Exploring Novel Approaches for Gene Target Selection & Trait Validation in Crops

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*Under the Private Securities Litigation Reform Act of 1995
“Yield10 develops high value seed yield traits for the agriculture and food industries”

- Efficiently developing superior traits for corn, soy, canola, wheat, rice and camelina
- The “Trait Factory” leverages 26 years of Met-Eng investment/achievements
- Optimizing photosynthesis and carbon efficiency to increase grain yield
- Strong leadership and R&D team, operates in Boston, USA and Saskatoon, Canada
Yield10 combines advanced discovery with genome editing to develop valuable traits.

TODAY
- Increased seed yield
- Increased oil content
- Improved stress resistance
- Increased biomass yield

TOMORROW
- Specialty oils and PHA biomaterials
- Winter cover crops

FUTURE
- Elimination of synthetic chemicals
- Disruptive seed sector business models
GRAIN, A Component of The Trait Factory

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

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 - ?

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Today

GRAIN: A Design-Build-Test-Learn Cycle for Traits

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

The “Trait Factory”
- Regulated traits 3-8 years
- Non-regulated traits 3-5 years
Increasing Crop Yield Will Require Gene Combinations

Step-change increases in seed yield will likely require increased photosynthetic carbon fixation and delivery of the increased fixed carbon to the seed

- Identifying the right combination of genes is a key task
CROP SPECIFIC GENOMICS DATA

- 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

GRAIN 3.0

Actionable gene modifications leading to valuable, novel yield traits
Metabolic Modeling Component

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)

**INPUTS**
- light
- CO₂
- NO₃⁻
- etc.

**LEAF**
- sugars
- amino acids

**SEED**
- CO₂
- seed 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
Overexpression of novel carbon fixation pathway in *Camelina sativa* increases seed yield

- Modeling predicts **112%** increase in maximum theoretical seed yield with novel pathway under photorespiratory conditions
- Experimental results show increase up to **128%**

**Camelina greenhouse study: Seed yield in best plants**

- **Control**
- **JS11** +128%
- **LX03** +73%
- **LG08** +116%
- **LU03**

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

**Equation:** 

NET: $2 \text{CO}_2 + 2 \text{HCO}_3^- \rightarrow \text{oxaloacetate}$
Transcriptome-based regulatory association networks convert big data into actionable gene targets

Complex transcriptomics data

Gene interaction network
 Identify functional modules related to trait

OUTPUT 1
Gene 1
Gene 2
Gene 3

OUTPUT 2
Gene a
Gene q
Gene b
Gene m
Gene d
Gene f
Gene s
Gene t
Gene k
Gene y
Gene z

Transform

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

Increased Yield
Regulatory Modeling Validation: Proof Points Achieved

Work with switchgrass

- Identify candidate global regulatory genes to increase photosynthesis and biomass yield

Functional modules of interest for increased biomass yield in switchgrass are enriched in genes related to photosynthesis and central carbon metabolism.
Transgenic plants produced
- *genes expressed from strong promoter active in green tissue*

Increases in aboveground and root biomass observed

Work funded by DOE-EERE

For more data, see: Ambavaram et al., Novel transcription factors PvBMY1 and PvBMY3 increase biomass yield in greenhouse-grown switchgrass (Panicum virgatum L.), 2018, Plant Science

\[ n=4 \text{ plants, asterisks indicate levels of significance; } * P \leq 0.01, ** P \leq 0.05 \]

Leaves & stems, 75%-100% increase
Roots, 85-140% increase

Leaves & stems, 100-160% increase
Roots, ~40% increase
Various photosynthetic parameters measured. Primary difference observed in electron transport rate around photosystem I and II [ETR(I) and ETR(II)]

Photosynthesis rate measurements using Dual-PAM-100 (Heinz Walz GmbH) in 2 month old plants with light adapted leaves on a sunny morning

Leaf used

2\textsuperscript{nd} leaf of a 2 month old plant

PPFD = photosynthetic photon flux density
Transcriptomics of high yielding switchgrass plants: A powerful resource for gene discovery

- Overexpression of **C4004** in switchgrass
  - Negative regulator of plant growth
  - Good editing target
  - Evaluating edits in rice and wheat

- Overexpression of **C4029** confers drought tolerance

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<tr>
<th>Control switchgrass</th>
<th>Transgenic C4004 Plants</th>
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2 month old plants 3 weeks without water

Biomass harvested Regrowth analyzed after 30 days
Combined modeling components to identify transcription factors (TFs) to increase oil and/or seed yield in industrial oilseed *Camelina sativa*

- 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
Known TFs Impact Oil and/or Seed Yield Validating Approach

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

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

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

**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

Wri1 mutant seed

Wild-type seed

Mutant (myb56-1)

Mutant (myb56-2)

Scale bars = 100 μM

Total fatty acid content (μg/seeds)

0 1.5 3 4.5 6 7.5

WT wr1-1

100 μM
Opportunities With GRAIN

- Development of advanced models
  - Camelina – in hand

- Develop crop-specific models
  - Canola
  - Soybean
  - Corn
  - Rice, wheat, potato, etc

- Identify gene targets for specific trait objectives
  - Targets for genome editing and transgenic approaches

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

Objective: Develop the best combination of gene edits to maximize oil/acre

C3008a
- Successful editing of all three copies of C3008a in Camelina
  - Camelina is an allohexaploid; each gene expected to be present in 3 copies
  - Received confirmation in 2017 that line is not regulated by USDA-APHIS
  - US field tests in progress

C3008a, C3008b, C3009
- Completed editing of three distinct genes of Camelina designed to increase oil
  - Simultaneous editing of 9 genes (3 target genes present in 3 copies each)
  - Received confirmation in 2018 that line is not regulated by USDA-APHIS
  - US field tests in progress

C3007 (BADC) and C3010
- Completed exclusive license to IP from University of Missouri (C3007 and C3010)
  - C3007 is a novel negative regulator of ACCase a key enzyme in fatty acid biosynthesis
  - Metabolix Oilseeds is currently editing the C3007 gene in Camelina and canola
Yield10 technologies enable multiple paths to value creation

North American Commodity Crops
- Accelerate deployment with Ag majors
- License agreements with milestones and participation in downstream economics

Specialty and Niche Crops including Nutritional Oils
- Focus on development of high value products in food and animal feed
- Participation in the downstream business

Technology Platforms
- “GRAIN” unique approach to identifying gene combinations for editing
- Non-dilutive funding and relationships with leading plant scientists
- R&D support for partner funded programs
QUESTIONS?

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