



## Breakthroughs in Plant Based PHB Production

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 VIRTUAL



2021 SYNTHETIC BIOLOGY:  
Engineering, Evolution & Design

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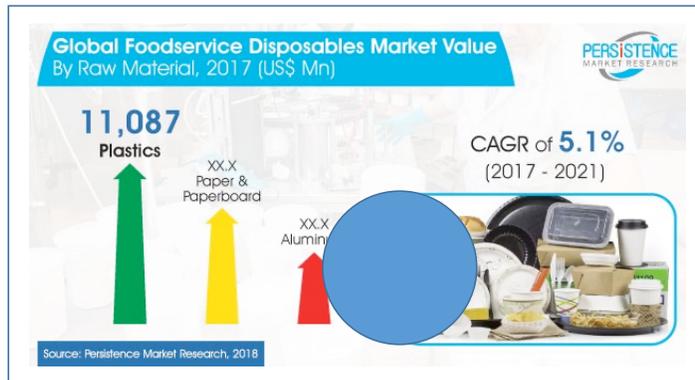
**\*Under the Private Securities Litigation Reform Act of 1995**

# PHA Bioplastics Opportunity

Plastics: Global Production, 350 Million TPY<sup>1</sup>, 4% Growth Rate, ~\$720 Billion by 2025<sup>2</sup>

- Increasing demand for biodegradable or bio-sourced plastics<sup>3</sup>
- Low-cost crop based PHA bioplastics – target markets
  - PHA biomaterials can functionally replace over 50% of today's plastics

## A. Current Materials



## B. PHA Replacements<sup>5</sup>



### Fermentation based PHA products

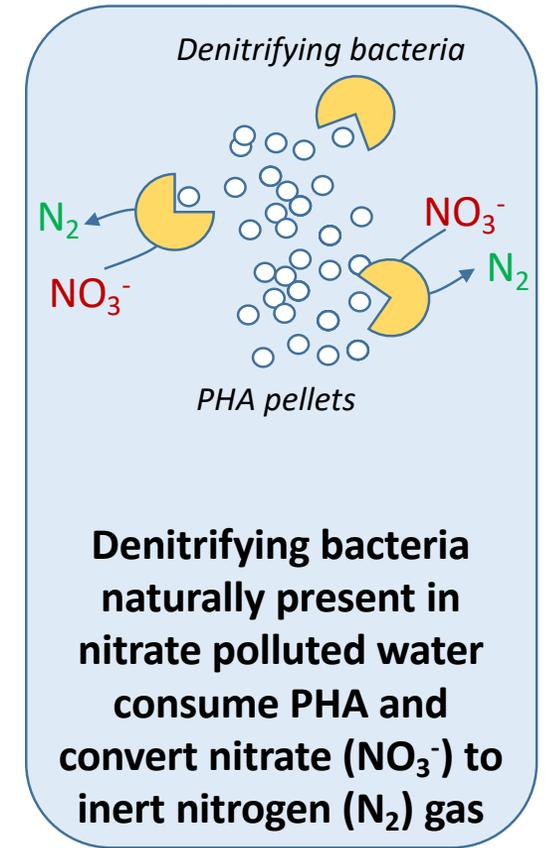
- Demonstrated functionality
- But 3- 5x more expensive

1. [https://www.plasticseurope.org/application/files/5715/1717/4180/Plastics\\_the\\_facts\\_2017\\_FINAL\\_for\\_website\\_one\\_page.pdf](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](http://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



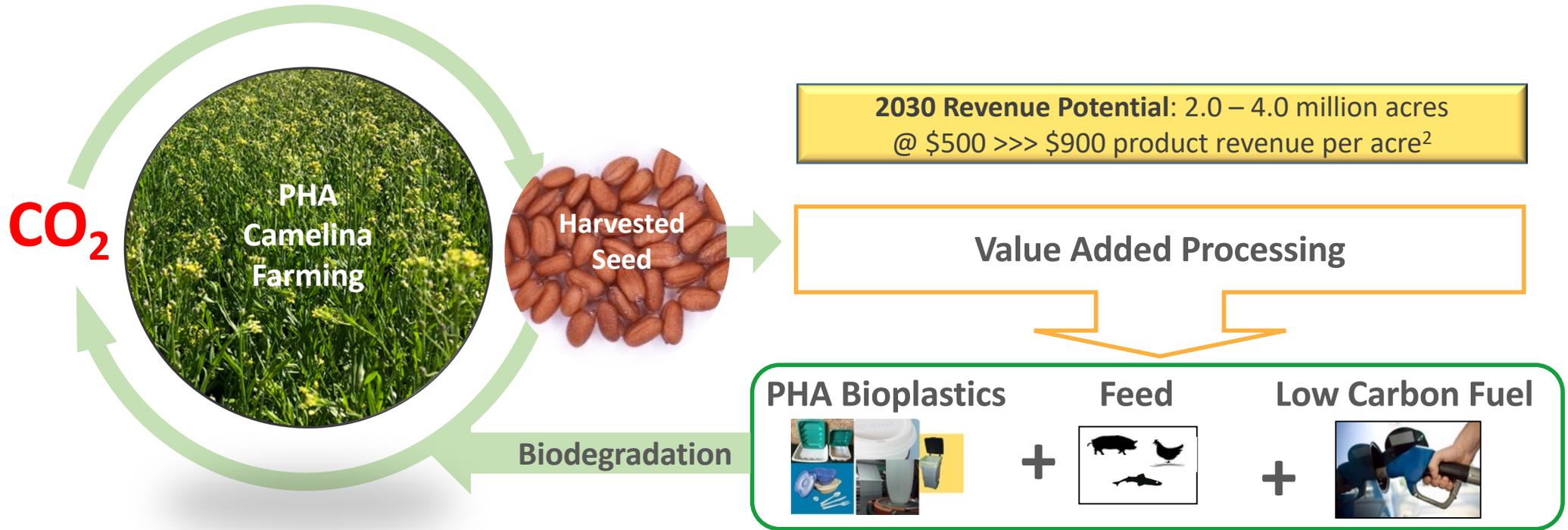
Camelina field plots at flowering



# Goal: Carbon Negative - Zero Waste Bioplastics

Yield10 genetically programmed Camelina to produce PHA Bioplastics in the seed

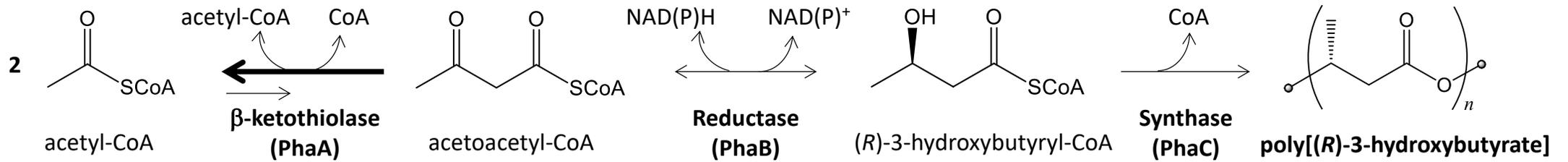
Addressable Market > \$200 billion<sup>1</sup>



<sup>1</sup> ~25% of plastics production, 50% of plastics used in single use packaging. <sup>2</sup> 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

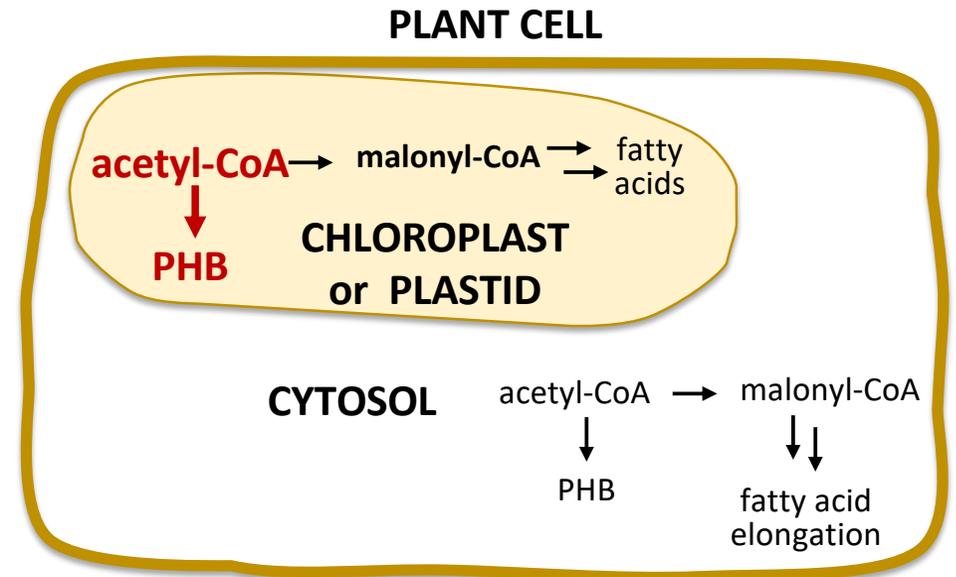
# PHB Pathway – Substrate Acetyl-CoA Well Suited to Oilseeds

## Bacterial PHB biosynthetic pathway



Engineering production in chloroplasts/seed plastids has yielded high levels of PHB in plants, but often with impaired growth<sup>1</sup>

- Little reported success with cytosolic production (highest reported level 0.34% dry cell weight<sup>2</sup>)



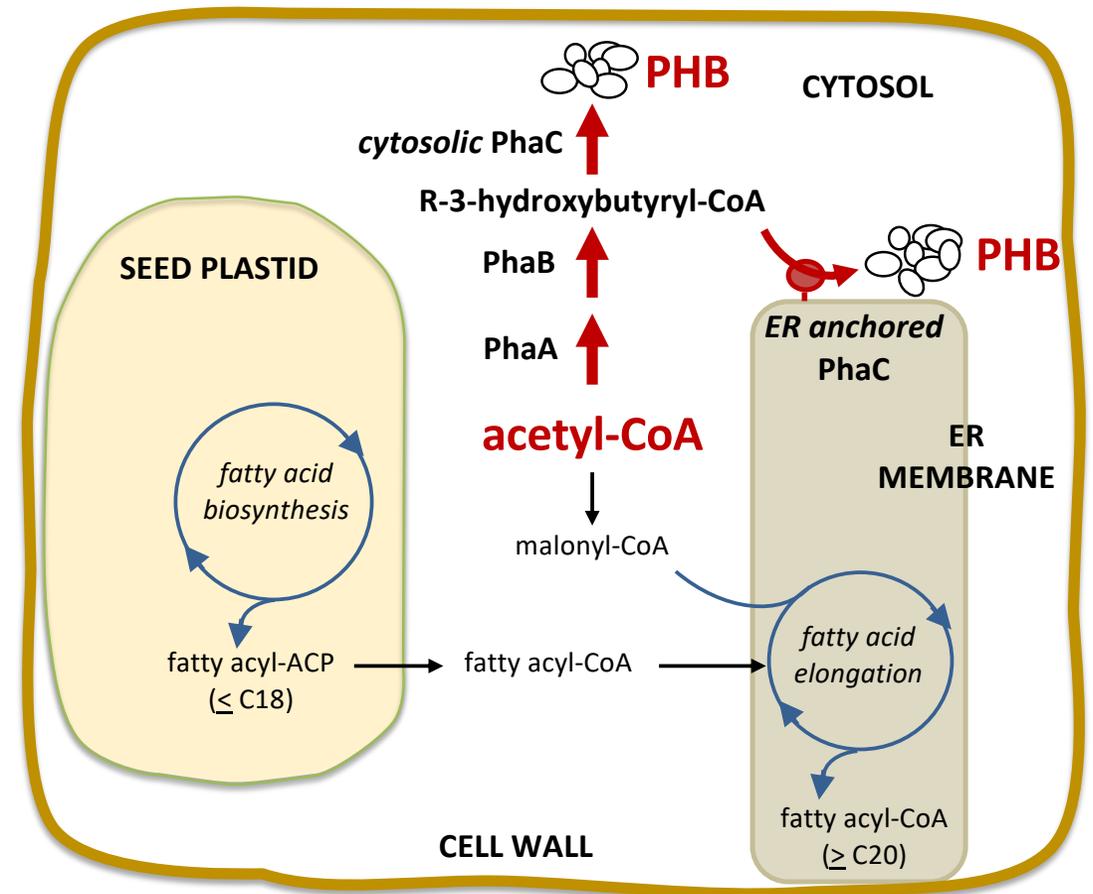
<sup>1</sup>Yield10 reference for production of PHB in *Camelina* seed plastids, Malik et al., 2015, *Plant Biotechnol. J.* 13, 675.

<sup>2</sup>Production 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

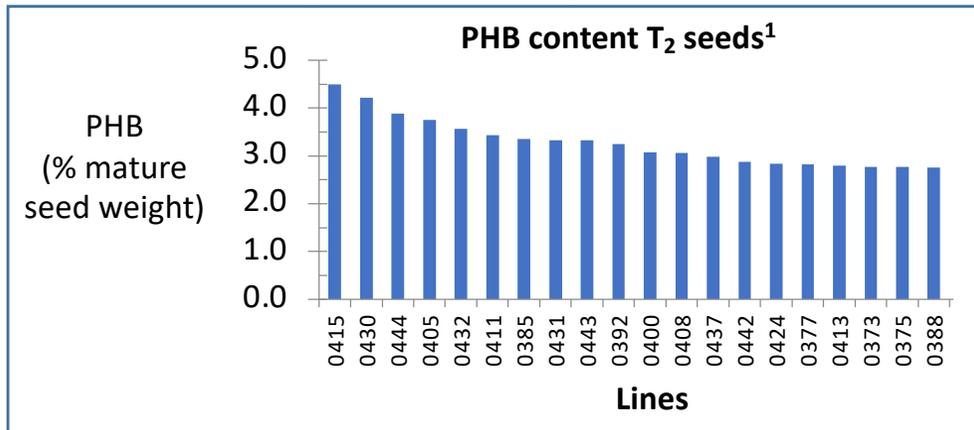


# T<sub>2</sub> Seed PHB Content and Survival of Seedlings

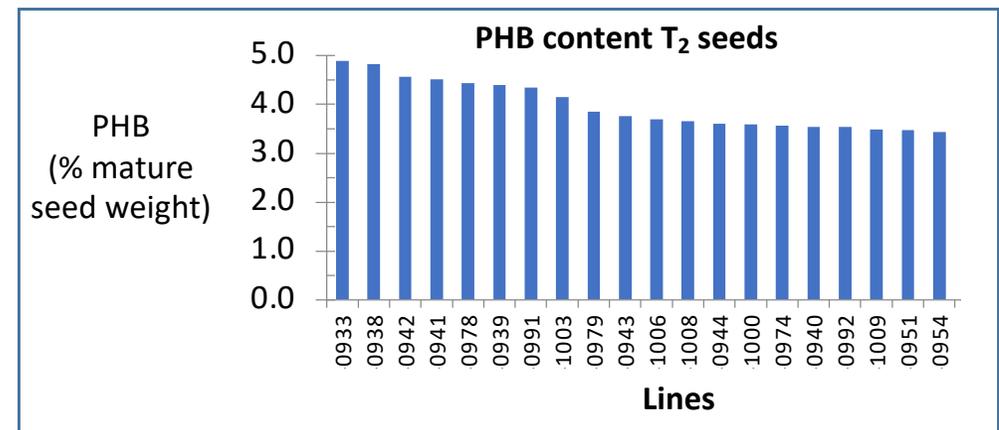
Second generation (T<sub>2</sub>) seeds contained up to 14x reported<sup>1</sup> highest level of cytosolic PHB

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

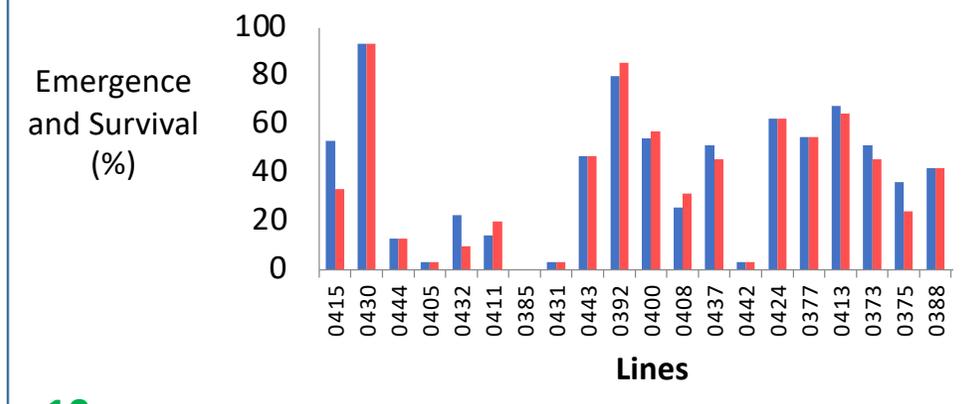
**Cytosolic PHA synthase**



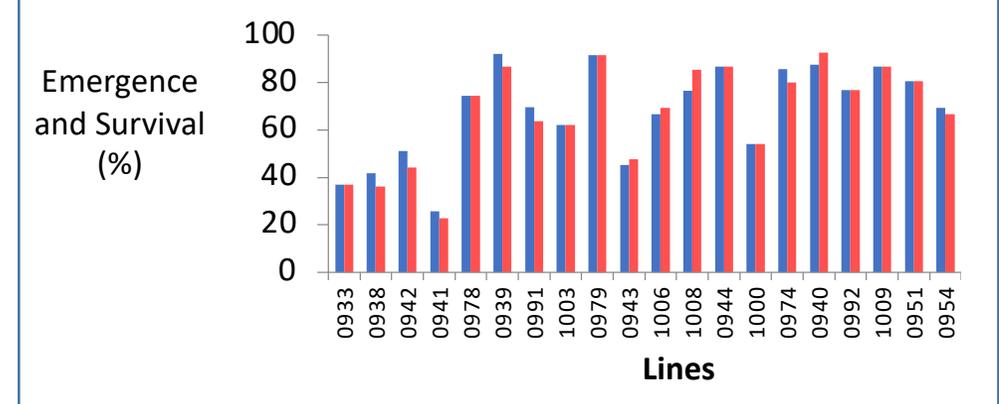
**ER anchored PHA synthase**



**Emergence (■) and Survival (■) of T<sub>2</sub> Seedlings**



**Emergence (■) and Survival (■) of T<sub>2</sub> Seedlings**



<sup>1</sup>Production 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



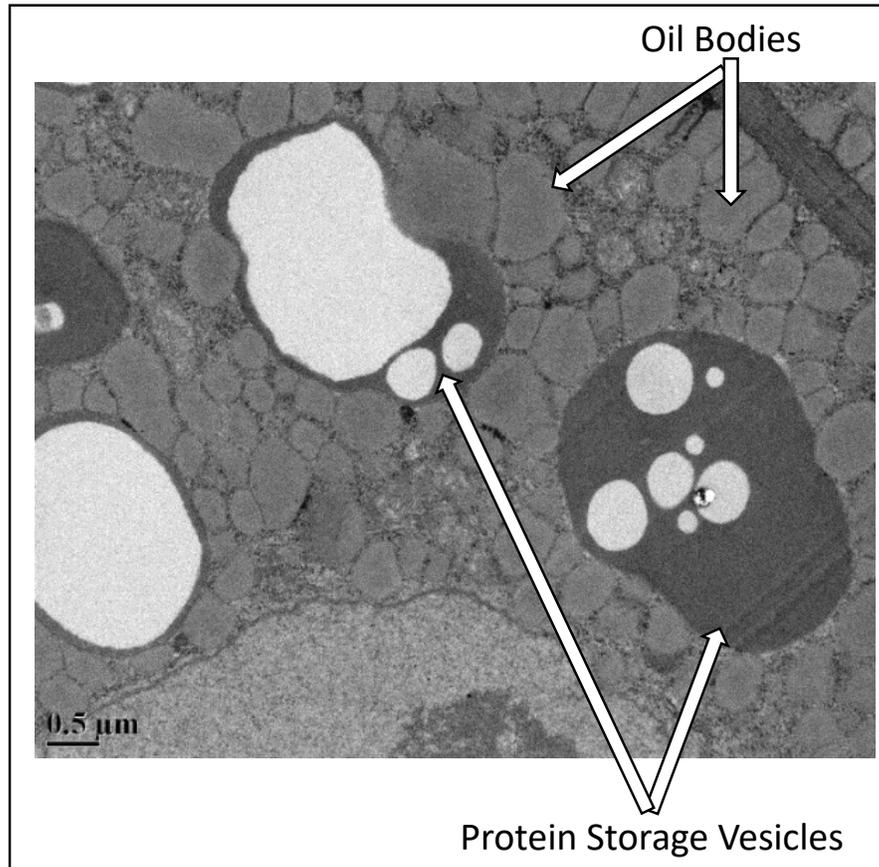
*Pursued only ER PhaC lines in later generations. PHB production more stable in ER PhaC lines.*

# PHB Polymer Accumulates as Granules in Seed

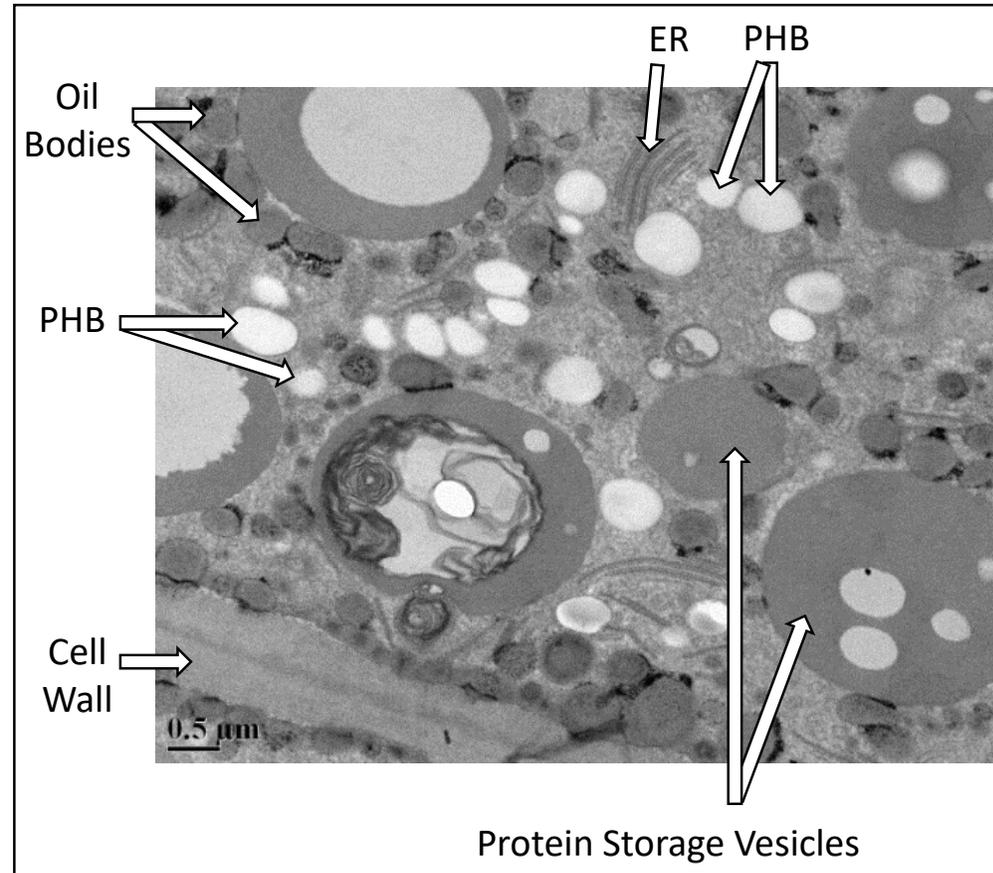


## Transmission electron microscopy (TEM) of cotyledon in imbibed seeds

Wild-type control



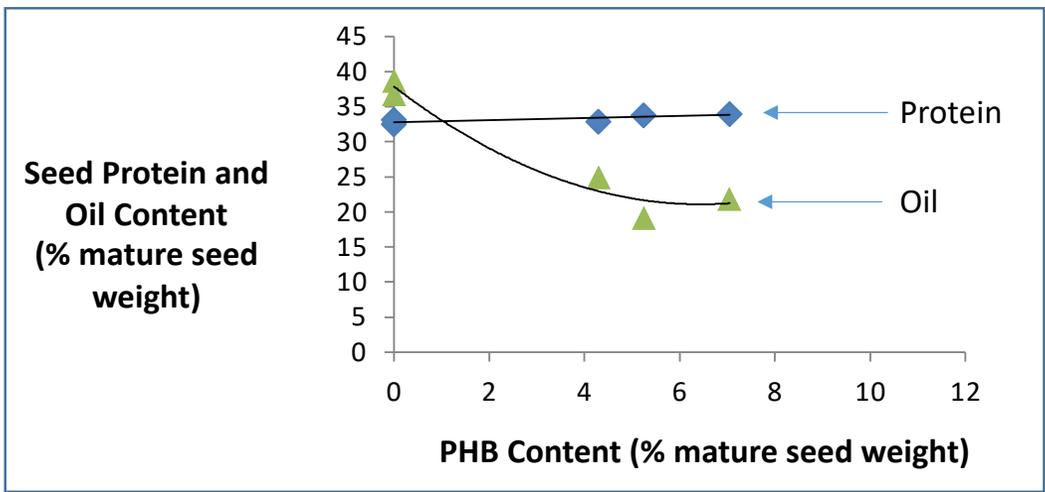
ER targeted synthase line



# PHB Produced at Expense of Oil



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

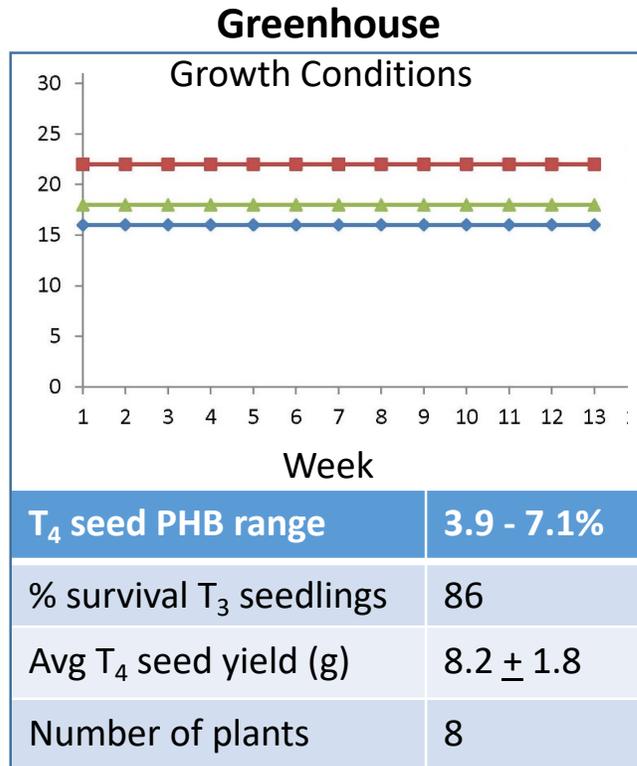


- *PHB has more value than oil*
- Looking for genes to increase carbon to seed to boost oil using GRAIN modeling platform

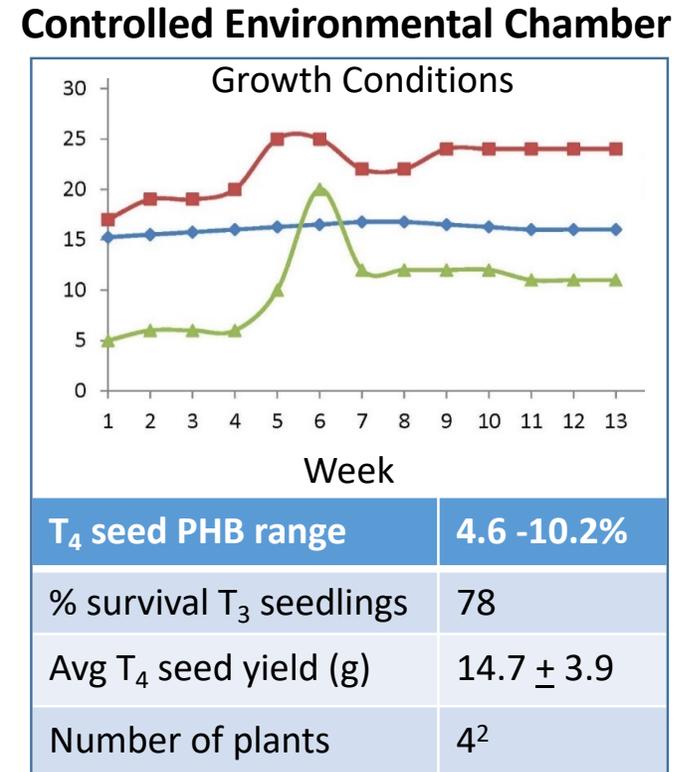
# PHB Production in Different Growth Conditions

Lines grown in greenhouse and controlled environmental chamber programmed to simulate average spring growth conditions<sup>1</sup>

- Results for best line shown



◆ Day length (h)  
■ Day temp (°C)  
▲ Night temp (°C)



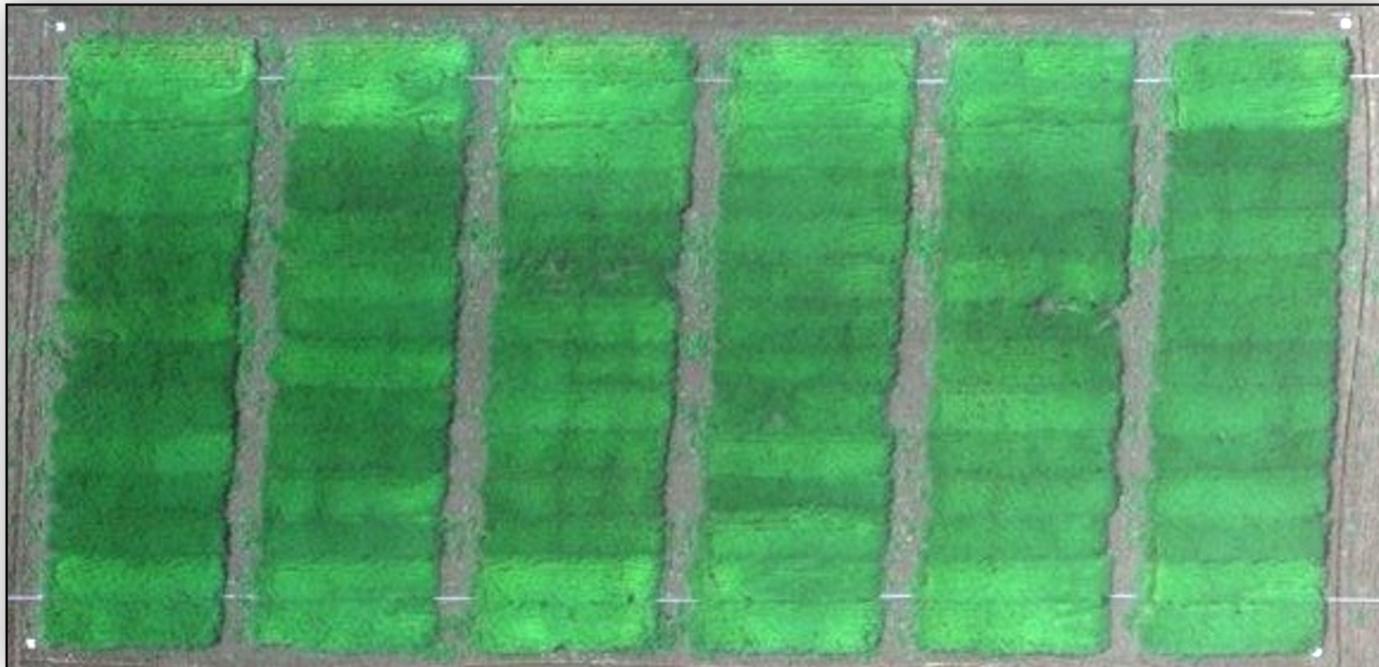
**Up to 10.2% PHB obtained in seeds of homozygous line**

14 <sup>1</sup>Temperature 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. <sup>2</sup>Size 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 billion<sup>1</sup>**

## 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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