

# Yield10 Bioscience, Inc.

**Breakthroughs in plant based PHB production** Kristi Snell, CSO and VP of Research



Sept 16, 2022

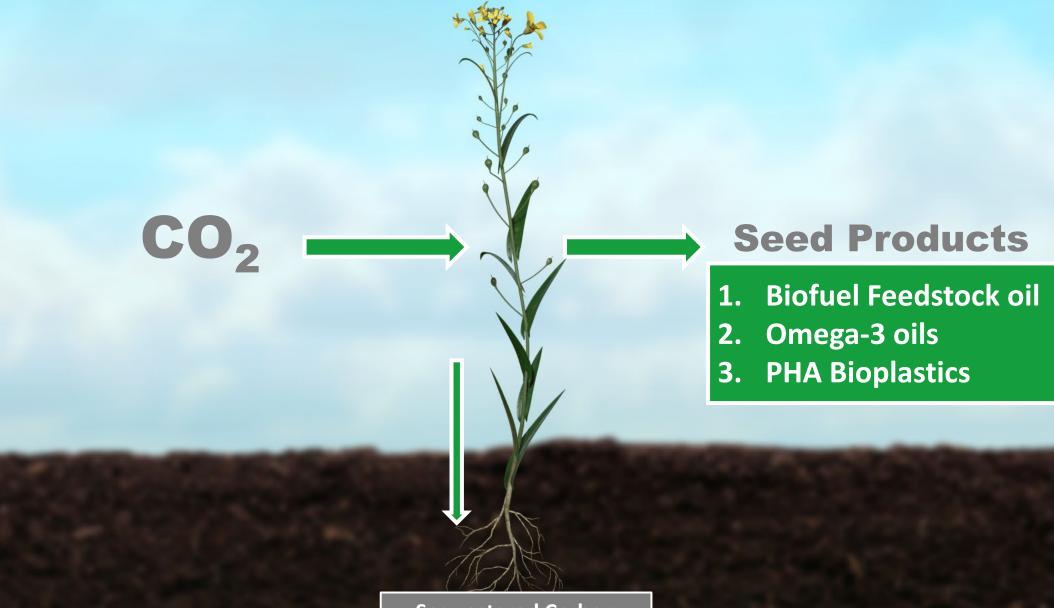
Sustainable Growth Starts with a Seed



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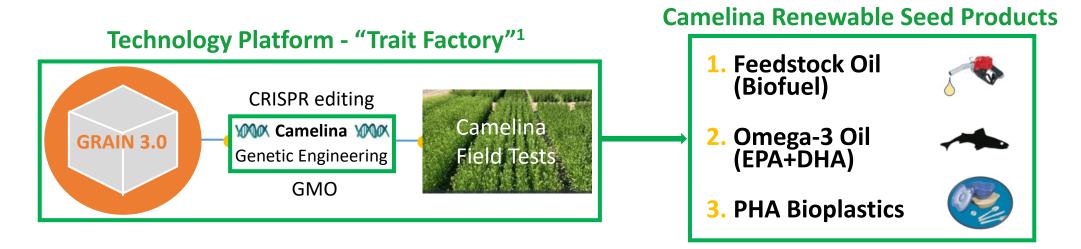
Because forward-looking statements are inherently subject to risks and uncertainties, some of which cannot be predicted or quantified and may be beyond the Company's control, you should not rely on these statements as predictions of future events. Actual results could differ materially from those projected 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.

### Yield10's Camelina Platform Oilseed Crop



### Yield10's Trait Factory and Business Models

### From Crop Science to Low Carbon Intensity (CI) Biofuels Feedstock Oil



### **Yield10: Biofuels Commercial Development Plan**

- Now: Launching proprietary Camelina with improved germplasm as low CI biofuels feedstock crop
- Next: Address growers needs with herbicide tolerance (*over-the-top weed control, tolerance to herbicide soil residues*) and disease resistance
- Medium to long-term: High-value Omega-3 (EPA+DHA) and PHA Bioplastic traits to significantly increase revenue per acre

<sup>1</sup>21 Patent Families Pending



# 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



Large scale winter Camelina growth



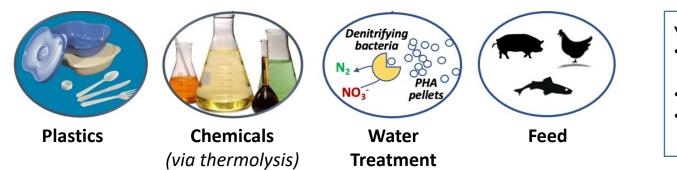
**Yield10 Camelina Review:** *Camelina sativa*, an oilseed at the nexus between model system and commercial crop. Malik et al., Plant Cell Rep., 2018

# PHA in Camelina: A Third Oilseed Coproduct



**Camelina Field** 

#### **PHA – Potential Markets**



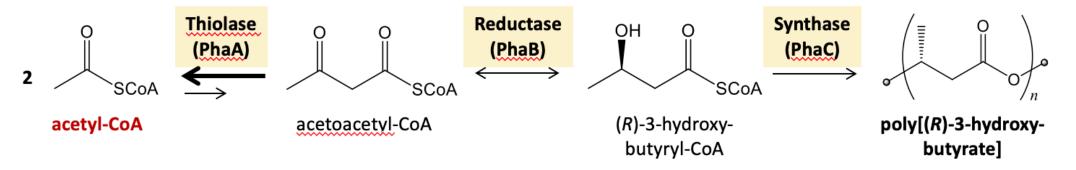
#### Yield10 references - PHB to chemicals

- Acrylates via metathesis of crotonates, Schweitzer & Snell, 2015, Org Process Res Dev, 19, 715.
- *n*-butanol, Schweitzer et al., 2015, Org Process Res Dev, 19, 710.
- Pyrolysis of P3HB/switchgrass Blends to produce crotonic acid, Mullen et al. **2014**, *J Anal Appl Pyrolysis*, 107, 40.

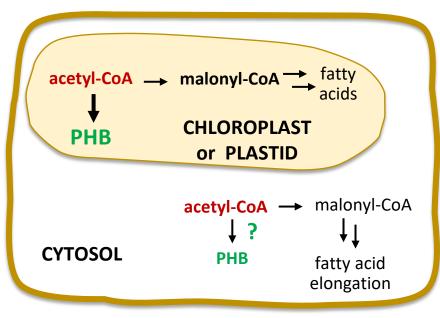


# PHB Pathway Well Suited to Oilseeds

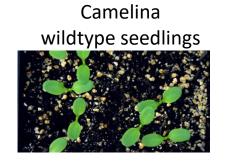
Native bacterial PHB biosynthetic pathway



**Engineered plant cell** 



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



Camelina seed specific plastid PHB producers

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

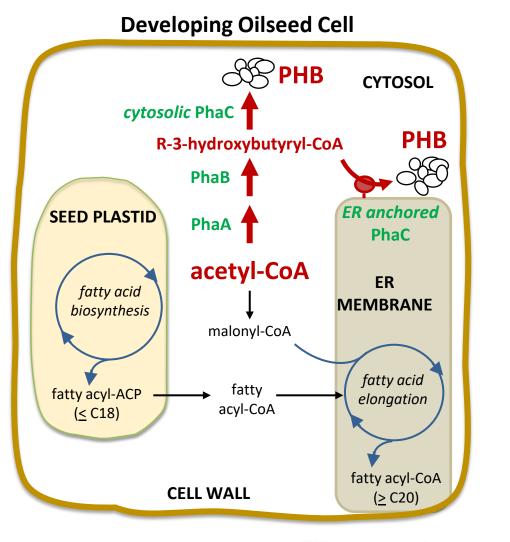
<sup>1</sup>Yield10 reference for production of PHB in Camelina seed plastids, Malik et al., 2015, *Plant Biotechnol. J.* 13, 675. <sup>2</sup>Production in cytosol of cotton fibers, John & Keller, 1996, *P. Natl. Acad. Sci. USA.* 93, 12768.

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

#### Two genetic constructs

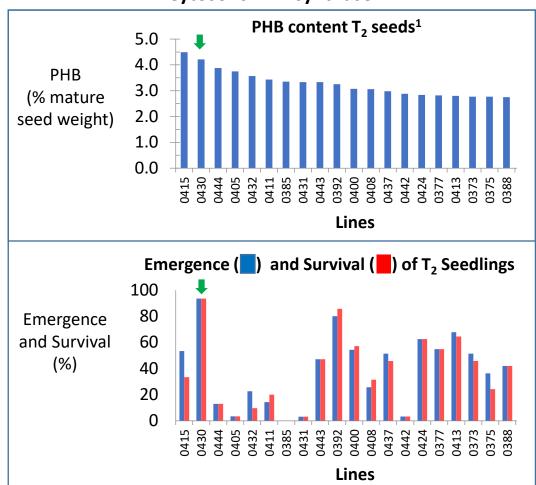
- <u>Construct 1</u>: All enzymes targeted to cytosol
- <u>Construct 2</u>: PhaA, PhaB targeted to cytosol; PhaC targeted to cytosolic face of endoplasmic reticulum (ER)
- Seed-specific expression constructs transformed into Camelina, lines isolated



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

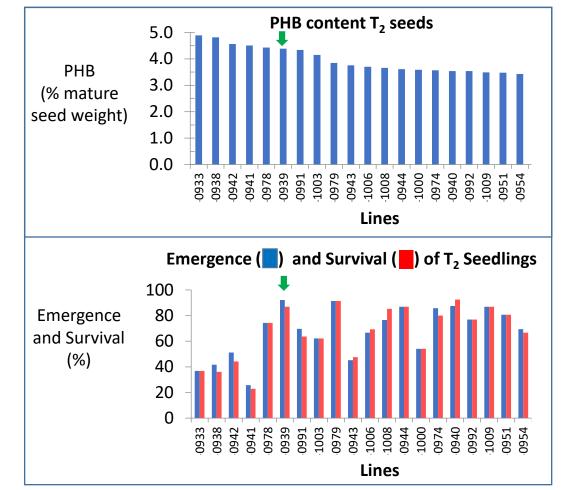
#### Second generation (T<sub>2</sub>) seeds contained up to 14x reported<sup>1</sup> highest level of cytosolic PHB

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



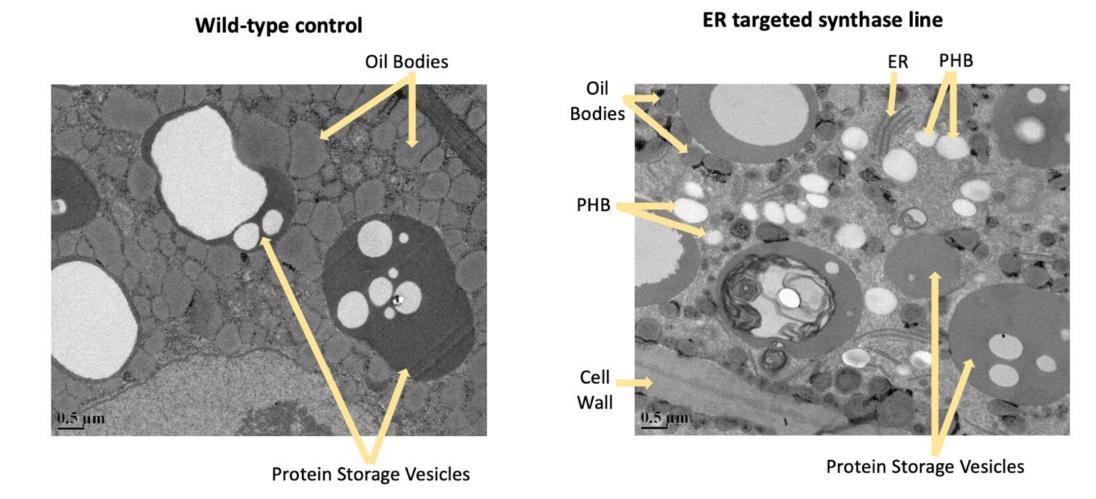


**ER anchored PHA synthase** 



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

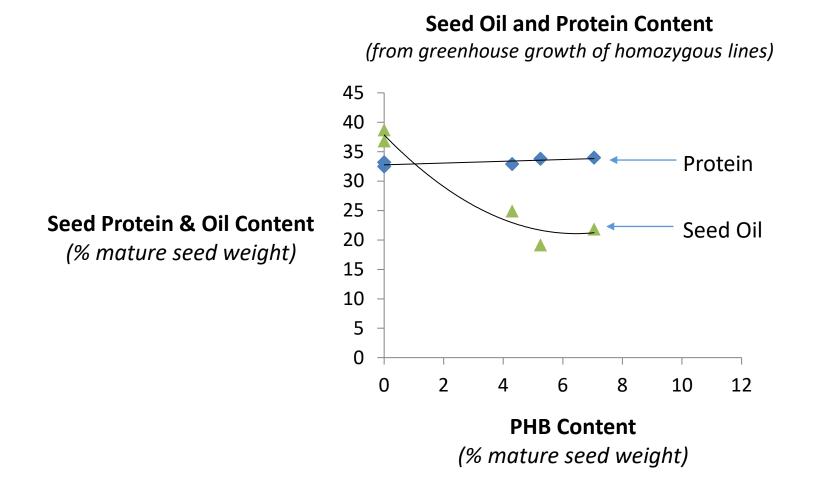
### Transmission electron microscopy (TEM) of cotyledon in imbibed seeds



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### PHB Produced at Expense of Oil



### 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.

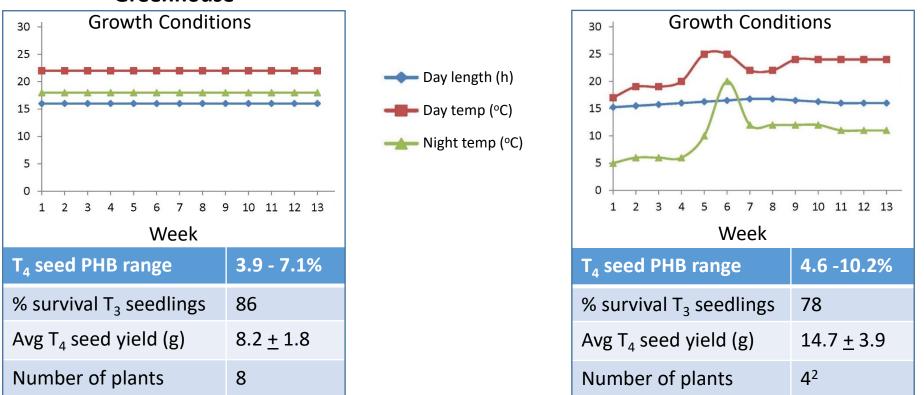


# PHB Production in Different Growth Conditions

Greenhouse

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

Results for best line shown



#### **Controlled Environmental Chamber**

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Up to 10.2% PHB obtained in seeds of homozygous line in chamber - 30X highest previously reported level <sup>3</sup>

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

# Field Trials of Prototype ER Targeted PHB Lines

**2020** Replicated field plots, line sorting



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

**2021** 0.2 acre seed scale up



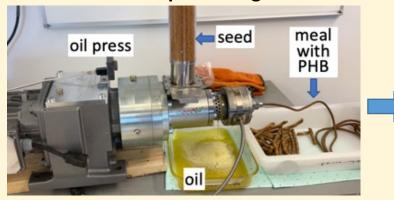
Drone photo, U.S. scale up site Plants produced 6% PHB

2022 Acre-scale seed scale up



Maturing plants, U.S scale up site Seed harvested, PHB will be measured





Seed crushing with oil press, removal of oil

#### Prototype PHA Camelina products



PHB polymer<sup>1</sup>



Seed processing, product prototyping, sampling & other business development activities



<sup>1</sup>After solvent extraction of PHB containing meal

#### PHA Trait Leverages Elite Camelina Platform - Address High Value Markets

#### **PHA Market Opportunity**

Growing global demand for biobased polymers

#### **Development Highlights and Milestones**

- Pilot scale activities, prototype ER targeted PHB line
  - Produced seed at acre-scale. Will isolate PHA for process development and product sampling

#### • PHA trait optimization R&D targets

- Increase PHA content to 10-20% of seed weight
- Demonstrate co-polymer production
- Field test PHA winter Camelina lines
- Combine PHA trait with traits required for farmer to grow Camelina (*herbicide tolerance/disease resistance*)
- Pursue collaborations with industry







# Yield10 Bioscience, Inc.

**Breakthroughs in plant based PHB production** Kristi Snell, CSO and VP of Research

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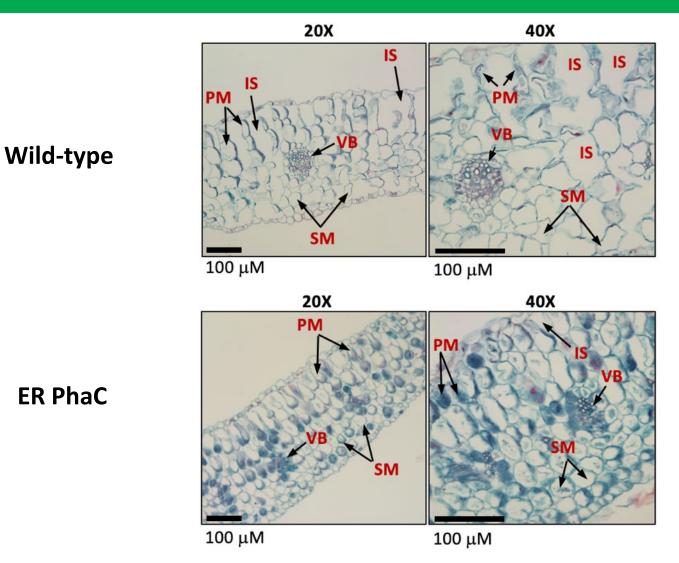


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### **Seedlings of Cytosolic PHB Producers**



Intercellular spaces significantly reduced in cotyledons of PHB producing lines

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

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