Yield10 BIOSCIENCE

Crop Trait Development: Target Selection and Validation using the GRAIN Platform

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

November 5th, 2019

7TH PLANT GENOMICS & GENE EDITING CONGRESS: USA
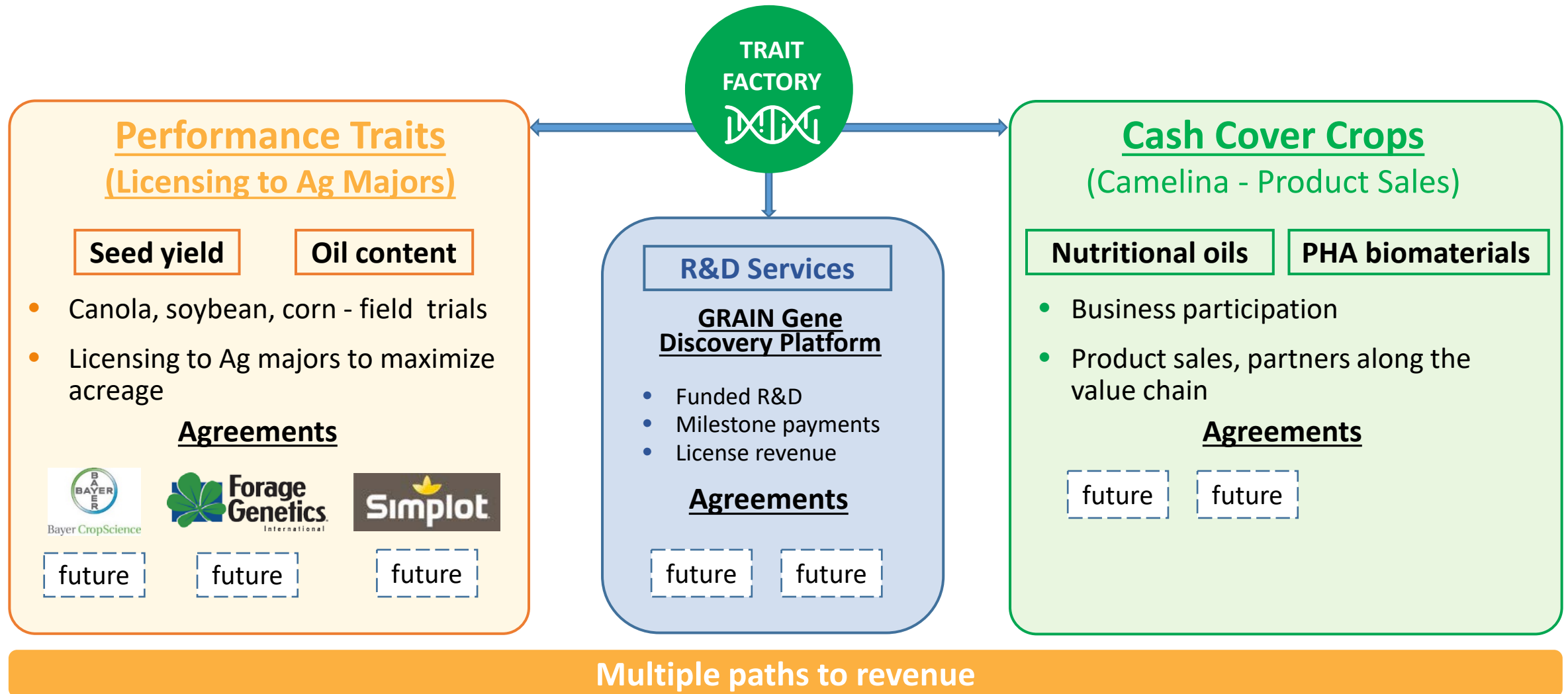### RALEIGH, USA
November 4-5 2019

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

Yield10 | BIOSCIENCE

Trait Factory – Products and Path to Revenue

Three potential revenue streams each with different commercialization paths

TRAIT FACTORY

Performance Traits
(Licensing to Ag Majors)

Seed yield	Oil content

- Canola, soybean, corn - field trials
- Licensing to Ag majors to maximize acreage

Agreements

Bayer CropScience   Forage Genetics International   Simplot

future	future	future

R&D Services

GRAIN Gene Discovery Platform

- Funded R&D
- Milestone payments
- License revenue

Agreements

future	future

Cash Cover Crops
(Camelina - Product Sales)

Nutritional oils	PHA biomaterials

- Business participation
- Product sales, partners along the value chain

Agreements

future	future

Multiple paths to revenue

Yield10 | BIOSCIENCE

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
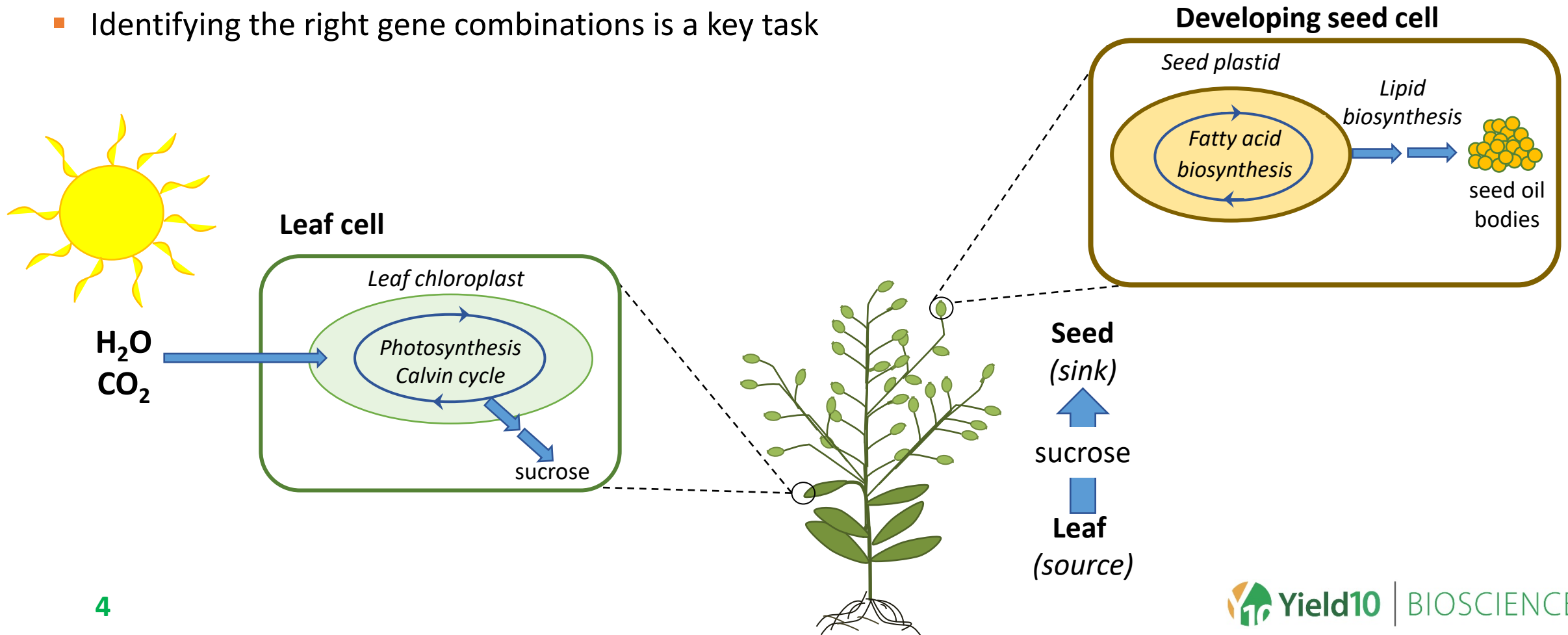
Canola

- Commodity food oil crop, 22 million acres harvested (20 million tonnes) in Canada in 2018[1]

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/

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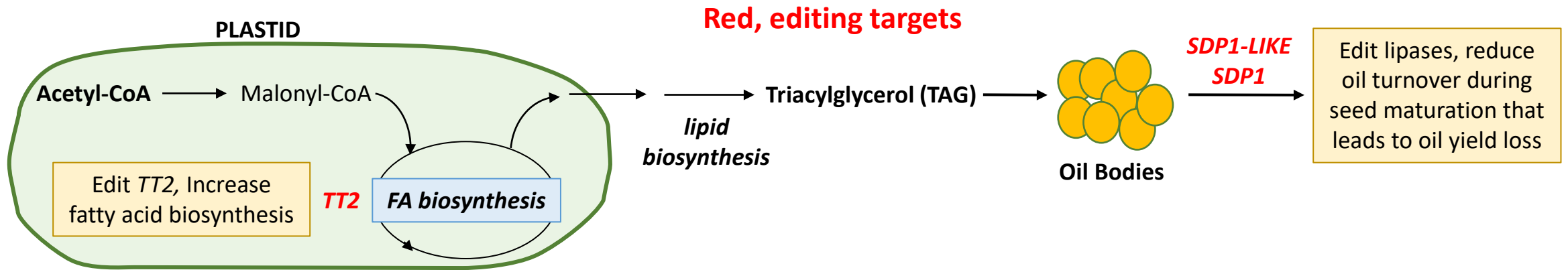model system and commercial crop. Malik et al., Plant Cell Rep., 2018

Yield10 | BIOSCIENCE

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 "Am I Regulated Letter?" to USDA-APHIS to determine regulatory status of trait

- Field test to determine trait performance

Yield10 | BIOSCIENCE

Editing of lipase genes (*SDP1, SDP1-LIKE*) and transparent testa2 (*TT2*) gene[1]

Red, editing targets

PLASTID

Acetyl-CoA → Malonyl-CoA → lipid biosynthesis → **Triacylglycerol (TAG)** → Oil Bodies → *SDP1-LIKE SDP1* → Edit lipases, reduce oil turnover during seed maturation that leads to oil yield loss

Edit *TT2*, Increase fatty acid biosynthesis

TT2 **FA biosynthesis**

1. Transparent testa2 (TT2) transcription factor target to upregulate fatty acid biosynthesis

- Negative regulation of embryo fatty acid biosynthetic enzymes
- Positive regulation of proanthocyanidin enzymes in seed coat

2. 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[2,3]
    - 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

Yield10 | BIOSCIENCE

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

- 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 *tt2* gene, loss of pigmentation in seed coat due to reduced expression of enzymes for proanthocyanidins

  - Unique distinction to track edited seed

Wild-type control   *tt2* 100% edited *(yellow seeded)*   *tt2* partial edits *(pigment retained)*

Yield10 | BIOSCIENCE

- **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

  - Further 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
	sdp1	*sdp1-like*	*tt2*					
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

X = edited allele   *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*
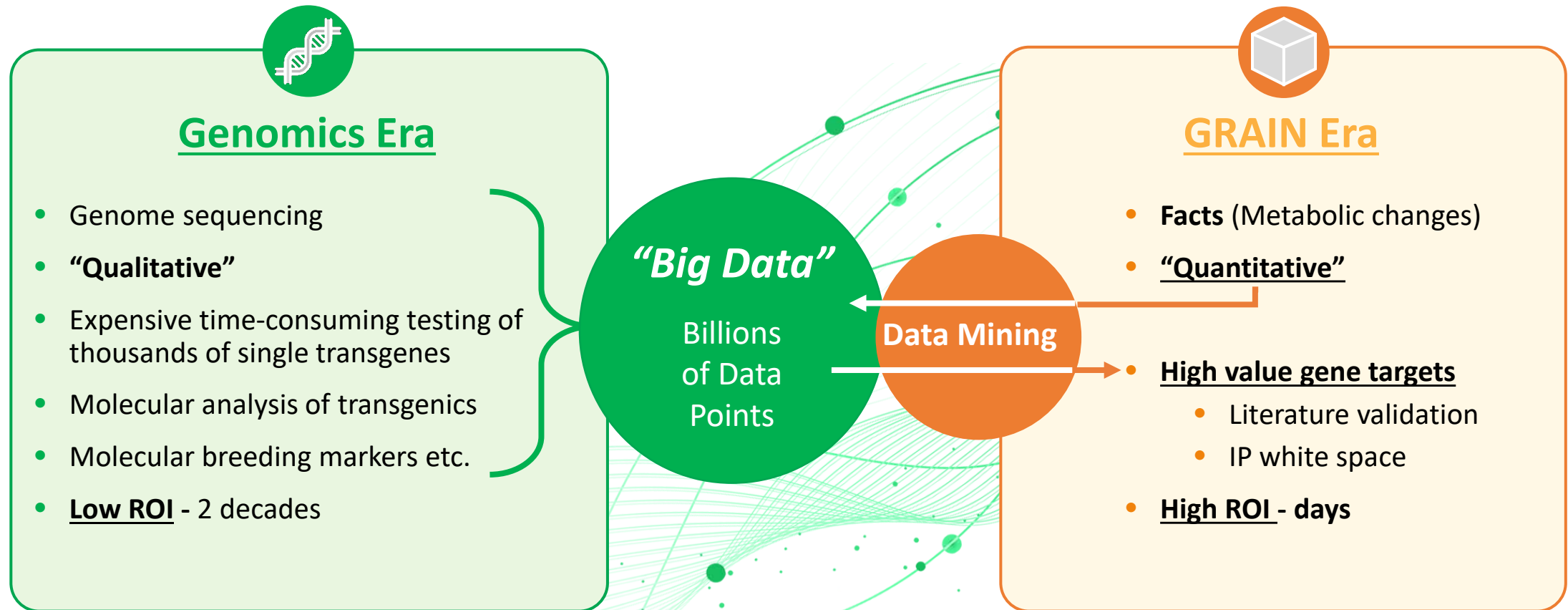
- Lines in field tests in 2019

  - Received 2018 confirmation that USDA-APHIS does not consider lines to be regulated pursuant to 7 CFR part 340*
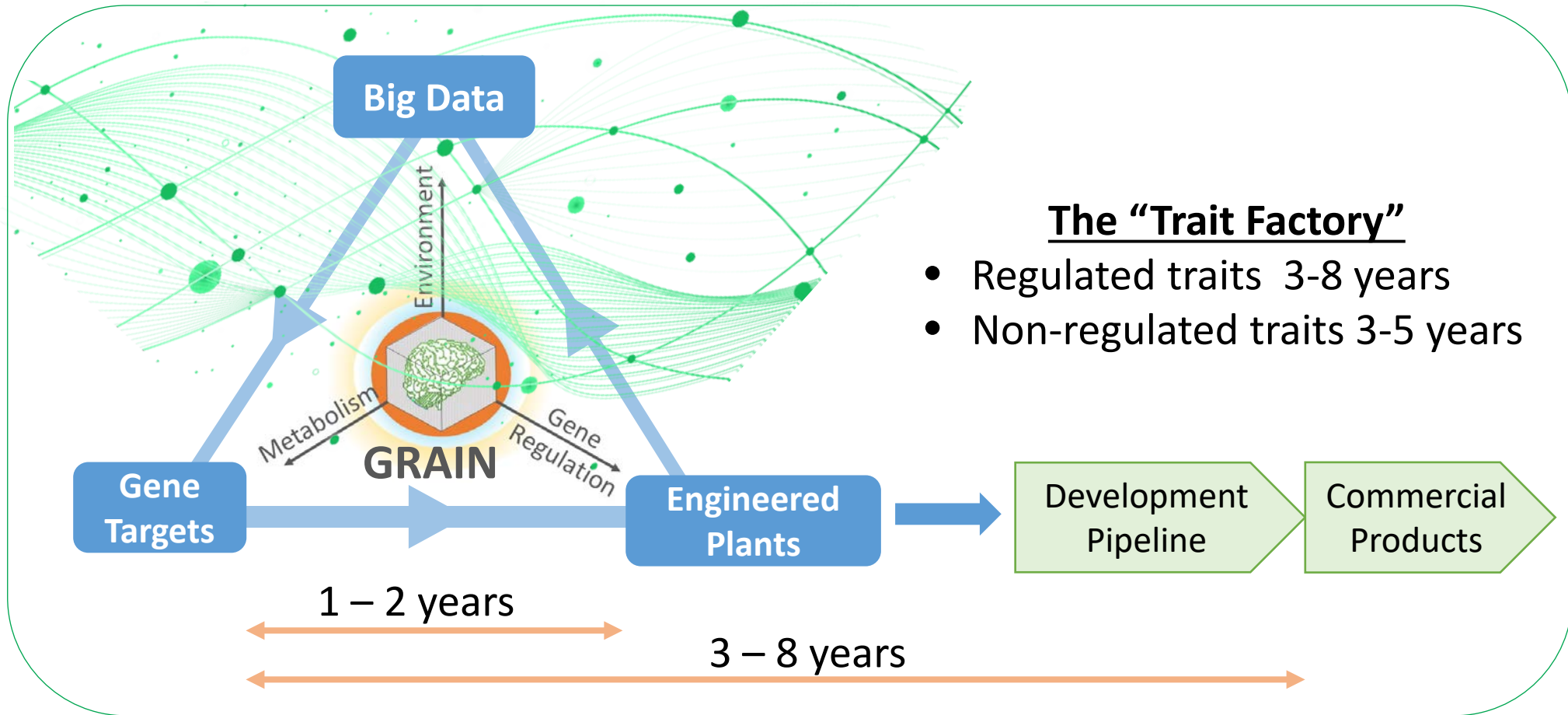
- **Opportunity exists to further engineer edited lines to increase seed yield**

Yield10 | BIOSCIENCE

- On some level a plant phenotype or trait reflects altered metabolism due to genetic variation
- GRAIN *(Gene Ranking Artificial Intelligence Network)* uses metabolism to rationally mine genomics data
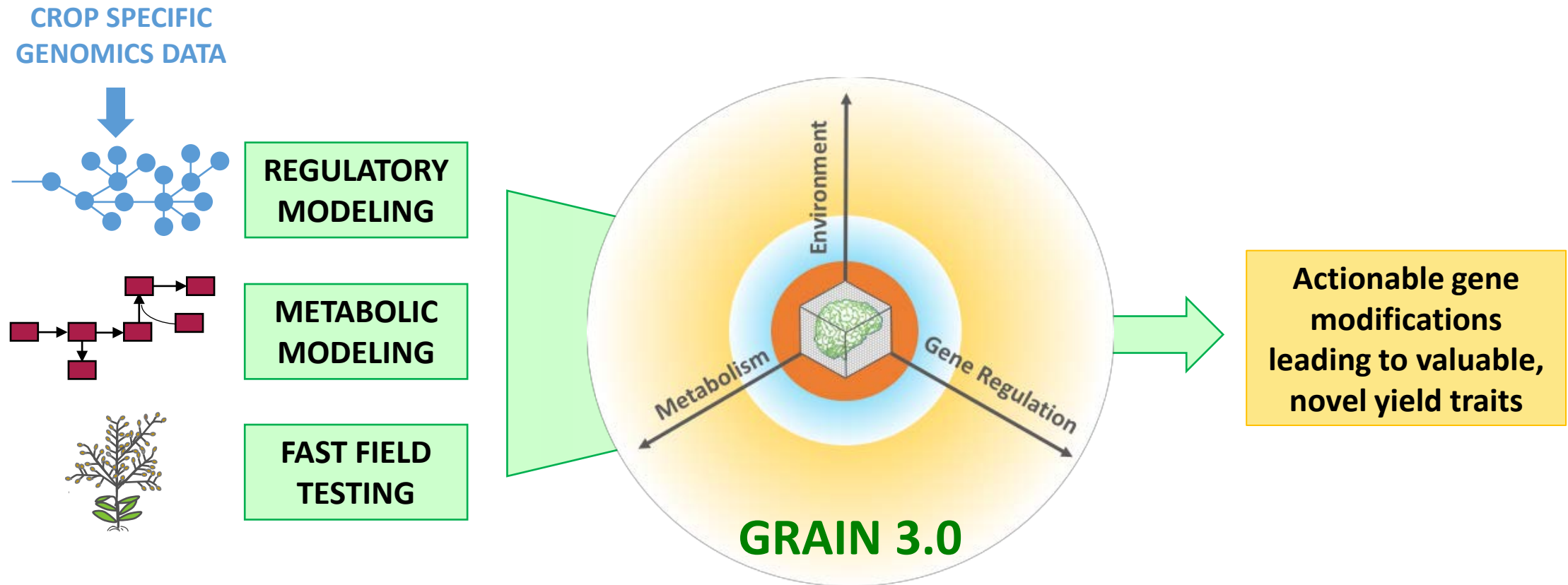
Genomics Era

- Genome sequencing
- **"Qualitative"**
- Expensive time-consuming testing of thousands of single transgenes
- Molecular analysis of transgenics
- Molecular breeding markers etc.
- **Low ROI** - 2 decades

"Big Data"
Billions of Data Points

Data Mining

GRAIN Era

- **Facts** (Metabolic changes)
- **"Quantitative"**
- **High value gene targets**
  - Literature validation
  - IP white space
- **High ROI - days**

Yield10 | BIOSCIENCE

The "Trait Factory"
- Regulated traits 3-8 years
- Non-regulated traits 3-5 years

Big Data

Environment

GRAIN

Metabolism

Gene Regulation

Gene Targets

Engineered Plants

Development Pipeline

Commercial Products

1 – 2 years

3 – 8 years

- Genome-editing is a key tool to deploy new traits and unlock value created using GRAIN

CROP SPECIFIC GENOMICS DATA

REGULATORY MODELING

METABOLIC MODELING

FAST FIELD TESTING

GRAIN 3.0

Environment

Metabolism

Gene Regulation

Actionable gene modifications leading to valuable, novel yield traits
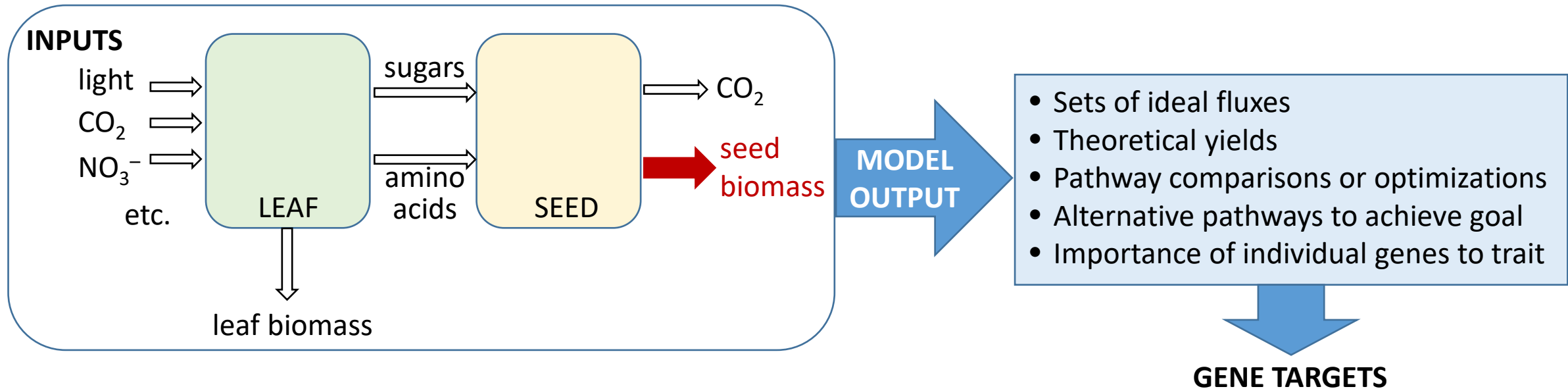
- Regulatory and metabolic modeling components, each component can generate its own set of gene targets
- Combining components provides unique tool where metabolic information can inform genomics decisions

12

Yield10 | BIOSCIENCE

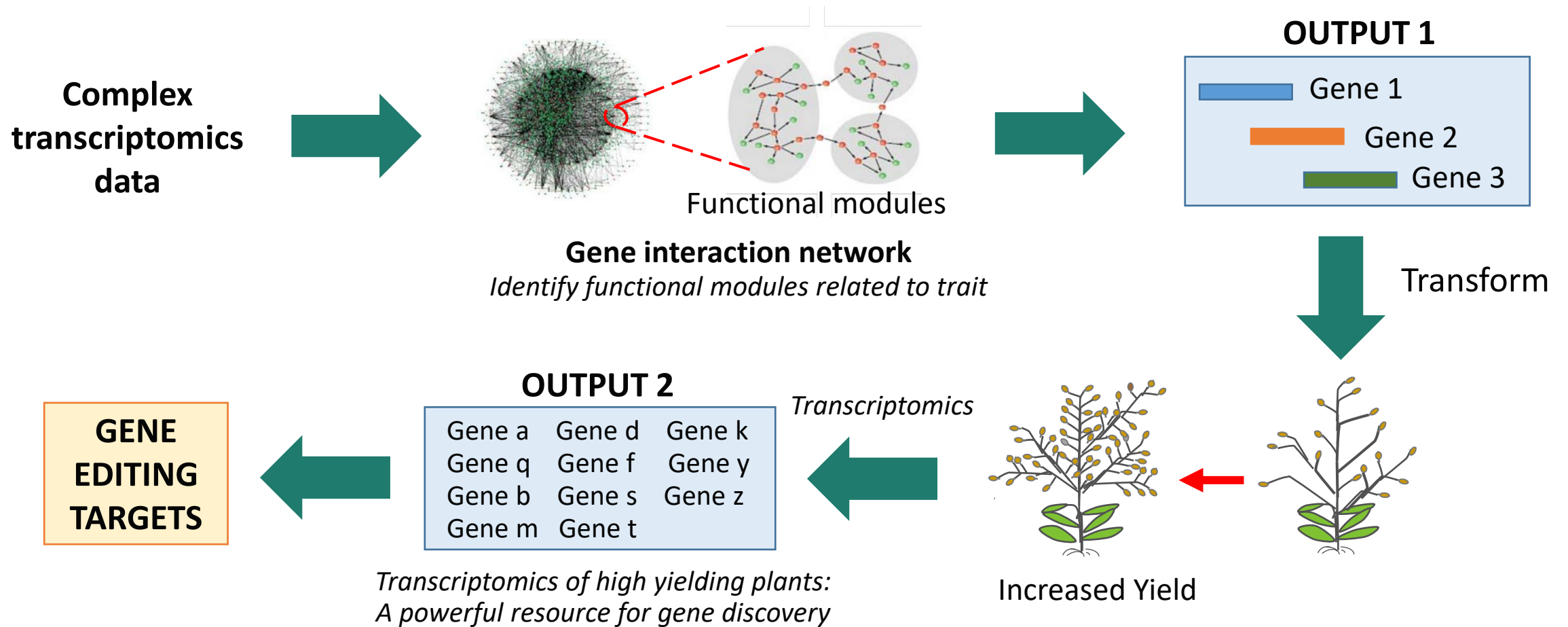## Metabolic modeling component based on flux balance analysis

- Contains all enzymatic reactions, transport functions, and cofactors (ATP, ADP, NADPH….) in plant system

- Incorporates thermodynamic information to avoid solutions that are thermodynamically unfavorable

- Define problem (e.g. maximize seed biomass production)

METABOLIC MODEL

INPUTS

light ⇒
CO_2 ⇒
NO_3^- ⇒
etc.

LEAF —sugars→ **SEED** —→ CO_2

LEAF —amino acids→ SEED ⟹ **seed biomass**

LEAF ⇒ leaf biomass

MODEL OUTPUT ⟹

- Sets of ideal fluxes
- Theoretical yields
- Pathway comparisons or optimizations
- Alternative pathways to achieve goal
- Importance of individual genes to trait

⟹ **GENE TARGETS**

Yield10 Review Paper on Metabolic Engineering in Plants: Skraly et al., Metabolic engineering to increase crop yield: From concept to execution, 2018, Plant Science

Yield10 | BIOSCIENCE

Transcriptome-based regulatory association networks convert big data into actionable gene targets

Complex transcriptomics data

Functional modules

Gene interaction network
Identify functional modules related to trait

OUTPUT 1

Gene 1
Gene 2
Gene 3

Transform

OUTPUT 2

Gene a	Gene d	Gene k
Gene q	Gene f	Gene y
Gene b	Gene s	Gene z
Gene m	Gene t	

Transcriptomics

GENE EDITING TARGETS

Transcriptomics of high yielding plants: A powerful resource for gene discovery

Increased Yield

Yield10 transcriptome-based regulatory association network proof of concept in switchgrass. Ambavaram et al., 2018, Plant Science, 273, 100.

Yield10 | BIOSCIENCE

Combined modeling components to identify transcription factors (TFs) to increase oil and/or seed yield in industrial oilseed *Camelina sativa*

Camelina Genomics Data

REGULATORY MODELING

METABOLIC MODELING

GRAIN 3.0
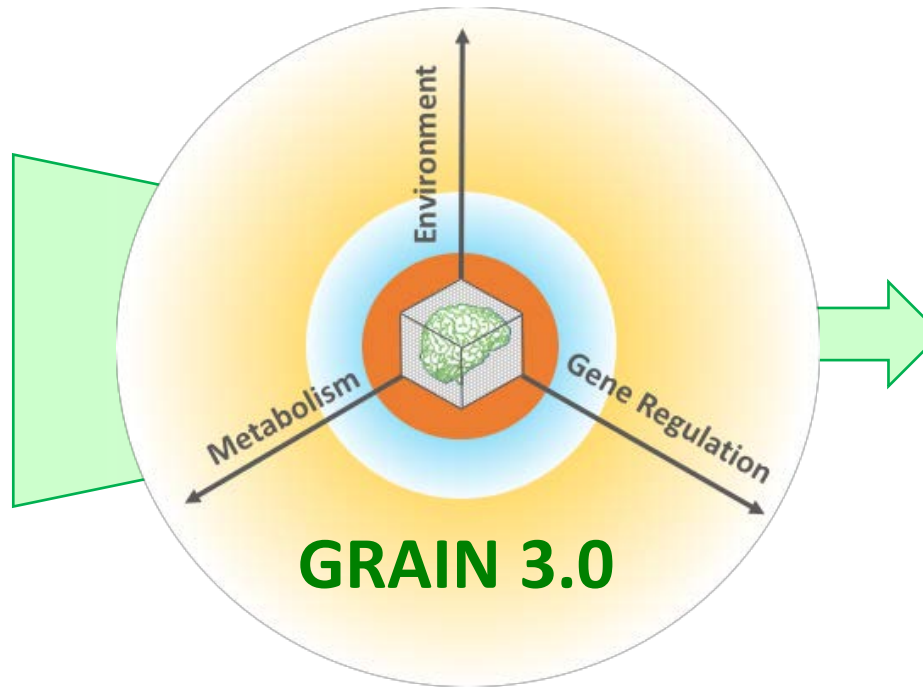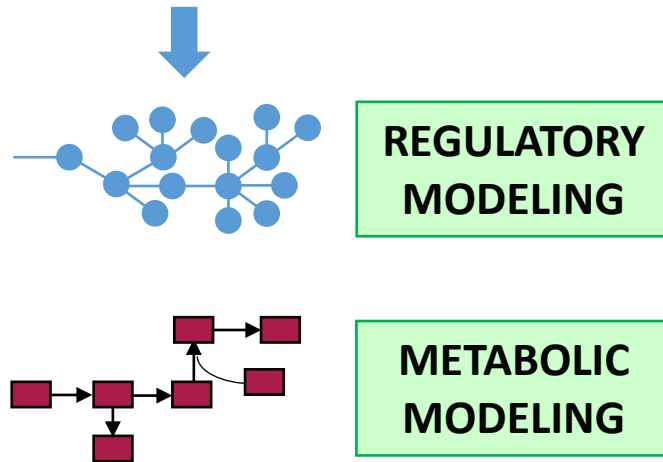
Prioritized List of Gene Targets

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
- **Many uncharacterized genes identified, IP white space**
- Currently pursuing multiple uncharacterized TFs
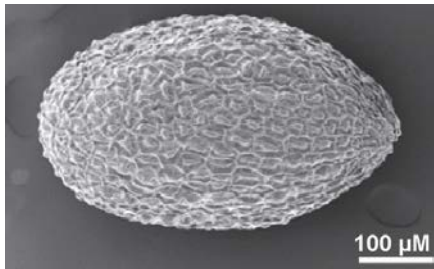
Yield10 | BIOSCIENCE

PLOS ONE

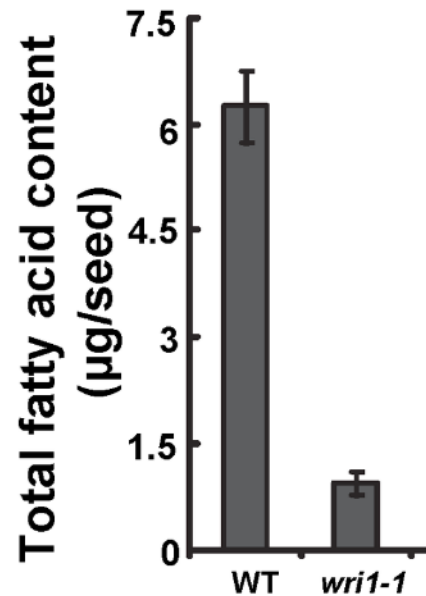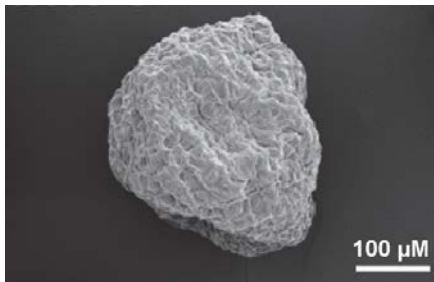**WRINKLED1, A Ubiquitous Regulator in Oil Accumulating Tissues from *Arabidopsis* Embryos to Oil Palm Mesocarp**

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

JIPB / Journal of Integrative Plant Biology
Serving the Plant Science community Since 1952 (Formerly Acta Botanica Sinica)

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

16

Yield10 | BIOSCIENCE

Conclusions

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

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

- Yield10 is using GRAIN platform to identify gene targets for traits, including increased oil content/seed yield
- GRAIN 3.0 uses metabolism to rationally mine genomics data to identify unique genes

Case study of Camelina line with multiple genome edits to increase oil yields

- Edited *SDP1, SDP1-LIKE*, and *TT2* gene targets, additional targets in progress
  - Significant increases in seed size and/or seed oil content can impact overall seed yield
  - Edited *sdp1, sdp1-like*, and *tt2* lines shifted carbon partitioning to oil at expense of total seed
  - *Suggests not enough carbon/reducing power to 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

Potential to combine (stack) edits with oil composition traits (e.g. high oleic, omega fatty acids)

Yield10 | BIOSCIENCE

QUESTIONS?

For more information visit www.yield10bio.com