



Breakthroughs in Plant Based PHB Production

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 VIRTUAL

 **SEED**
2021 SYNTHETIC BIOLOGY:
Engineering, Evolution & Design

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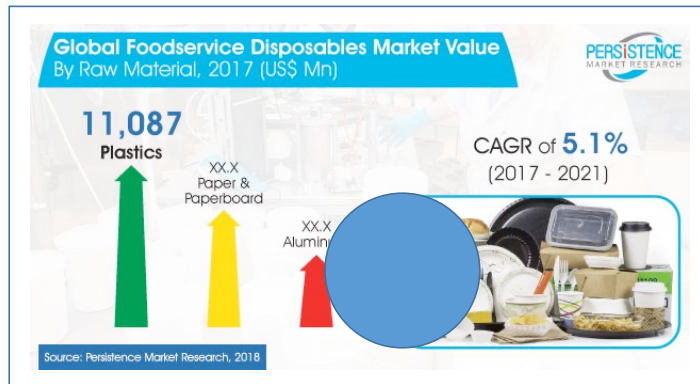
***Under the Private Securities Litigation Reform Act of 1995**

PHA Bioplastics Opportunity

Plastics: Global Production, 350 Million TPY¹, 4% Growth Rate, ~\$720 Billion by 2025²

- Increasing demand for biodegradable or bio-sourced plastics³
- Low-cost crop based PHA bioplastics – target markets
 - PHA biomaterials can functionally replace over 50% of today's plastics

A. Current Materials



B. PHA Replacements⁵



Fermentation based PHA products

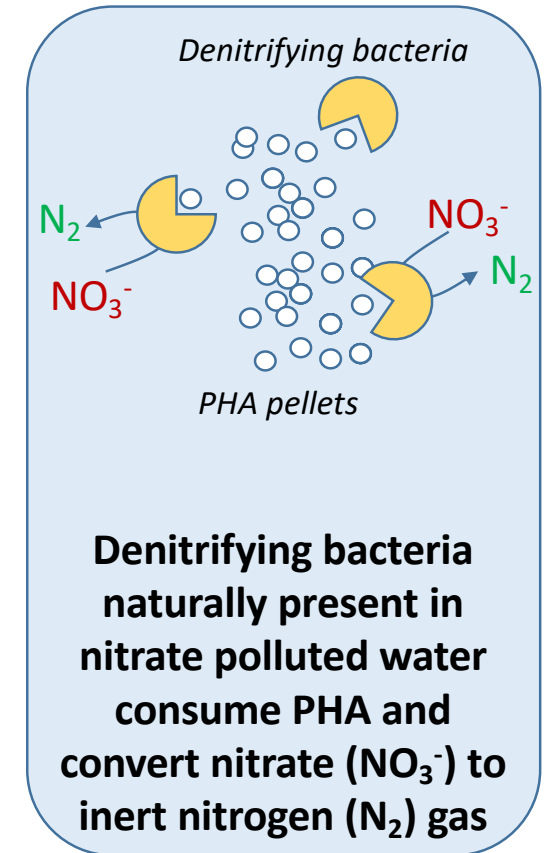
- Demonstrated functionality
- But 3- 5x more expensive

1. https://www.plasticseurope.org/application/files/5715/1717/4180/Plastics_the_facts_2017_FINAL_for_website_one_page.pdf
2. <https://www.prnewswire.com/news-releases/plastics-market-size-worth-usd-721-14-billion-by-2025--cagr-4-0-grand-view-research-inc-300801897.html>
3. www.European-bioplastics.org/market
4. <https://www.persistencemarketresearch.com/market-research/foodservice-disposables>
5. [Yield10 corporate archives](#)

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
 - Plastics, renewable chemicals, water treatment, animal feed ingredient
- Barriers:
 - Market adoption has been severely restricted by high cost
 - Production by fermentation: cost too high for most applications

Water treatment application





PHA Camelina

Mission:

Low-cost, large-scale
Carbon Negative
- Zero Waste Bioplastics -

Why Camelina?

- Promising oilseed crop
 - seed oil levels ~ 40% of seed weight
 - does not outcross with canola
- Good platform for specialty/niche crops for high value products
- Both spring and winter varieties available
 - winter varieties, potential use as cover crop for corn and soybean acres
- Camelina producing specialty products will increase value proposition for farmers

Greenhouse grown Camelina



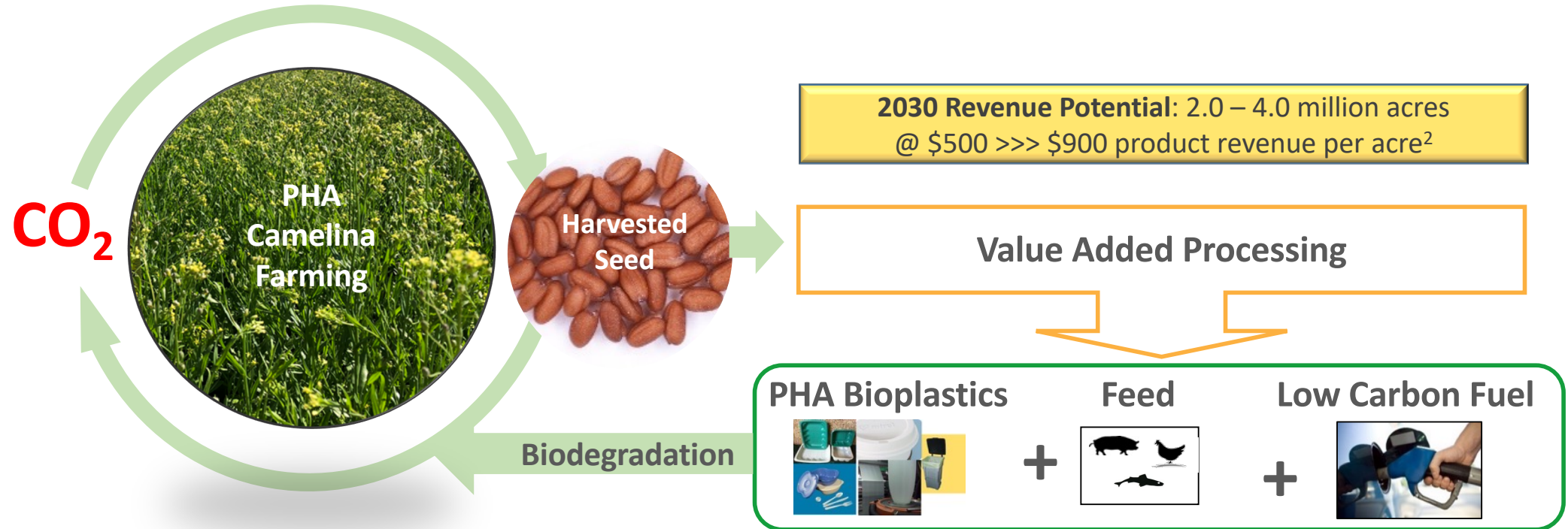
Camelina field plots at flowering



Goal: Carbon Negative - Zero Waste Bioplastics

Yield10 genetically programmed Camelina to produce PHA Bioplastics in the seed

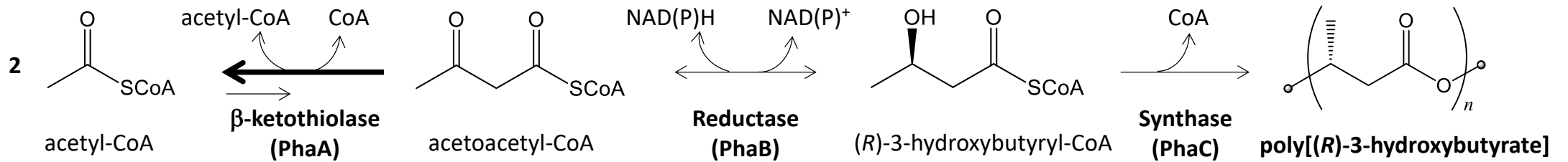
Addressable Market > \$200 billion¹



¹ ~25% of plastics production, 50% of plastics used in single use packaging. ² Estimates of market opportunity are based on industry sources as well as management's analysis, financial estimates and timelines for market introduction and adoption. >>> Technology Improvements, increased yield and oil/or PHA seed content

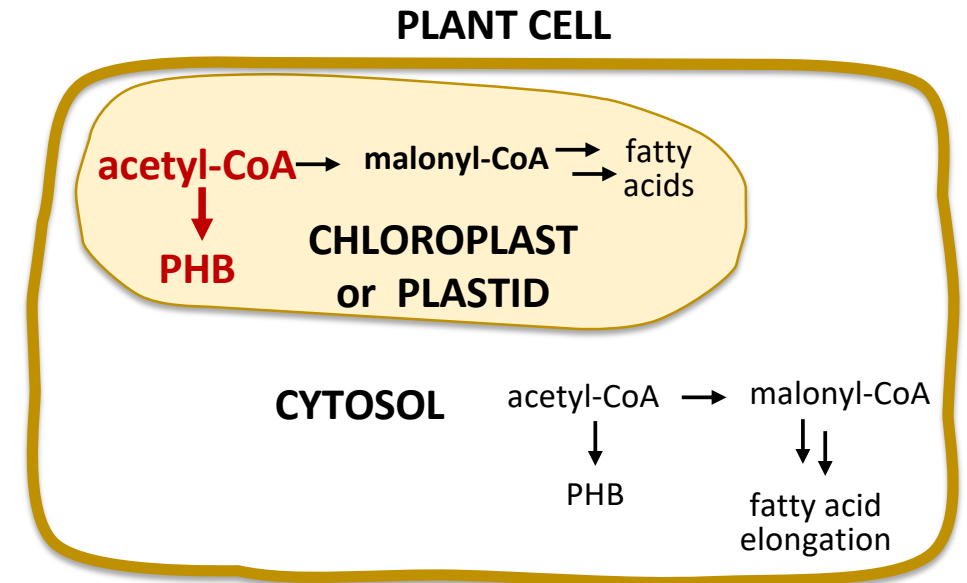
PHB Pathway – Substrate Acetyl-CoA Well Suited to Oilseeds

Bacterial PHB biosynthetic pathway



Engineering production in chloroplasts/
seed plastids has yielded high levels of PHB
in plants, but often with impaired growth¹

- Little reported success with cytosolic production (highest reported level 0.34% dry cell weight²)



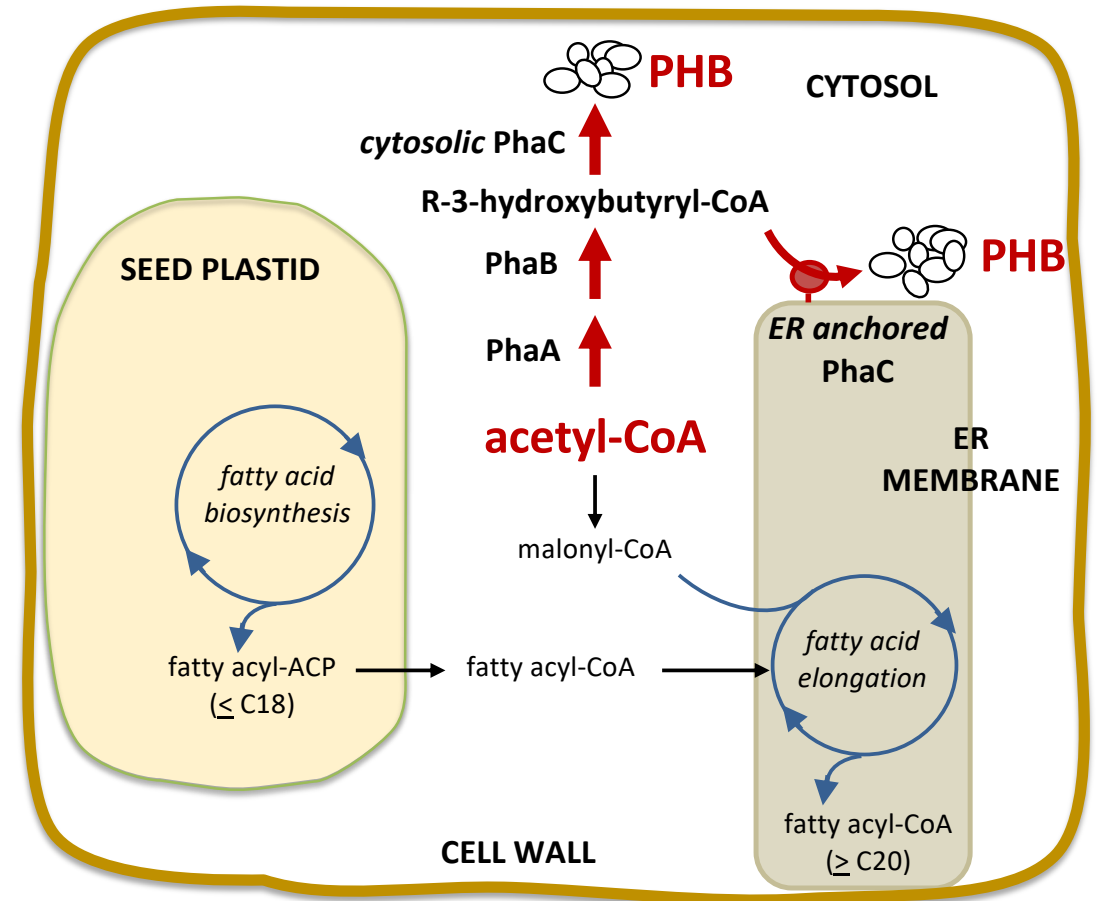
¹Yield10 reference for production of PHB in Camelina seed plastids, Malik et al., 2015, *Plant Biotechnol. J.* 13, 675.

²Production in cytosol of cotton fibers, John & Keller, 1996, *P. Natl. Acad. Sci. USA*. 93, 12768.

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

- Two genetic constructs
 - All enzymes targeted to cytosol
 - PhaA and PhaB enzymes targeted to cytosol; PhaC anchored to the cytosolic face of the endoplasmic reticulum (ER)
- Camelina plants transformed, lines isolated

DEVELOPING OILSEED CELL

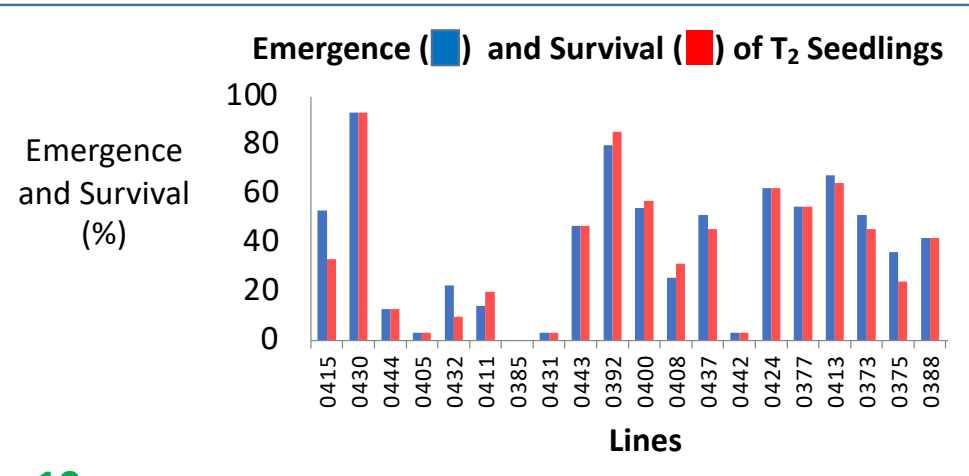
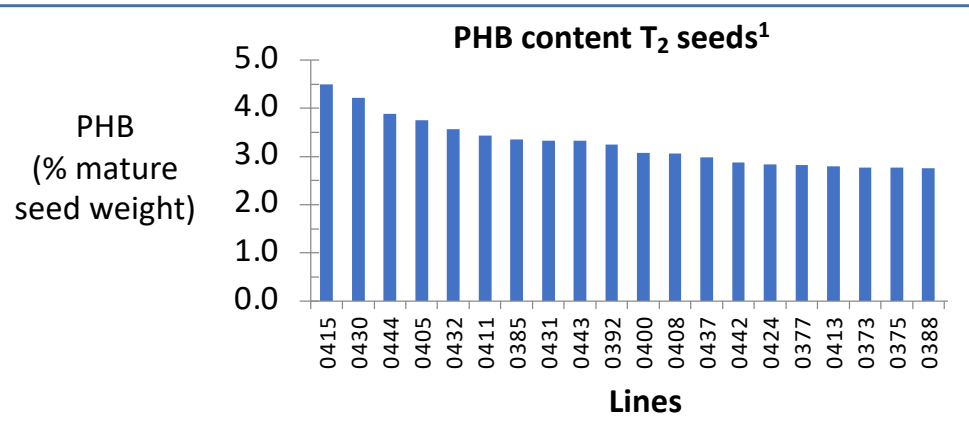


T₂ Seed PHB Content and Survival of Seedlings

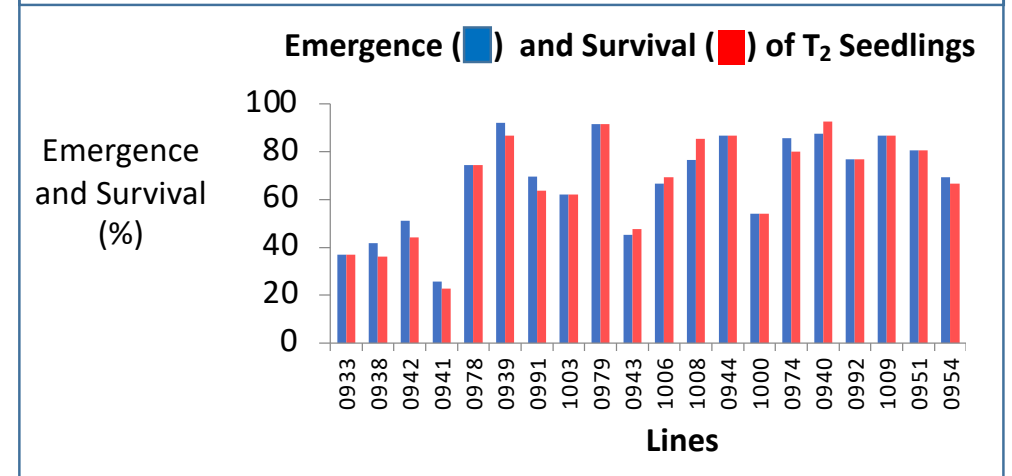
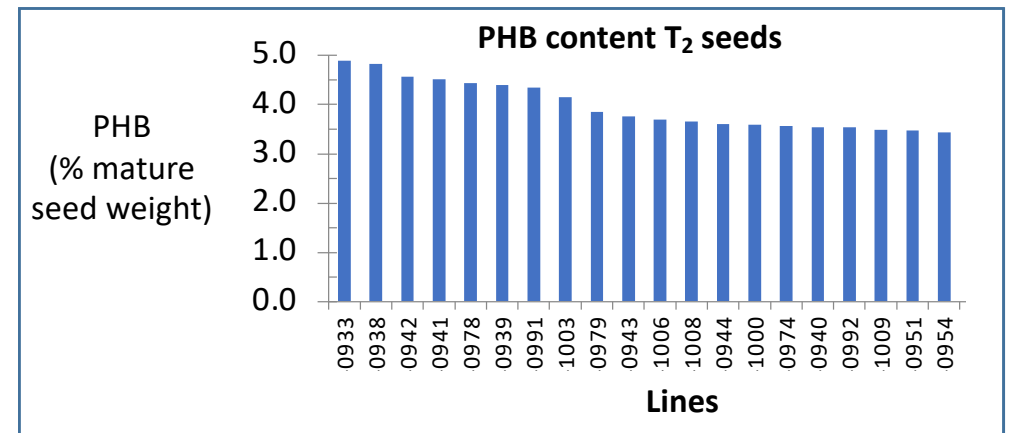
Second generation (T₂) seeds contained up to 14x reported¹ highest level of cytosolic PHB

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

Cytosolic PHA synthase



ER anchored PHA synthase



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

Phenotype of 7 day old seedlings

Wild-type



Cytosolic PhaC
4.5% PHB
53% emergence
33% survival



ER PhaC
4.4% PHB
92% emergence
87% survival

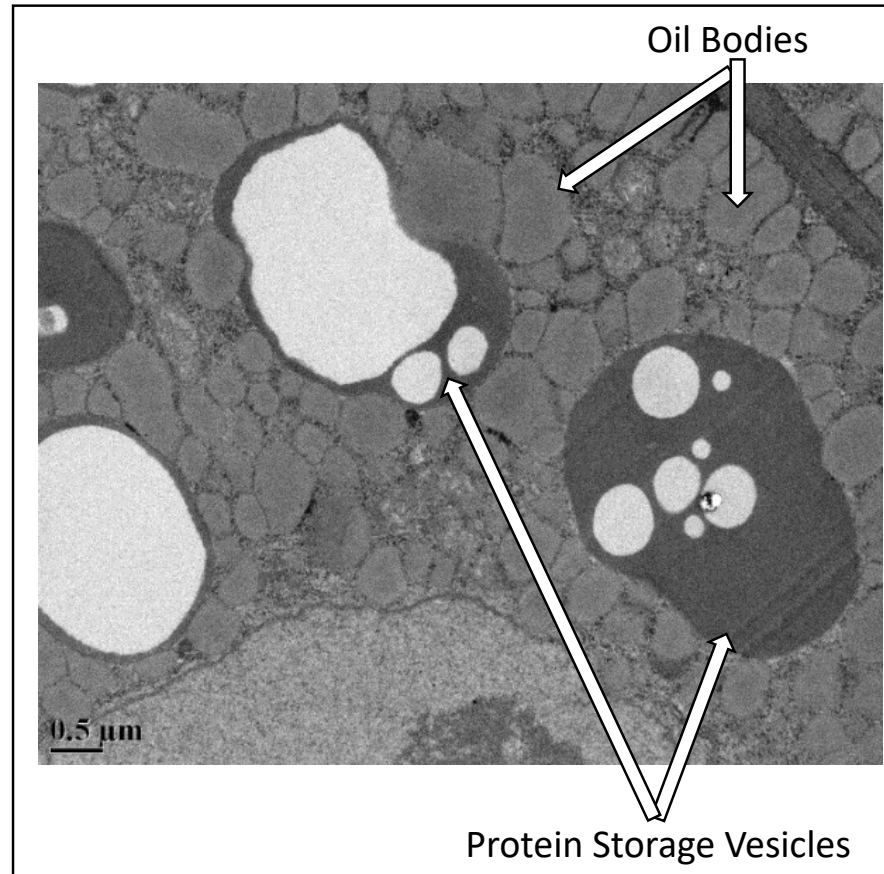


Pursued only ER PhaC lines in later generations. PHB production more stable in ER PhaC lines.

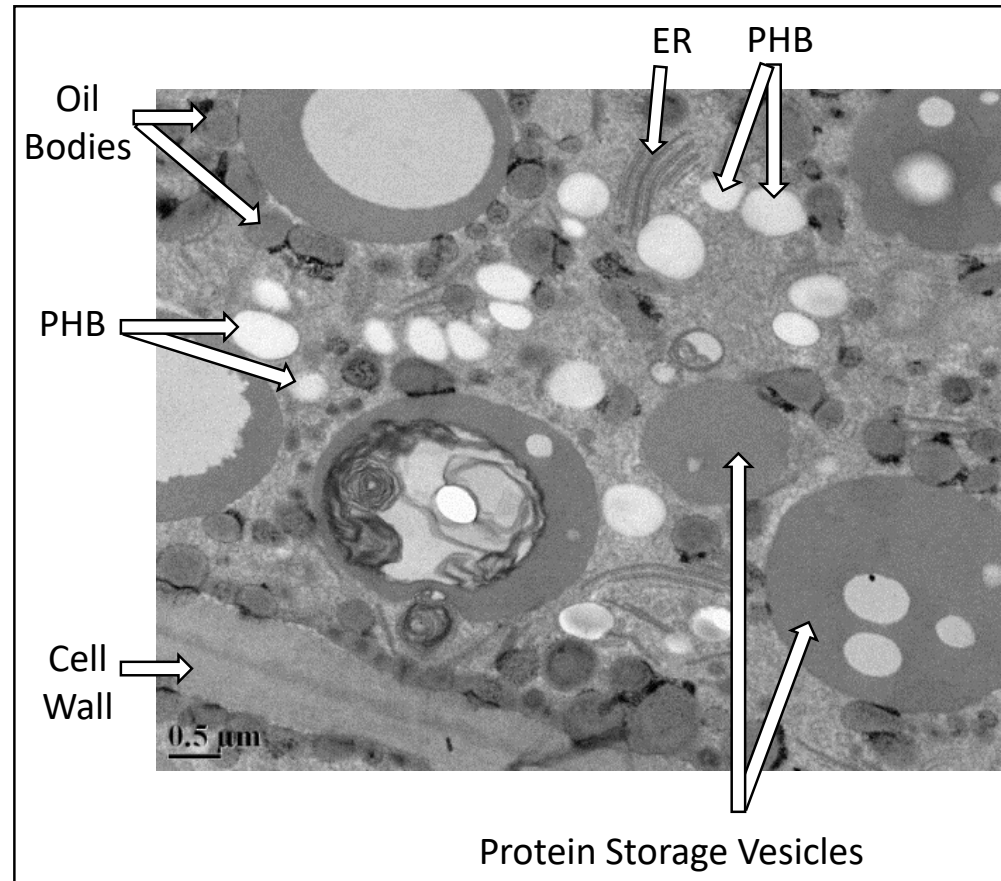
PHB Polymer Accumulates as Granules in Seed

Transmission electron microscopy (TEM) of cotyledon in imbibed seeds

Wild-type control

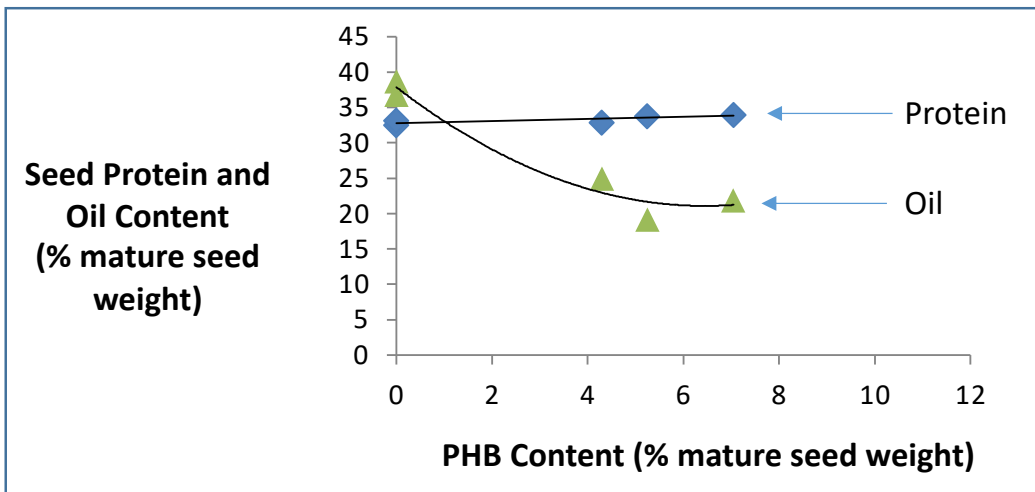


ER targeted synthase line



PHB Produced at Expense of Oil

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

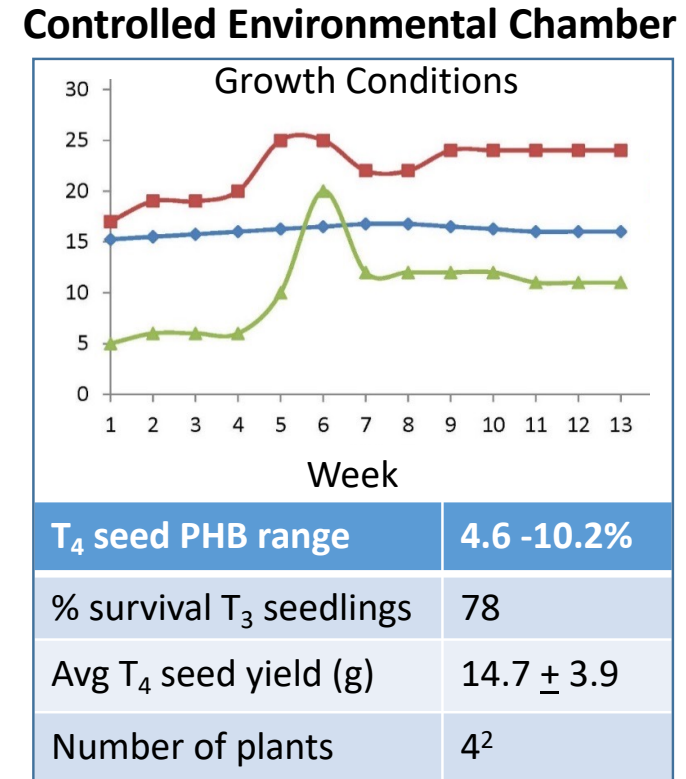
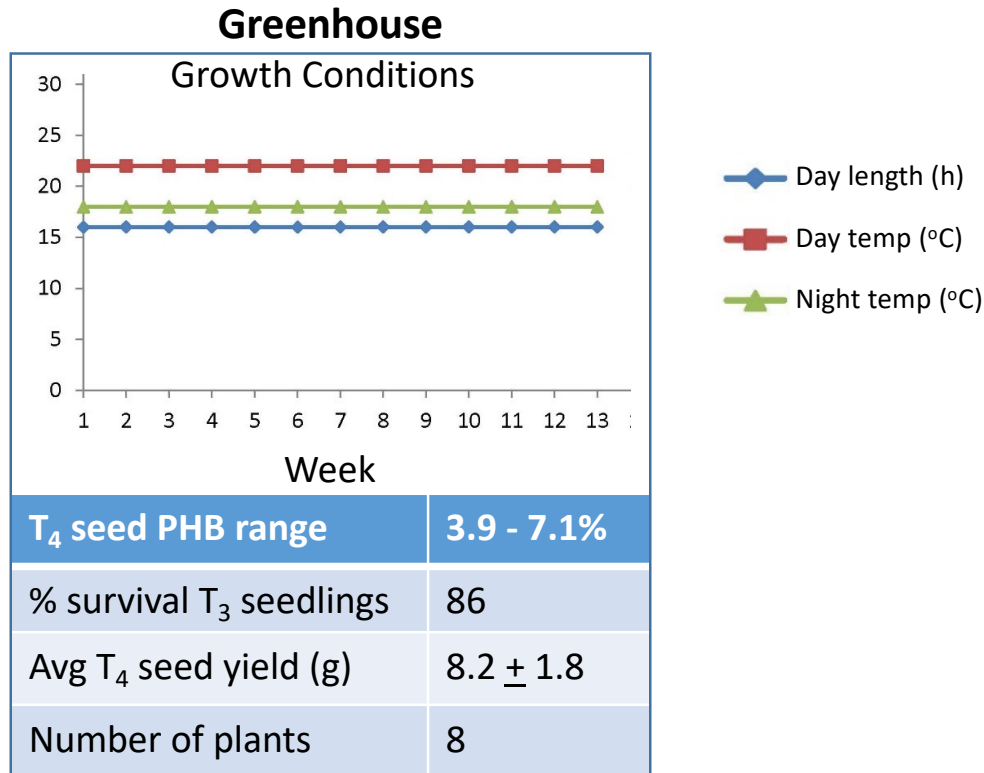


- *PHB has more value than oil*
- Looking for genes to increase carbon to seed to boost oil using GRAIN modeling platform

PHB Production in Different Growth Conditions

Lines grown in greenhouse and controlled environmental chamber programmed to simulate average spring growth conditions¹

- Results for best line shown



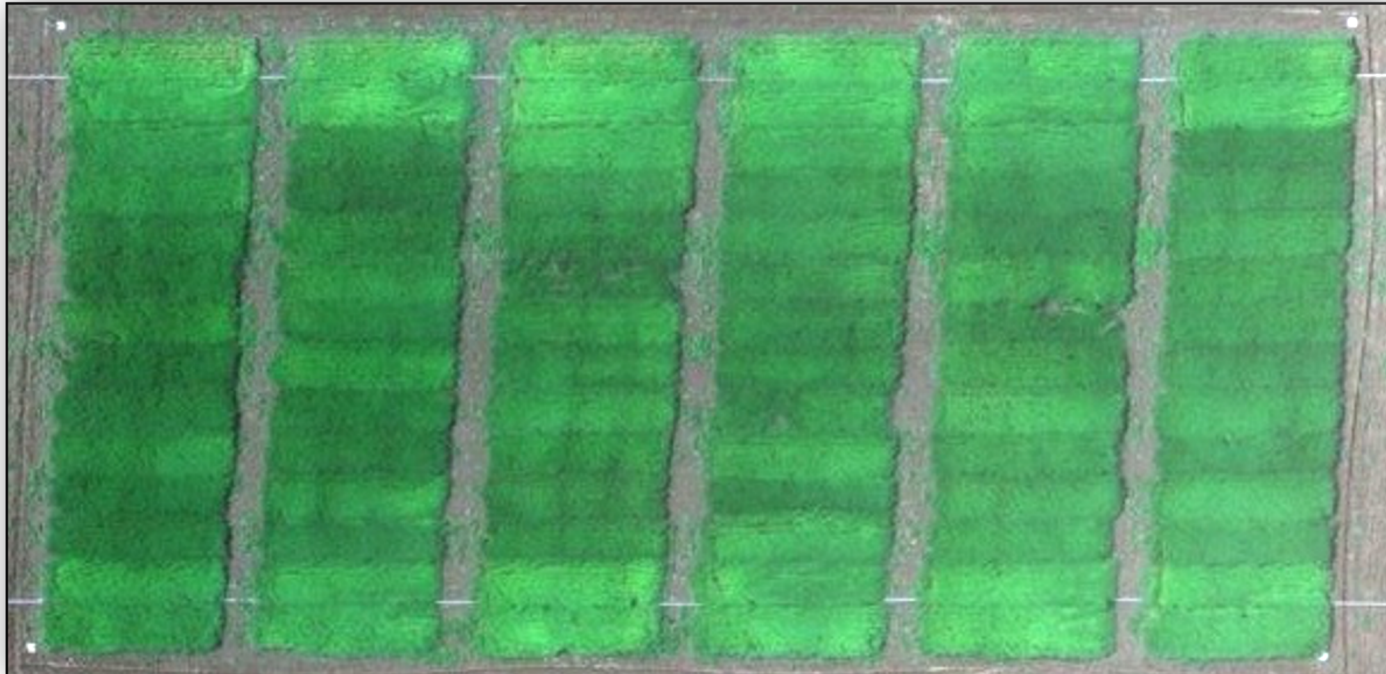
Up to 10.2% PHB obtained in seeds of homozygous line

14 ¹Temperature settings in the controlled environmental chamber adapted from average weekly historical data between early May and late July for Saskatoon, Saskatchewan, Canada, an area suitable for *Camelina* growth. ²Size of growth chamber limited number of replicates

2020 PHA Field Trials

- Conducted field tests of PHA Camelina in 2020 season
 - Small replicated plots of multiple lines with ER targeted PhaC (PHA synthase)
- Proof-of-concept milestone for producing PHA in field grown Camelina
 - Up to 6% PHB produced in seeds of Camelina in the field

Drone photo of PHA Camelina replicated plots at 2020 U.S. field test site



Selected two PHA Camelina lines for further scale up in 2021

Two separate 0.2 acre plots recently planted in U.S.

- Further seed scale up
- Seed processing and product prototyping, sampling and other business development activities



PHA Development Program Status

Addressable Market

\$200 billion¹

2030 Potential Revenue

PHA 2.0 – 4.0 million acres
@ \$500 >>> \$900 product revenue per acre

- Developed new technology solution to produce PHA in Camelina, patent application in 2019
- Conducted field tests of PHA Camelina in 2020 season
- Proof-of-concept milestone for producing PHA in field grown Camelina achieved – up to 6% PHB in mature seed
- Selected two PHA Camelina lines for further scale up in 2021
- Elite PHA line development ongoing
 - Goal systematically increase PHA seed content to increase harvest value



PHA Camelina plants at 2020 U.S. Field Test Site



Sample PHA resin pellets produced by Metabolix



QUESTIONS?

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