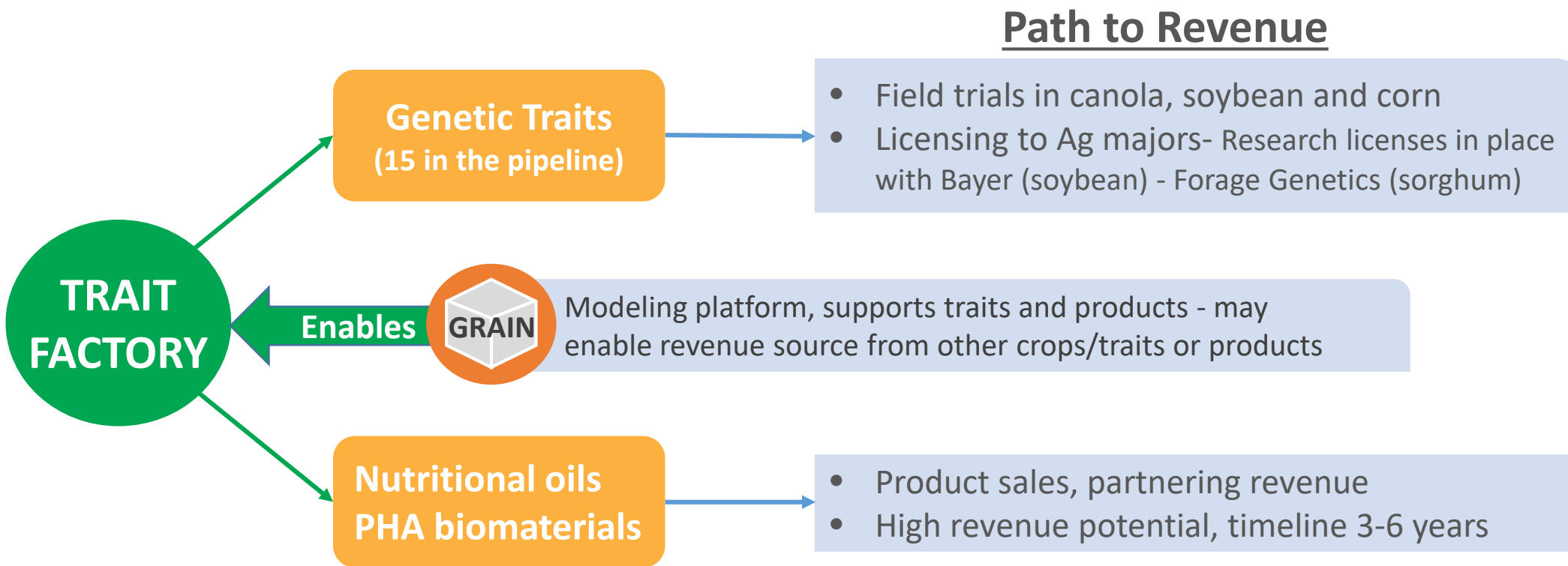


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Bayer CropScience



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

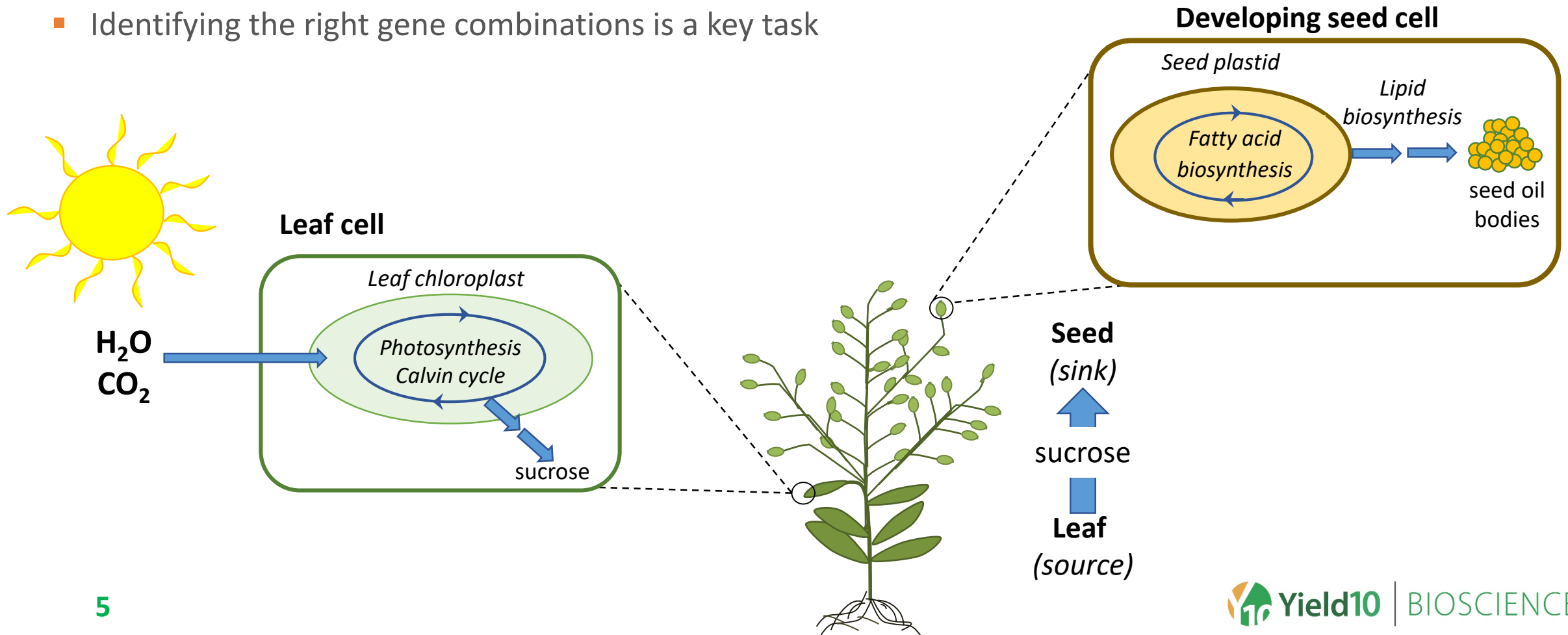
¹ <https://www.canolacouncil.org/markets-stats/statistics/harvest-acreage/>

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



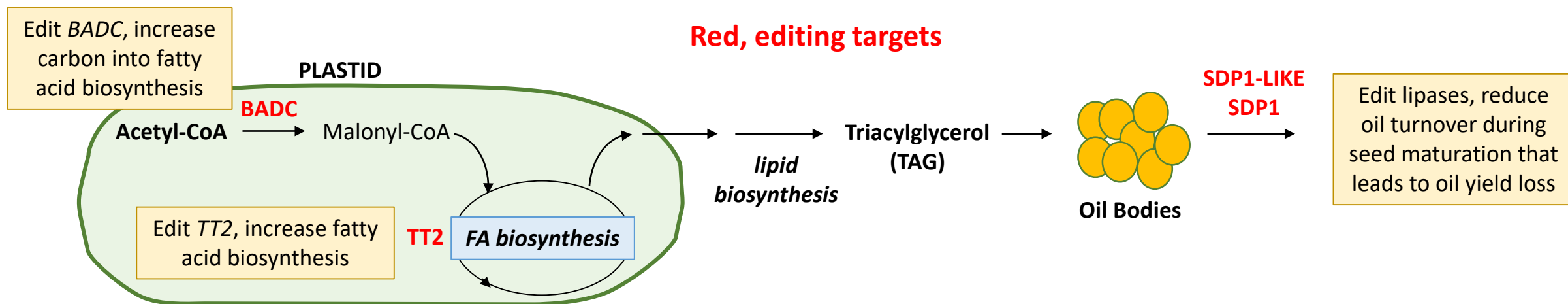
Traits for Boosting Seed Oil Content

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



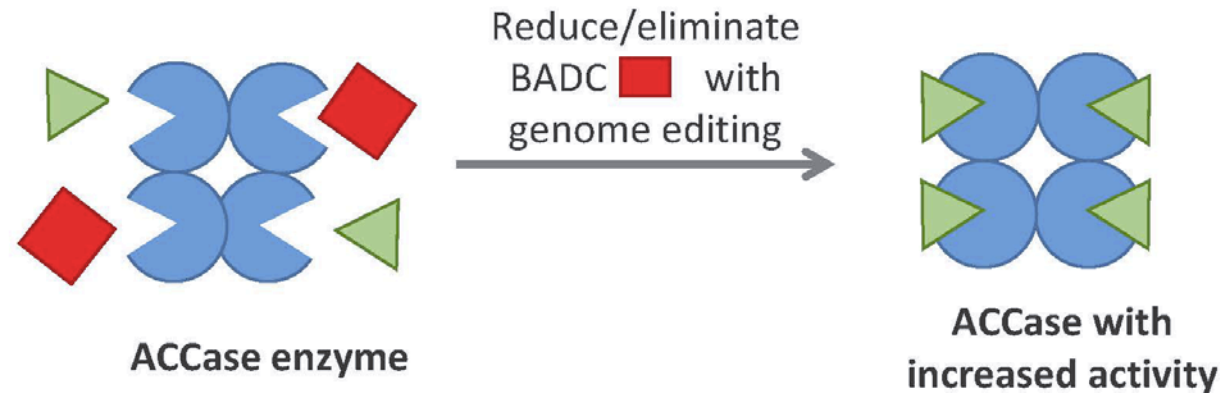
- **BADC** = biotin/lipoyl attachment domain containing protein - a negative regulator of acetyl-CoA carboxylase
- **TT2** = transparent testa2 - transcription factor that reduces expression of fatty acid biosynthetic genes
- **SDP1** (sugar-dependent 1 protein) and **SDP1-LIKE** (sugar-dependent 1-like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

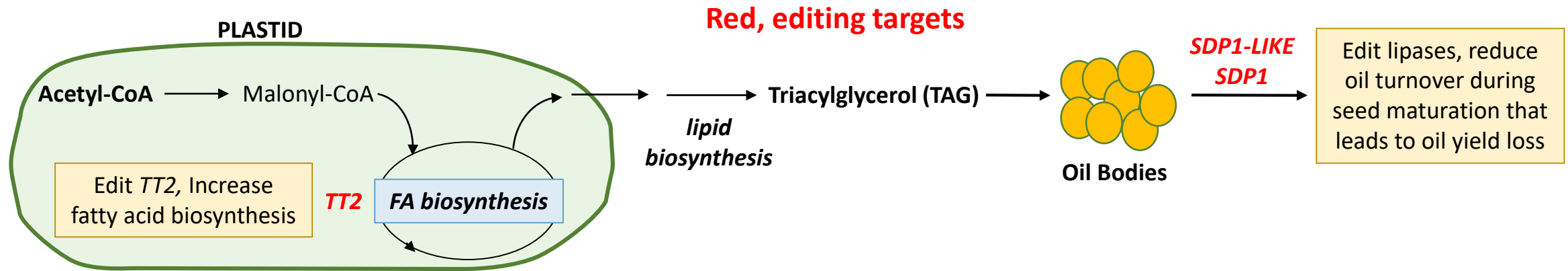
- Identified multiple *BADC* genes in Camelina and canola
 - Camelina 3 *BADC* genes (9 alleles total)
 - Canola 3 *BADC* genes (6 alleles total)
- Obtained stable edits for select *badc* genes/gene combinations in Camelina and canola
 - **Complete editing of all alleles was not obtained, possibly lethal**
- 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.

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 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

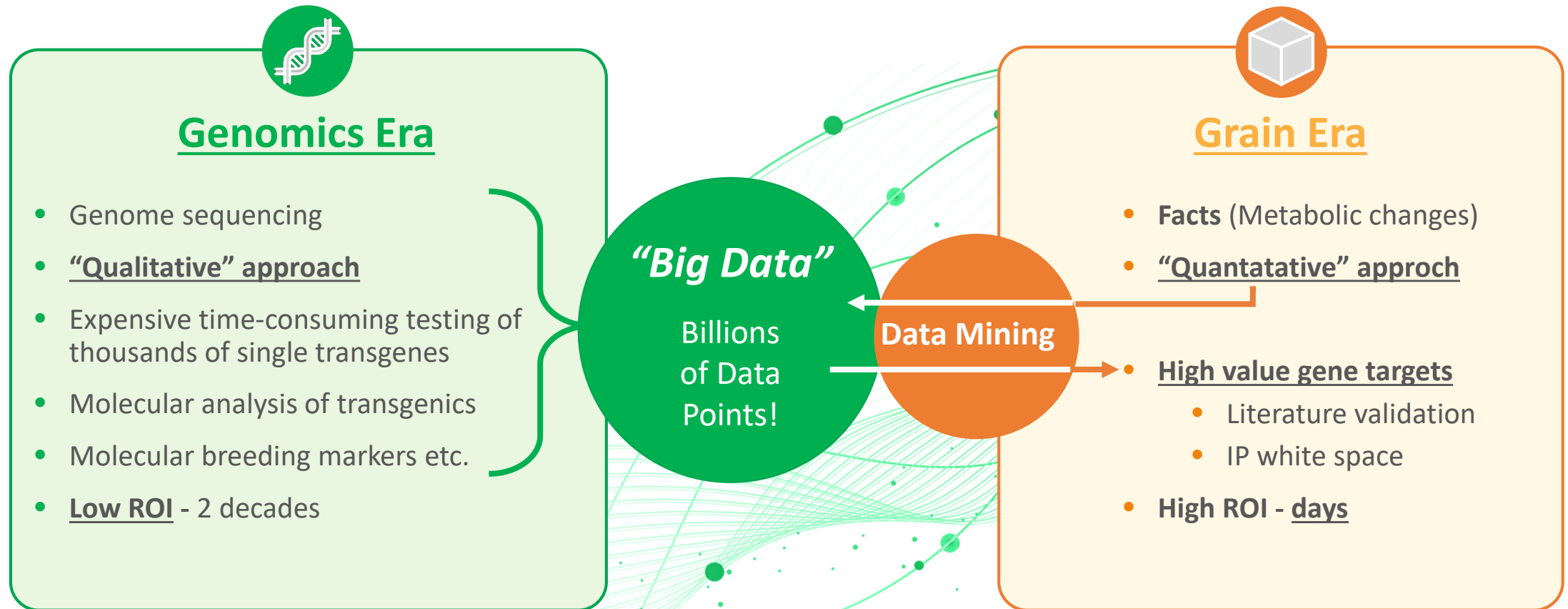
Line 3 = *sdp1* (100%), *sdp1-like* (100%), *tt2* (100%) edits

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

Line 4 = 100% *sdp1-like* edits

GRAIN 3.0 (Gene Ranking Artificial Intelligence Network)

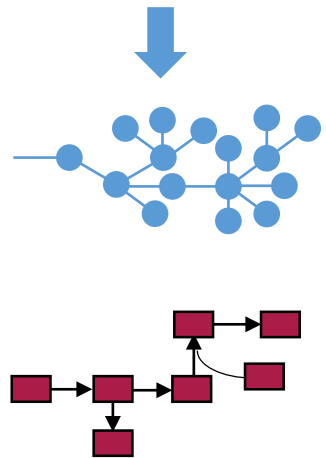
- On some level a plant phenotype or trait reflects altered metabolism due to genetic variation
- GRAIN uses metabolism to rationally mine genomics data



GRAIN 3.0: Using Metabolism to Rationally Mine Genomics Data

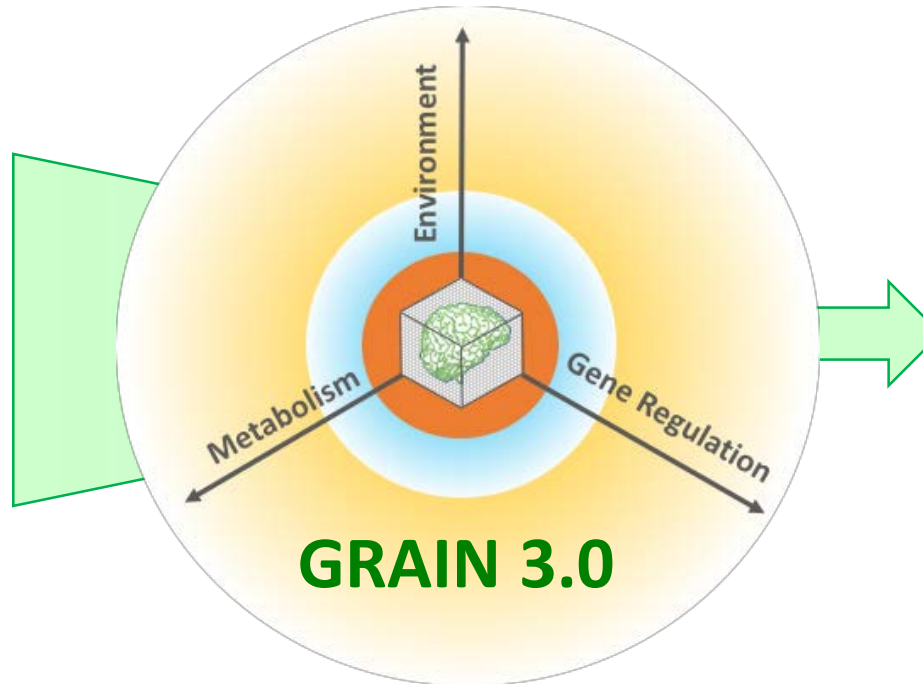
Combined regulatory and metabolic modeling components to identify transcription factors (TFs) to increase seed and oil yield in *Camelina*

Camelina Genomics Data



REGULATORY
MODELING

METABOLIC
MODELING



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

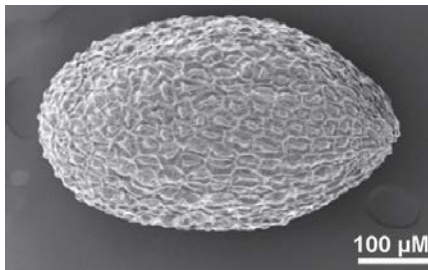


WRINKLED1, A Ubiquitous Regulator in Oil Accumulating Tissues from *Arabidopsis* Embryos to Oil Palm Mesocarp

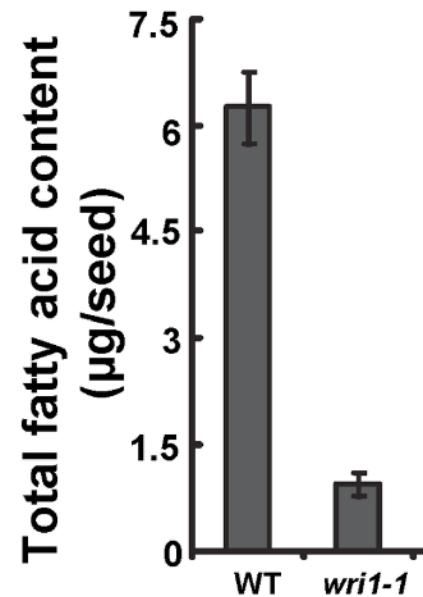
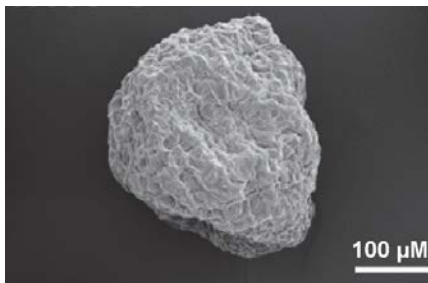
Wei Ma , Que Kong, Vincent Arondel, Aruna Kilaru, Philip D. Bates, Nicholas A. Thrower, Christoph Benning, John B. Ohlrogge

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*

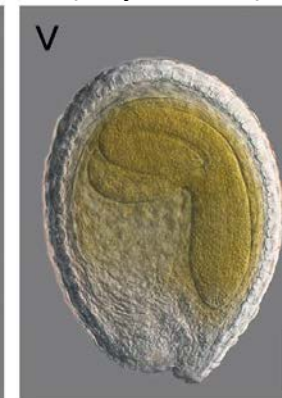
Yanjie Zhang, Wanqi Liang, Jianxin Shi, Jie Xu, Dabing Zhang 

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



QUESTIONS?

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