Breakthroughs in Plant Based PHA Bioplastic Production

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Sustainable Growth Starts with a Seed

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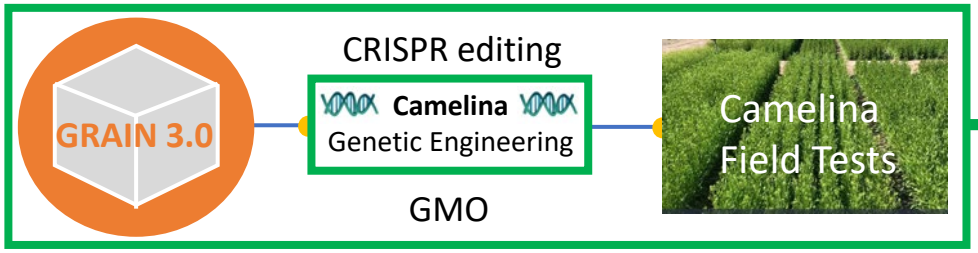
*Under the Private Securities Litigation Reform Act of 1995

Yield10's Trait Factory and Business Models

From Crop Science to $200 Billion Total Addressable Market

Technology Platform - "Trait Factory"[1]



Camelina Seed Products

1. **Feedstock Oil (Biofuel)**
2. **Omega-3 Oil (EPA+DHA)**
3. **PHA Bioplastics**

+

Trait Licensing[2]

Bayer CropScience
GDM
Forage Genetics International
Simplot

[1]21 Patent Families Pending
[2]Research License Agreements, 3rd party R&D to create option value for Yield10 gene traits on over 400 million acres of major crops (soybean, corn, canola, etc.)

Why Camelina?

- Promising oilseed crop
  - seed oil levels ~ 40% of seed weight
  - does not outcross with canola
- Excellent platform crop for novel high value seed products
- Both spring and winter varieties
  - winter varieties, potential use as cover crop for corn and soybean acres
- Camelina producing specialty products: ⬆ value proposition for farmer

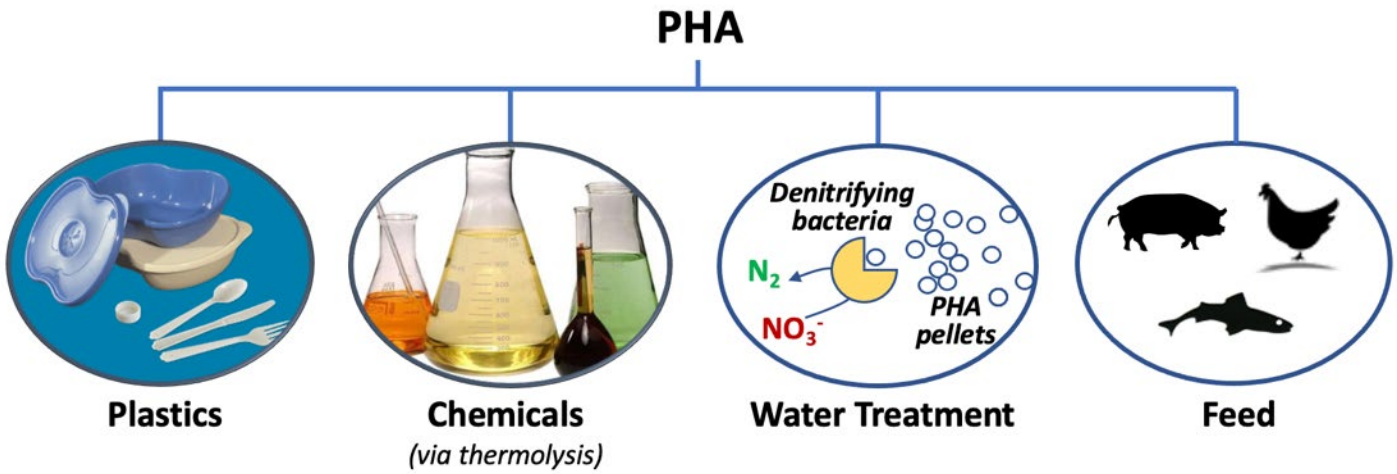
Greenhouse grown Camelina

Camelina field plots at flowering

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

Polyhydroxyalkanoate (PHA) Biomaterials

- Renewable, biodegradable class of biomaterials produced by some microorganisms as reservoir of stored carbon and energy
- Fully degradable in all biologically active environments
- Unique features of polymers will allow use in multiple applications



PHA Bioplastics Opportunity

Plastics: Global Production, 350 Million TPY, 4% Growth Rate, ~$720 Billion by 2025

- Increasing demand for biodegradable or bio-sourced plastics
- Low-cost crop PHA bioplastics –functionally replace over 50% of todays plastics
- Barriers: Market adoption has been severely restricted by high cost
  - *<u>Production by fermentation</u>: cost too high for most applications*

A. Current Materials

B. PHA Replacements[1]

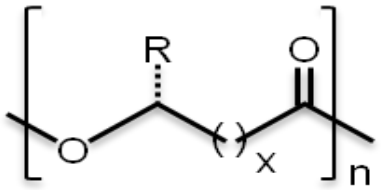
Fermentation PHA products
- *Demonstrated functionality*
- *But 3- 5x more expensive*

1. Yield10 corporate archives

PHA Camelina

Mission:

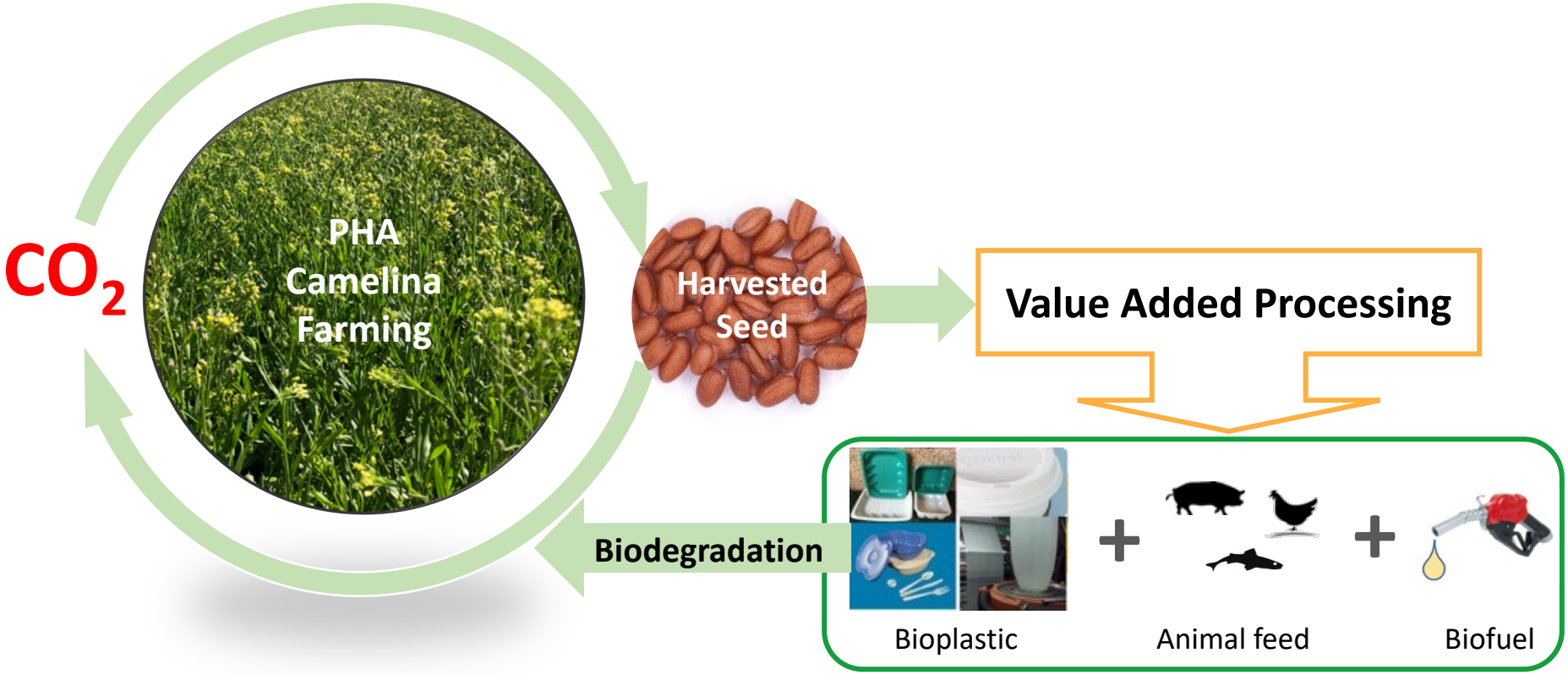
Low-cost, large-scale,
Carbon Negative,
Zero Waste Bioplastics
produced in Camelina seeds



PHA Bioplastics

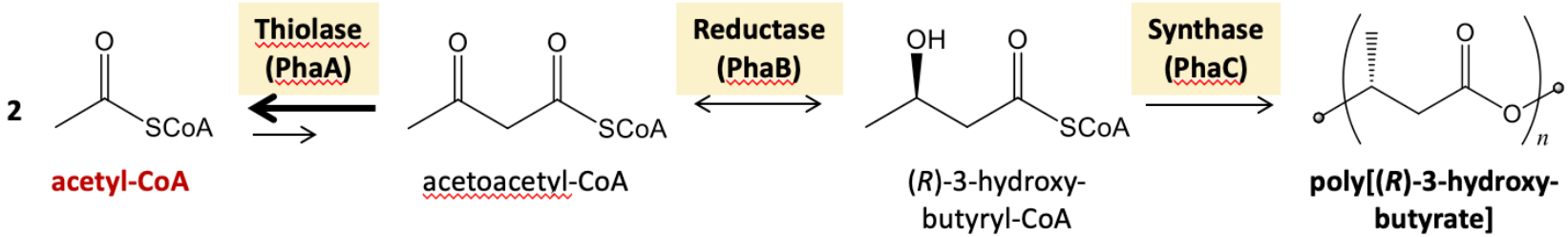
Goal: Carbon Negative - Zero Waste Bioplastics

Yield10 genetically programmed Camelina to produce PHA Bioplastics in the seed

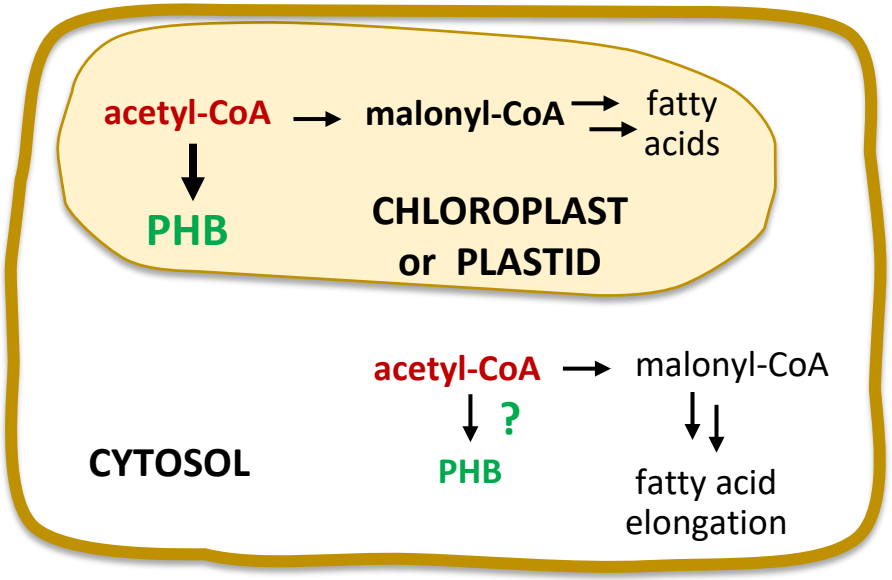


PHB Pathway Well Suited to Oilseeds

Native bacterial PHB biosynthetic pathway



Engineered plant cell



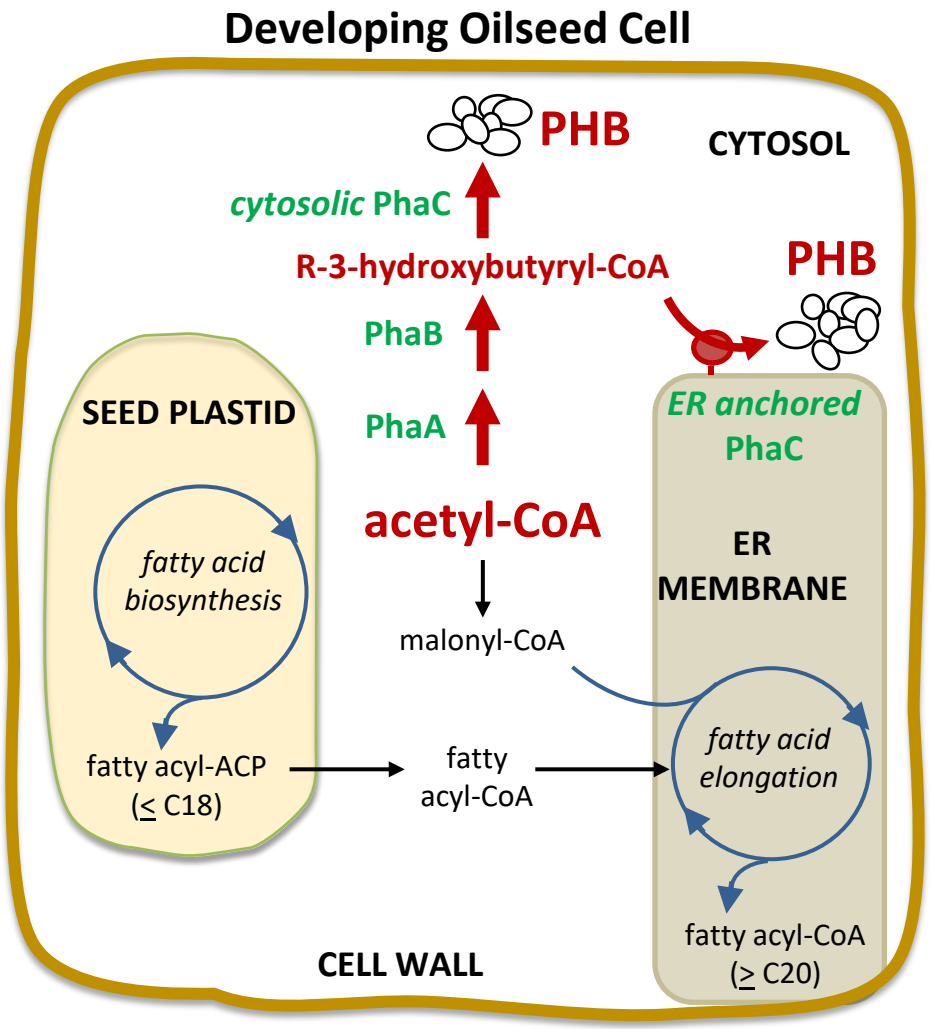
- Production in chloroplasts/seed plastids has yielded high levels of PHB in plants, but often with impaired growth[1]
- Little reported success with cytosolic production (*highest reported level 0.34% dry cell weight*[2])

[1]**Yield10 reference for production of PHB in Camelina seed plastids, Malik et al.,** 2015, *Plant Biotechnol. J.* 13, 675. [2]**Production in cytosol of cotton fibers, John & Keller,** 1996, *P. Natl. Acad. Sci. USA.* 93, 12768.

Revisit Production of PHB in Cytosol of Seed

Capture portion of acetyl-CoA in cytosol of seed for production of PHB

- Two genetic constructs
  - Construct 1: All enzymes targeted to cytosol
  - Construct 2: PhaA, PhaB targeted to cytosol; PhaC targeted to cytosolic face of endoplasmic reticulum (ER)
- Seed-specific expression constructs transformed into Camelina, lines isolated



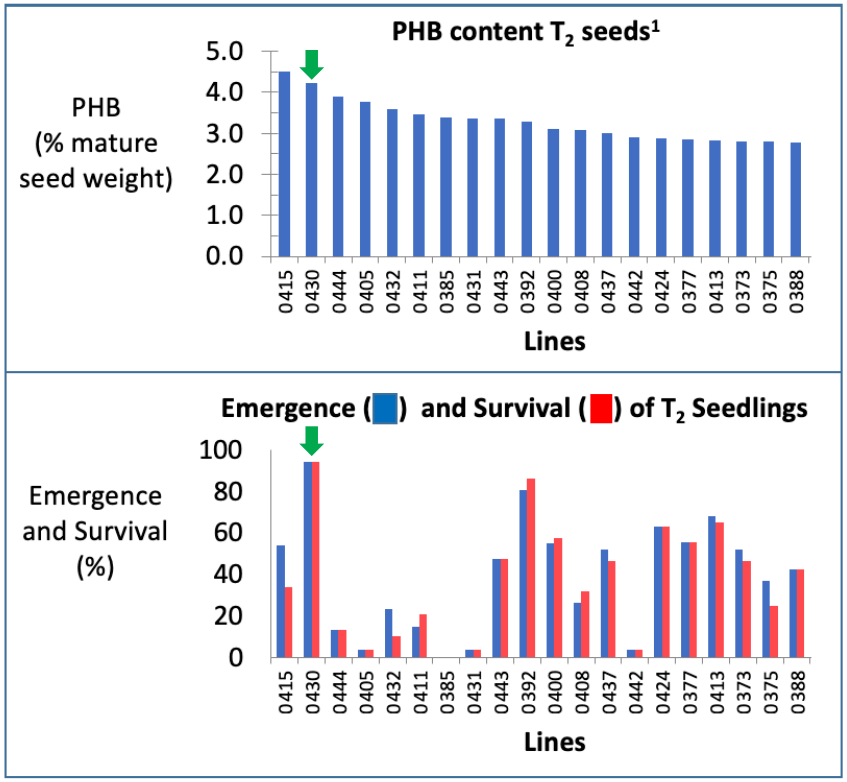
ketothiolase (PhaA), **reductase** (PhaB), **PHA synthase** (PhaC)

T_2 Seed PHB Content, Survival of Seedlings

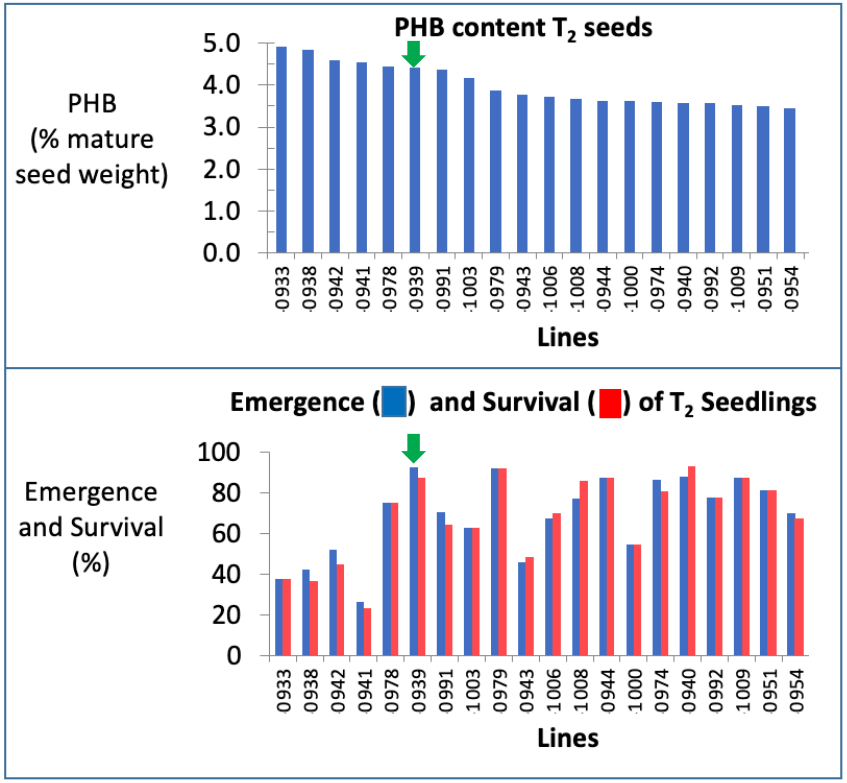
Second generation seeds: 14x previously reported[1] highest level of cytosolic PHB

- Some lines with good emergence & survival contained > 4% PHB *(mature seed weight)*

Cytosolic PHA synthase



ER anchored PHA synthase



[1]**Production in cotton fibers, 0.34% dry cell weight,**
John & Keller, 1996, *P. Natl. Acad. Sci. USA*. 93, 12768.

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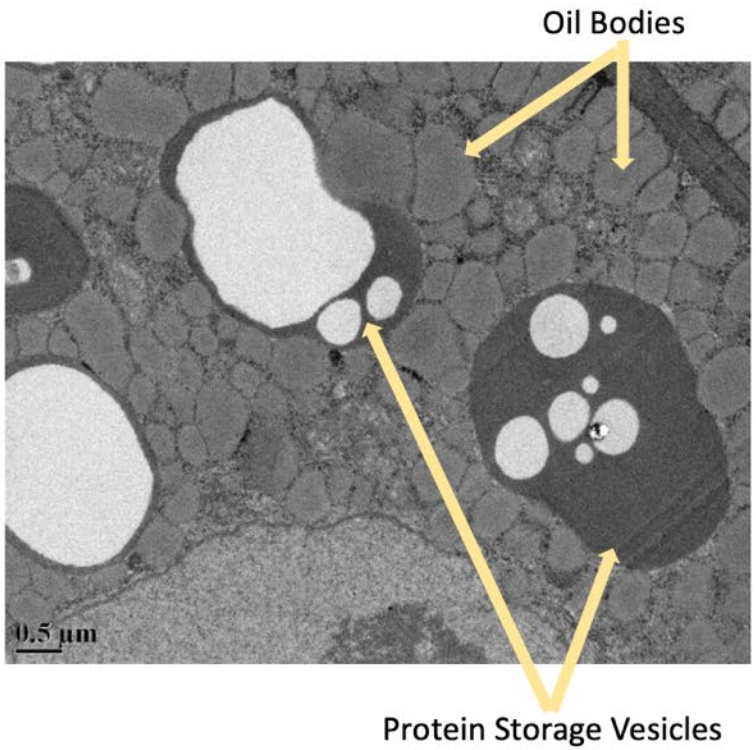
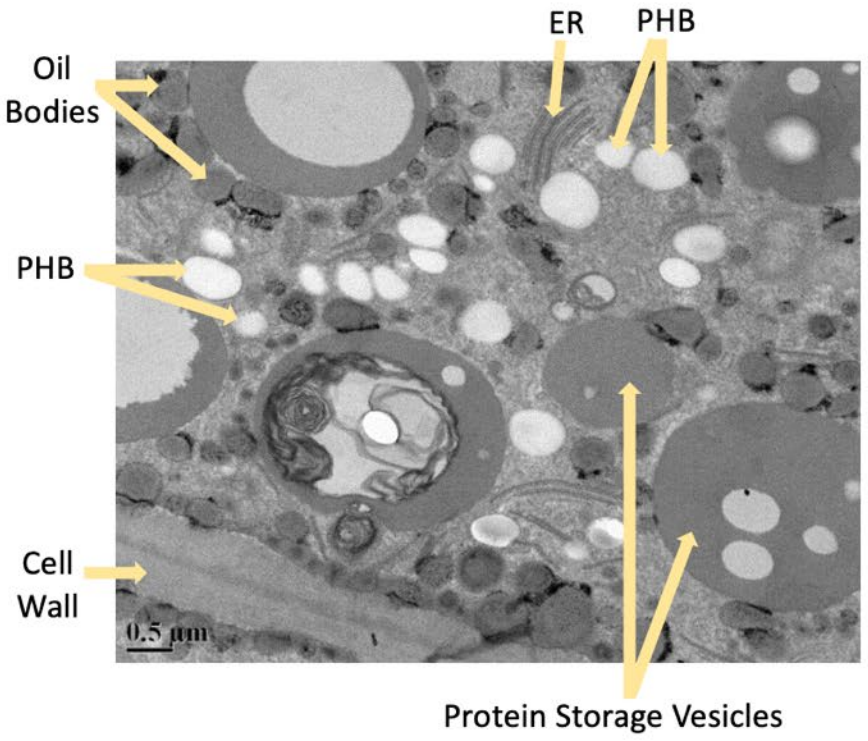
PHB Polymer Accumulates as Granules in Seed

Transmission electron microscopy (TEM) of cotyledon in imbibed seeds

Wild-type control

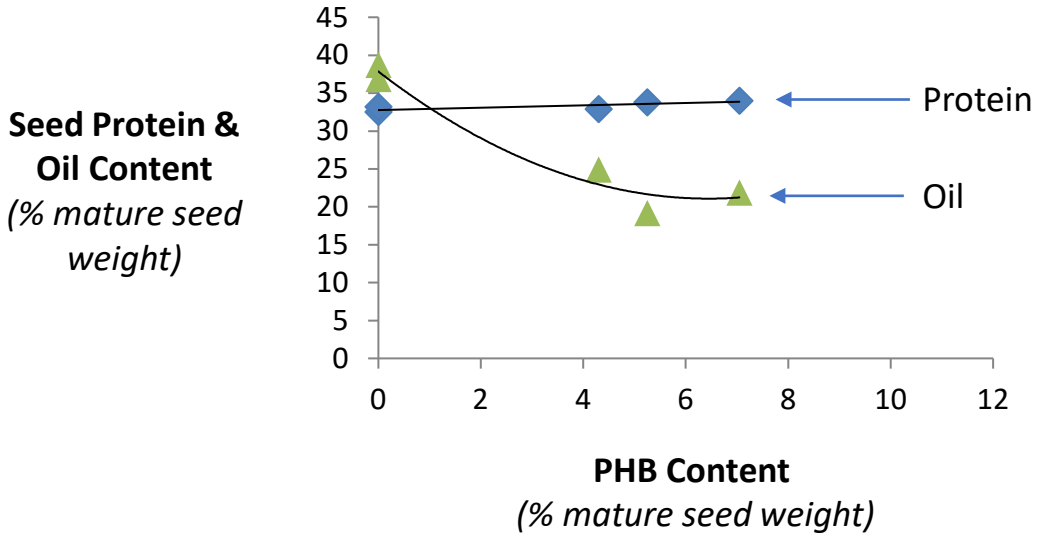


ER targeted synthase line



PHB Produced at Expense of Oil

Seed Oil and Protein Content
(from greenhouse growth of homozygous lines)



PHB has more value than oil

Looking for genes to increase carbon to boost oil using GRAIN modeling platform

Seedlings of Cytosolic PHB Producers

Cytosolic PHB production in seeds ➡ healthy seedlings with narrow cotyledons

Wild-type

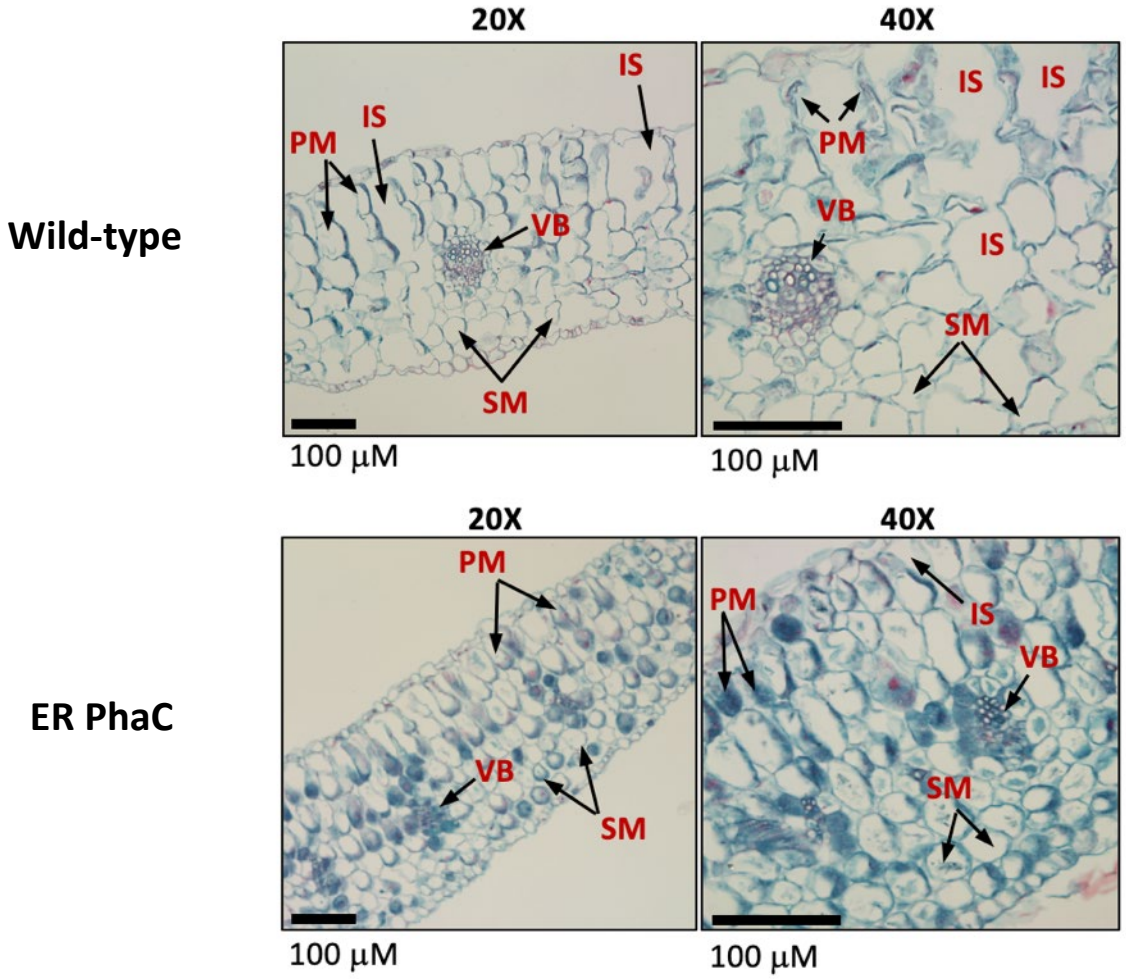
Cytosolic PhaC
4.5% PHB
53% emergence
33% survival

ER PhaC
4.4% PHB
92% emergence
87% survival

Pursued only ER PhaC lines in later generations. PHB production more stable in ER PhaC lines.

Seedlings of Cytosolic PHB Producers

Light microscopy of cotyledons



Intercellular spaces significantly reduced in cotyledons of PHB producing lines

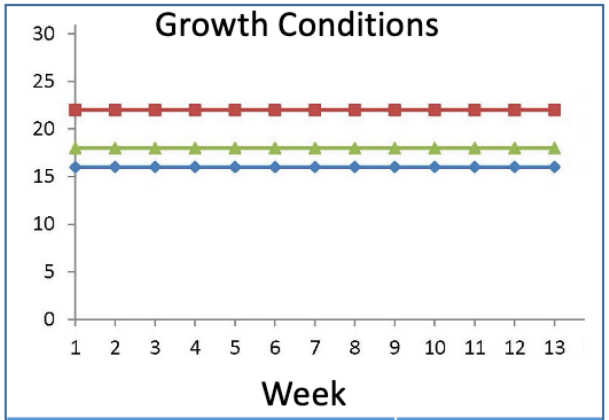
IS, intercellular space; PM, palisade mesophyll;
SM, spongy mesophyll; VB, vascular bundle

PHB Production in Different Growth Conditions

Lines grown in greenhouse and controlled environmental chamber programmed to simulate average spring growth conditions[1]

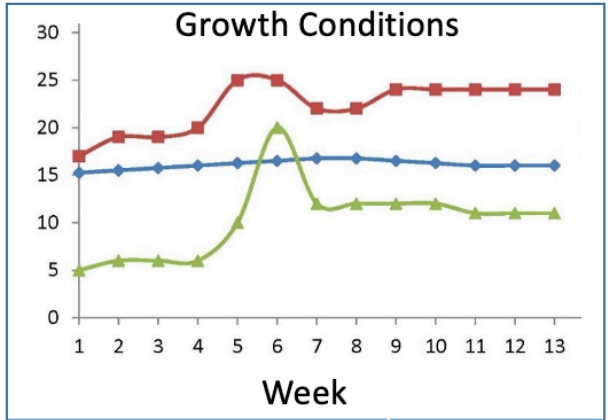
- Results for best line shown

Greenhouse



T_4 seed PHB range	3.9 - 7.1%
% survival T_3 seedlings	86
Avg T_4 seed yield (g)	8.2 ± 1.8
Number of plants	8

Controlled Environmental Chamber



T_4 seed PHB range	4.6 -10.2%
% survival T_3 seedlings	78
Avg T_4 seed yield (g)	14.7 ± 3.9
Number of plants	4[2]

Up to 10.2% PHB obtained in seeds of homozygous line in chamber
30X highest previously reported level[3]

[1]Temperature settings in controlled environmental chamber adapted from average weekly historical data between early May & late July for Saskatoon, Saskatchewan, Canada. [2]Size of growth chamber limited number of replicates. [3]Production in cytosol of cotton fibers 0.34% dry cell weight, John & Keller, 1996, P. Natl. Acad. Sci. USA. 93, 12768.

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Field Trials of Cytosolic PHB Producers

2020 - replicated field plots, line sorting

PHA Camelina plants at 2020 U.S. field test site
6% PHB produced in best line

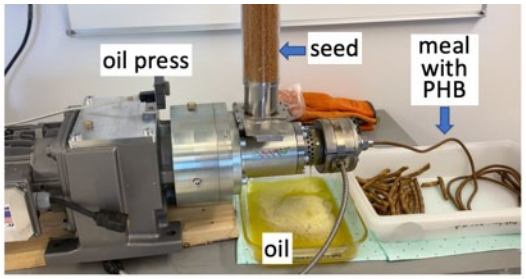
2021 - 0.2 acre seed scale up

Drone photo, 0.2 acre U.S. scale up site
Plants produced 6% PHB

2022 – Planting at acre-scale for larger scale sample production

- seed processing, product prototyping, sampling & other business development activities

Small scale seed processing from field



Seed crushing with oil press, removal of oil

PHB extraction

PHB precipitation

Prototype PHA Camelina products

PHB polymer

Seed oil

Protein rich meal

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PHA Development Program Status

- Developed new technology solution to produce PHA in Camelina
  - Patent application in 2019
- Conducted field tests of PHA Camelina in 2020 and 2021 seasons
  - Planting at acre-scale in 2022
- Proof-of-concept milestone for producing PHA in field grown Camelina achieved
  - Up to ~6% PHB in mature seed
- Elite PHA line development ongoing
  - Goal systematically increase PHA seed content to increase harvest value

- Up Next….
  - Engineering PHB copolymers

PHA Camelina plants at 2020 U.S. field test site

Sample PHA resin pellets produced by Metabolix

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Questions?

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