Breakthroughs in Plant Based PHB Production: Harnessing Nature to Heal Nature

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

Trait Factory – Products and Path to Revenue

Three potential revenue streams each with different commercialization paths

TRAIT FACTORY

Performance Traits (Licensing to Ag Majors)

Seed yield | **Oil content**

- Canola, soybean, corn - field trials
- Licensing to Ag majors to maximize acreage

Agreements

Bayer CropScience | Forage Genetics International | Simplot

future | future | future

R&D Services

GRAIN Gene Discovery Platform

- Funded R&D
- Milestone payments
- License revenue

Agreements

future | future

Cash Cover Crops (Camelina - Product Sales)

Nutritional oils | **PHA biomaterials**

- Business participation
- Product sales, partners along the value chain

Agreements

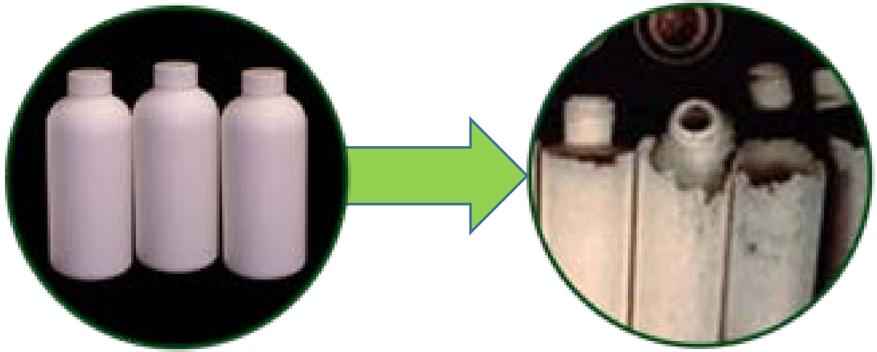
future | future

Multiple paths to revenue

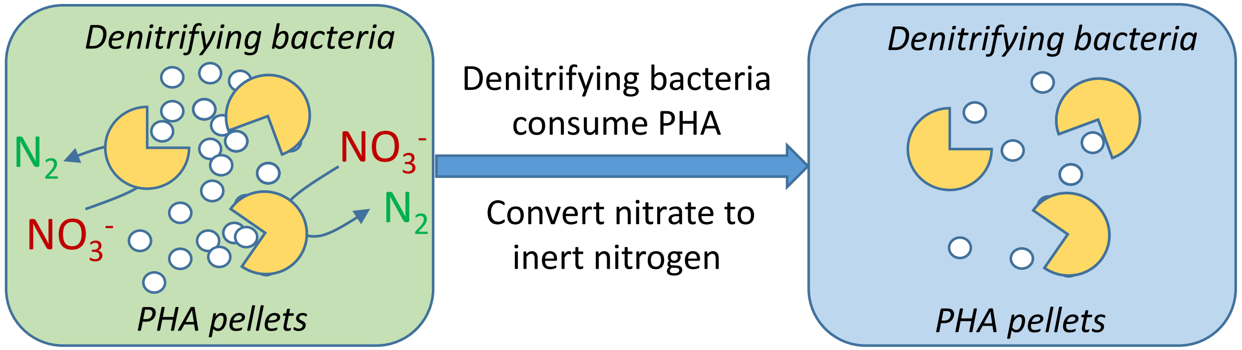
Polyhydroxyalkanoate (PHA) biomaterials

- Renewable, biodegradable class of biomaterials produced by some microorganisms as reservoir of stored carbon and energy
- Fully degradable in all biologically active environments (soil, rivers, oceans, compost, sewage, etc.)
  - Intracellular and extracellular depolymerases that degrade the polymer
- Unique features of polymers will allow use in multiple applications
  - Plastics applications have historically been targeted
  - ***Pellets for water treatment simpler first commercial product***

Renewable Biodegradable Plastics



Water Treatment Application



High nitrate = algal growth

Low nitrate = NO algal growth

Barriers to Commercialization of PHAs

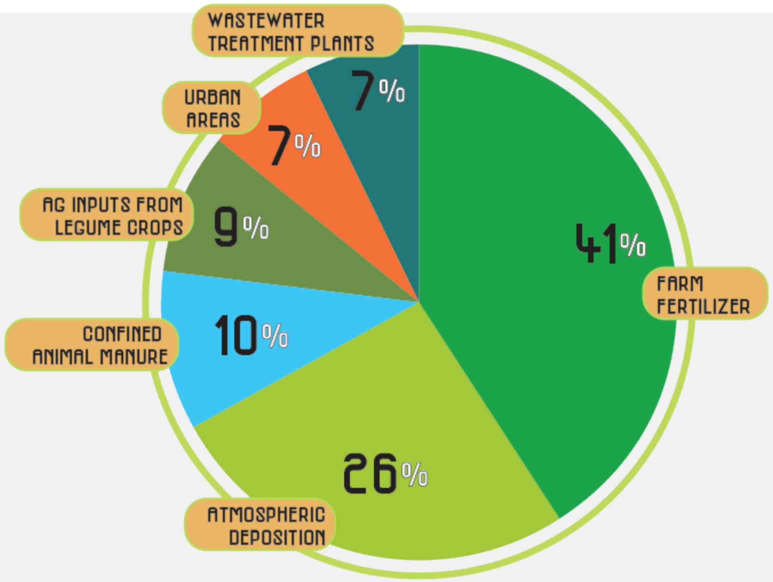
Market adoption has been severely restricted by high cost

- Production by bacterial fermentation:
  - Successful high-level production in bacteria through fermentation, cost too high for most applications

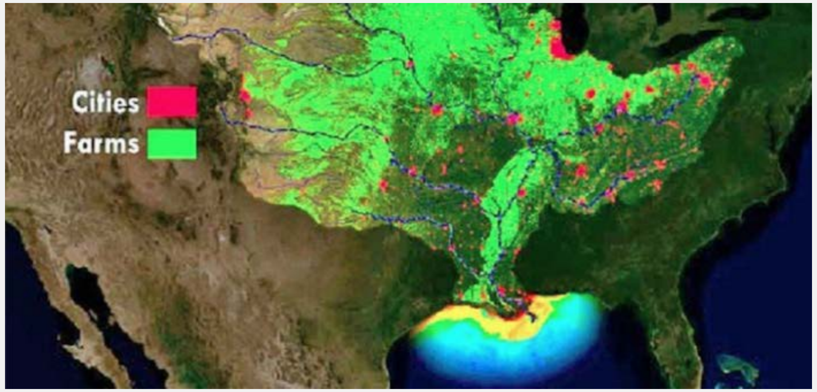
- Production in plants:
  - Highly favorable economics and lifecycle for large scale production – **BUT** – high level production in plants often impairs plant growth or seedling emergence/survival

Nutrient Run-Off Impacts Human Health and the Environment

Environment



Sources of Nitrogen Delivered to The Gulf of Mexico[1]



Dead zone in Gulf of Mexico linked to nutrient inputs from cities and farms in Mississippi River Basin[2]

- *Nutrient runoff into Gulf causes algal blooms*
- *Decomposition of algae creates low oxygen levels that kill fish and marine life*
- ***6,950 mile dead zone measured by NOAA in 2019[3]***

Human health

"Considering all studies, the strongest evidence for a relationship between drinking water nitrate ingestion and adverse health outcomes (besides methemoglobinemia) is for colorectal cancer, thyroid disease, and neural tube defects. Many studies observed increased risk with ingestion of water nitrate levels that were below regulatory limits." Mary H. Ward et., al. 2018, International Journal of Environmental Research and Public Health, 15, 1557.

1. https://www.usgs.gov/special-topic/water-science-school/science/nitrogen-and-water?qt-science_center_objects=0#qt-science_center_objects
2. https://www.workboat.com/news/coastal-inland-waterways/noaa-sees-very-large-dead-zone-for-gulf-of-mexico/
3. National Oceanic and Atmospheric Administration (NOAA). https://www.noaa.gov/media-release/large-dead-zone-measured-in-gulf-of-mexico

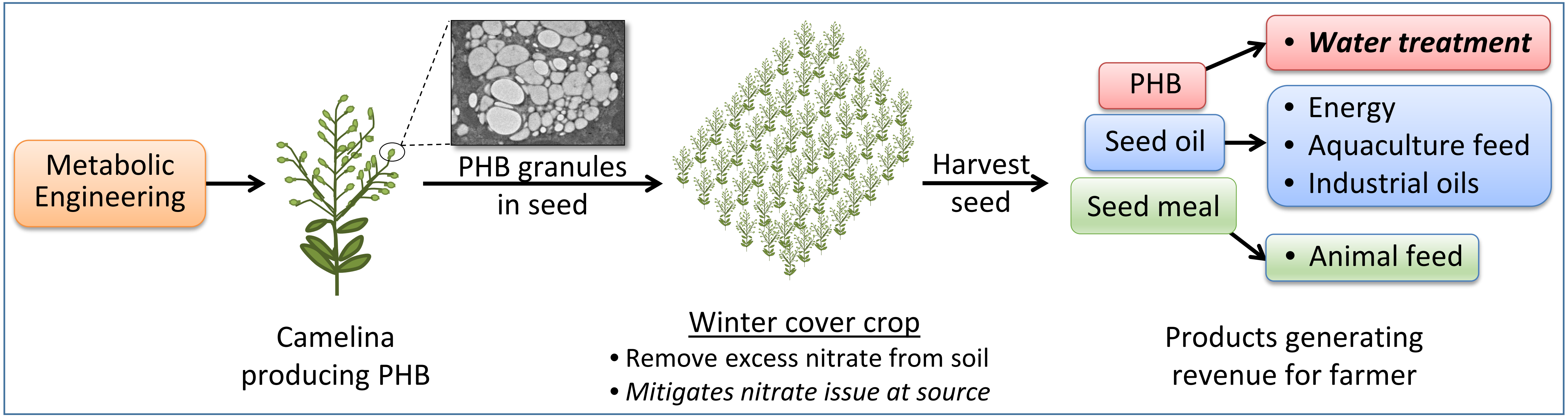
Production of Polyhydroxybutyrate (PHB) in Oilseeds

PHB cover crop: Harnessing nature to heal nature

Cover crop mitigates nutrient runoff in field, produces product for water treatment

Choice of crop – Camelina[1]

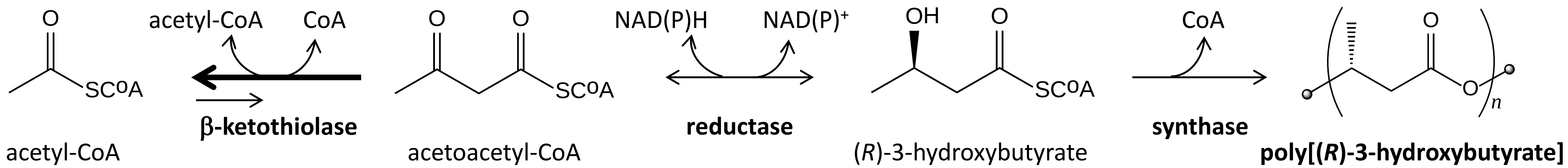
- Seed oil levels typically 40% of seed weight (depends on cultivar and growth conditions)
- Does not outcross with *Brassica napus* or other crop Brassicas
- Both spring and winter varieties available
  - *winter varieties cover crop after corn and soybean to prevent nitrogen runoff*



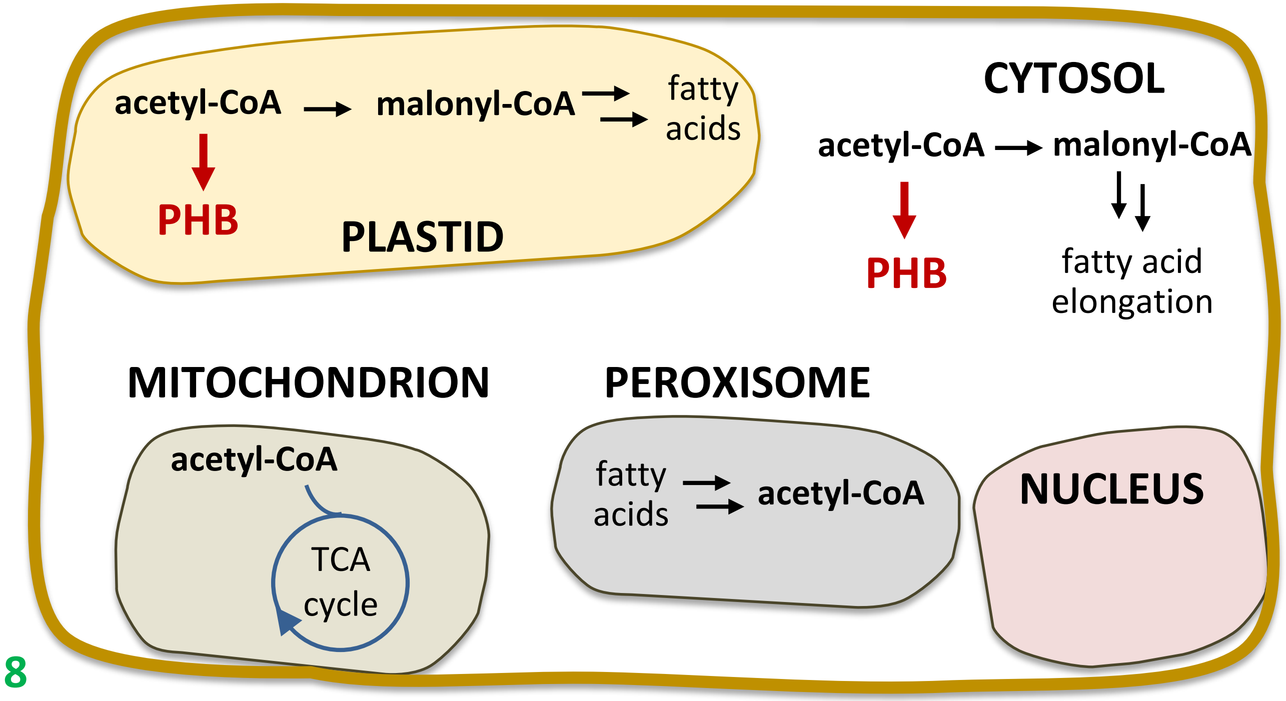
[1]**Yield10 Camelina Review:** Malik et al., 2018, Plant Cell Rep., 37, 1367

PHB pathway – substrate acetyl-CoA well suited to oilseeds

PHB biosynthetic pathway



Targeted sites for production of polymer in seeds



Chloroplasts/plastids have yielded highest levels of PHB in plants

- Little production in cytosol and peroxisome
- No production demonstrated in mitochondria

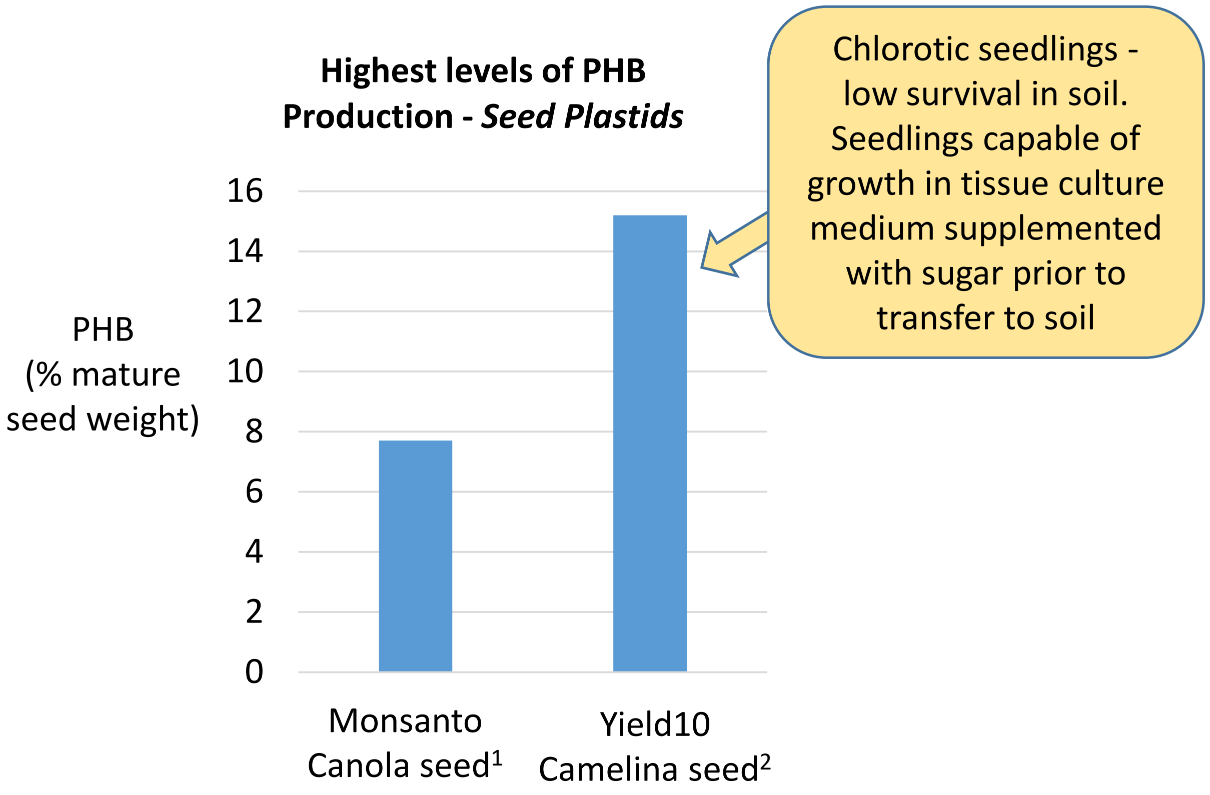
Yield10 Reviews

Snell et al., 2015, Production of novel biopolymers in plants: recent technological advances and future prospects, Curr. Opin. Biotech., 32C, 68.

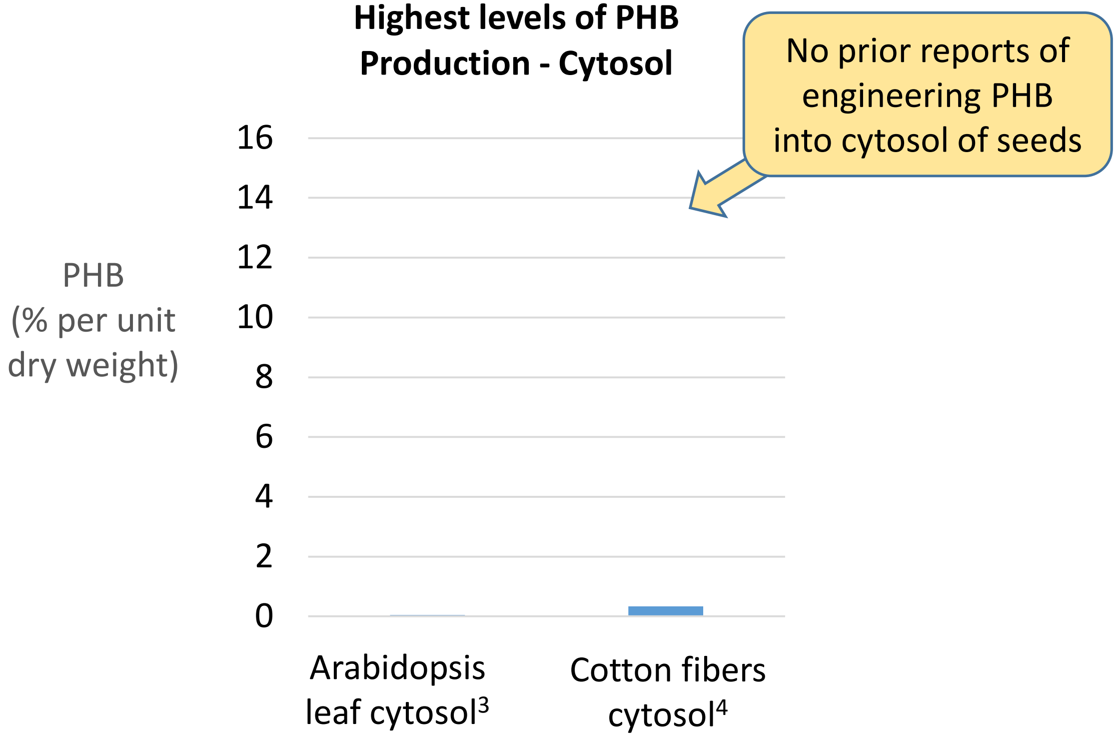
Somleva et al., 2013, PHA bioplastics, biochemicals, and energy from crops Plant Biotechnol. J., 11, 233

Prior Work: Production of PHB in Oilseeds

Prior work in seed plastids



Prior work in seed cytosol



References. [1]**Houmiel et al.**, 1999, Planta, 209, 547. [2]**Malik et al.**, 2015, Plant Biotechnol. J., 13, 675. [3]**Poirier et al.**, 1995, Int. J. Biol. Macromol, 17, 7. [4]**John & Keller**, Proc. Natl. Acad. Sci. USA, 93, 12768.

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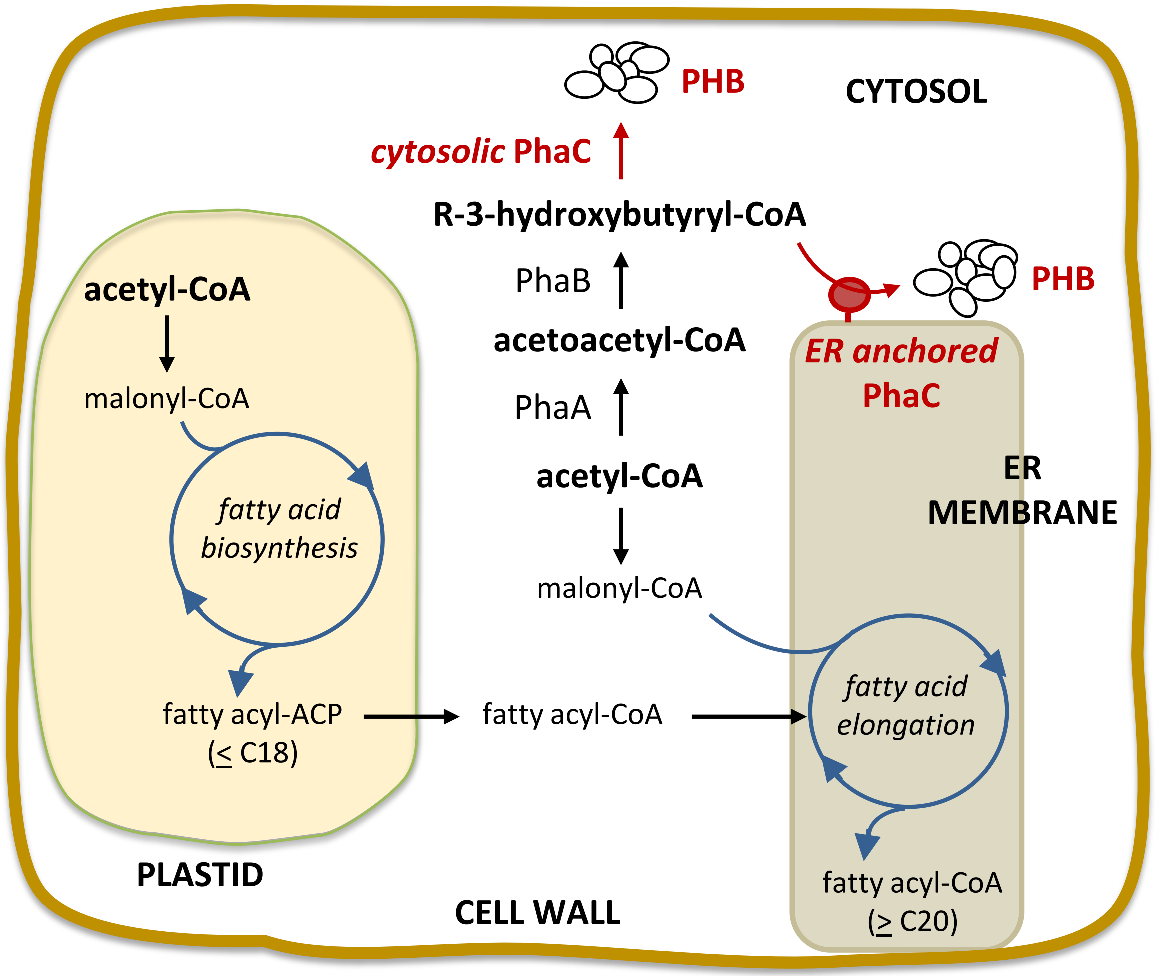
Revisit production of PHB in cytosol

Two genetic constructs created that differ in targeting of polymerization enzyme

- **Construct 1.** All enzymes targeted to cytosol
- **Construct 2.** Thiolase (PhaA) and reductase (PhaB) enzymes targeted to cytosol; PHA synthase (PhaC) anchored to the cytosolic face of the endoplasmic reticulum (ER)

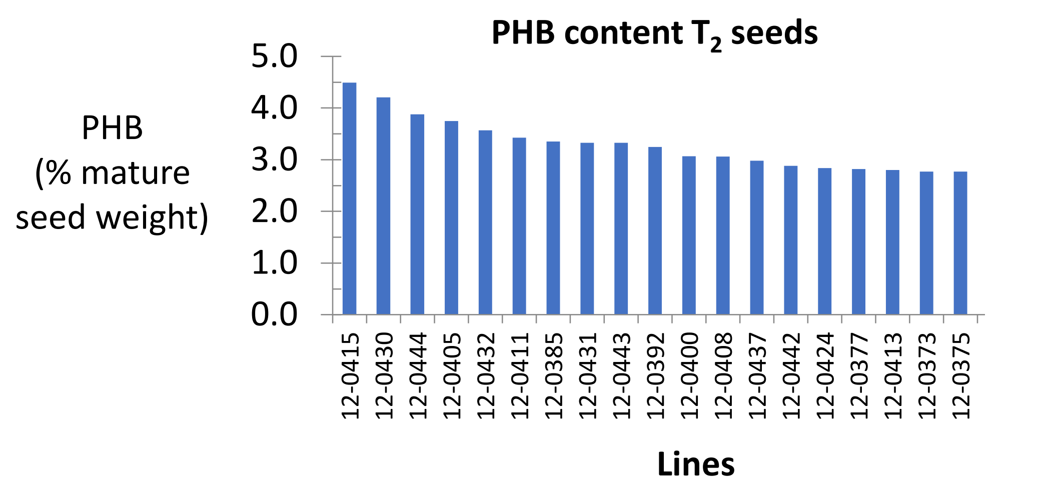
Camelina plants transformed, T_1 generation seeds isolated, T_1 plants grown in soil in greenhouse, T_2 generation seeds harvested

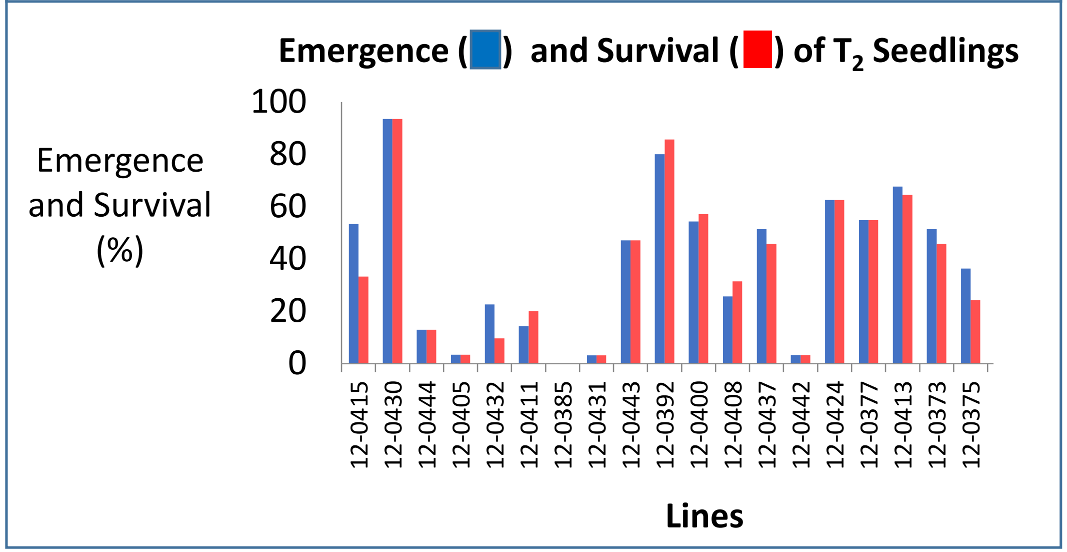
DEVELOPING OILSEED CELL



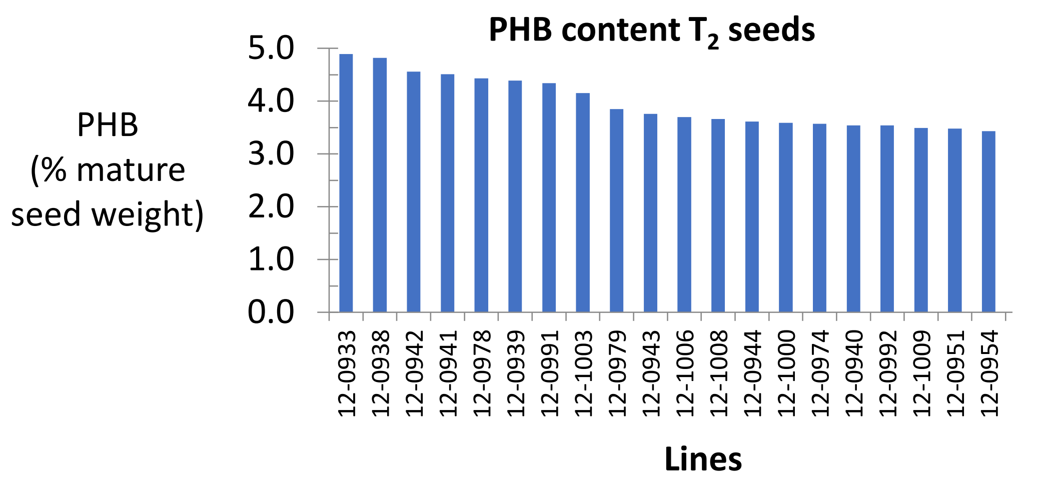
T_2 Generation Seed: PHB Content and Survival of lines

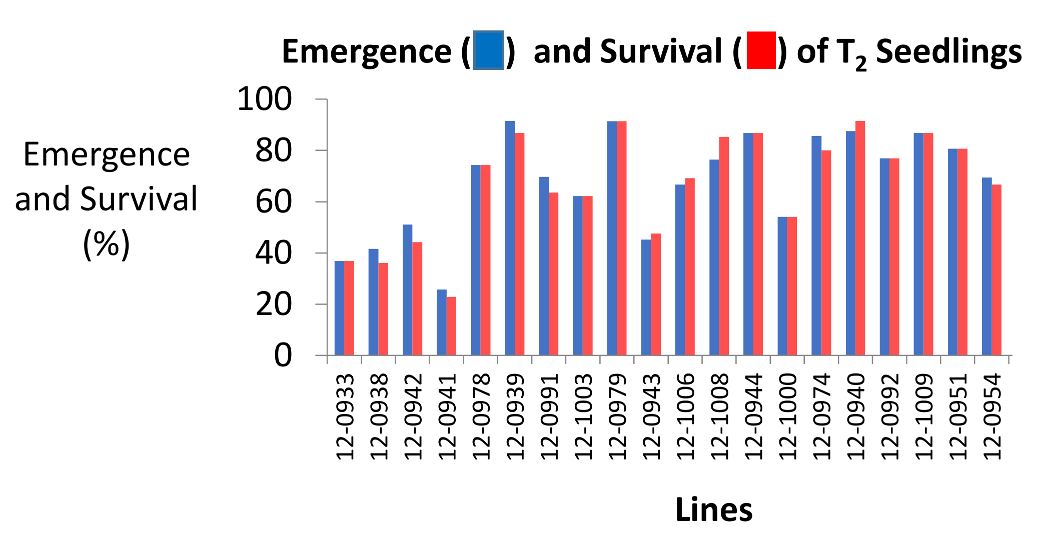
Cytosolic PHA synthase
(top 20 PHB producers)





ER anchored PHA synthase
(top 20 PHB producers)





Cytosolic PHB producers, healthy seedlings with narrow cotyledons

Phenotype of 7 day old seedlings

Wild-type

Cytosolic PhaC
4.5% PHB
53% emergence
33% survival

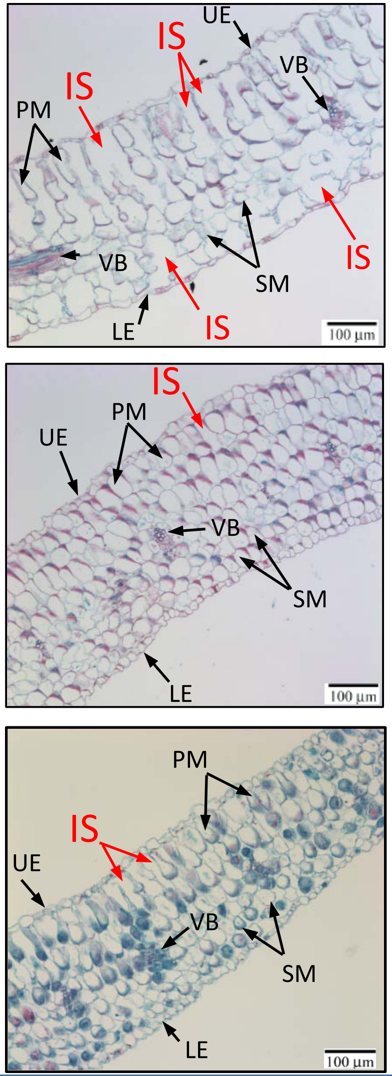
ER PhaC
4.4% PHB
92% emergence
87% survival

Light microscopy of cotyledon cross sections

Wild-type

Cytosolic PhaC

ER PhaC



<u>20X magnification</u>
IS = Intercellular space
VB = vascular bundle
PM = palisade mesophyll
SM = spongy mesophyll
UE = upper epidermis
LE = lower epidermis

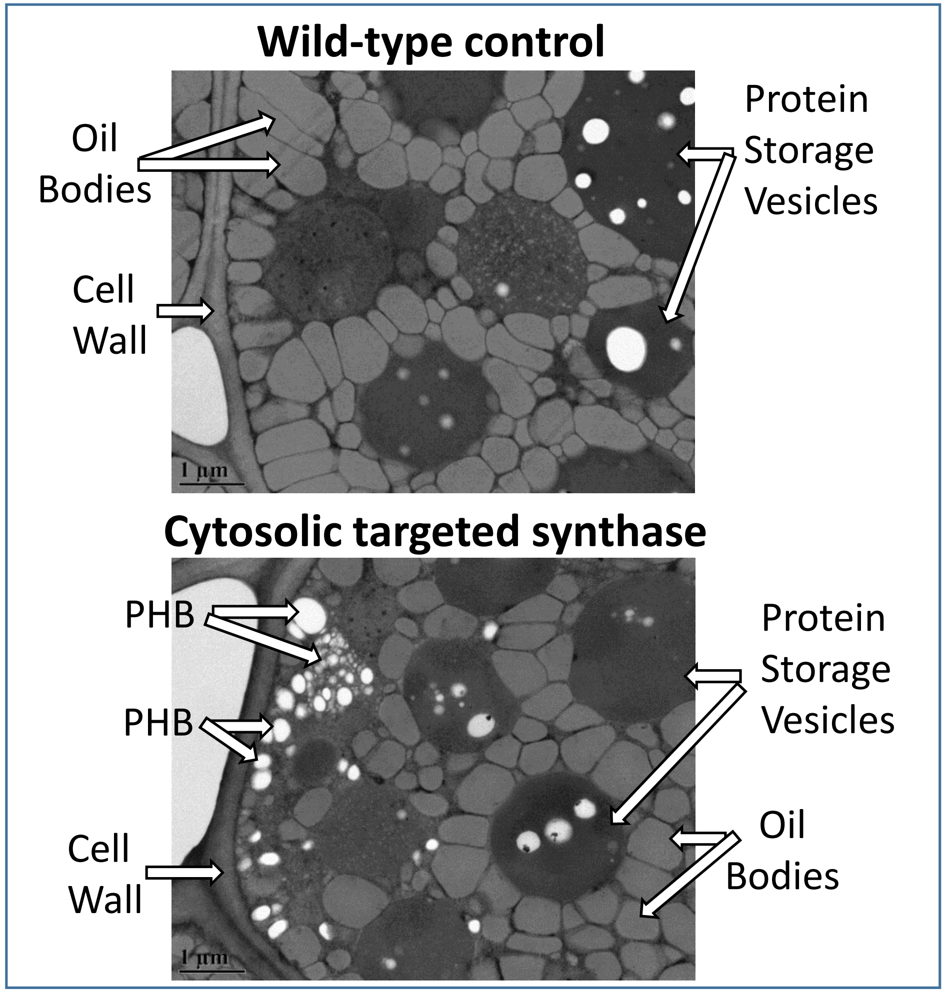
Light microscopy of representative samples.

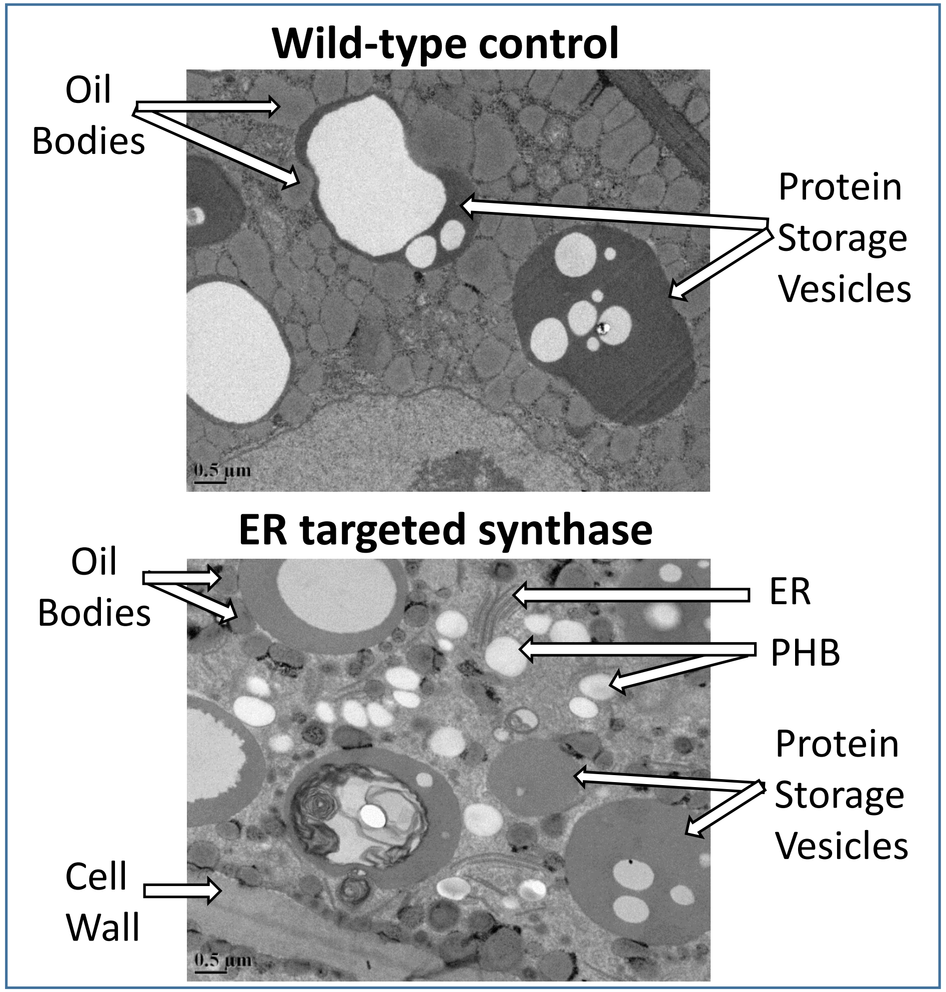
Visible differences in:
- size and presence of intercellular spaces (IS)
- elongation of palisade layer

Spatial Distribution of PHB Granules in Seeds

Transmission electron microscopy (TEM) of cotyledon in imbibed T_2 seeds

- Polymer accumulates as granules within seed





Polymer Production in Advanced Generations

ER targeted synthase showed clear advantage over cytosolic targeted synthase in homozygous lines

- **Cytosolic targeted synthase**: PHB production dropped from high of 4.5% PHB (T_2 seeds) to 2.9% PHB (T_3 seeds)
- **ER targeted synthase**: Homozygous lines producing T_3 seeds with 9.1% and 6.8% PHB isolated

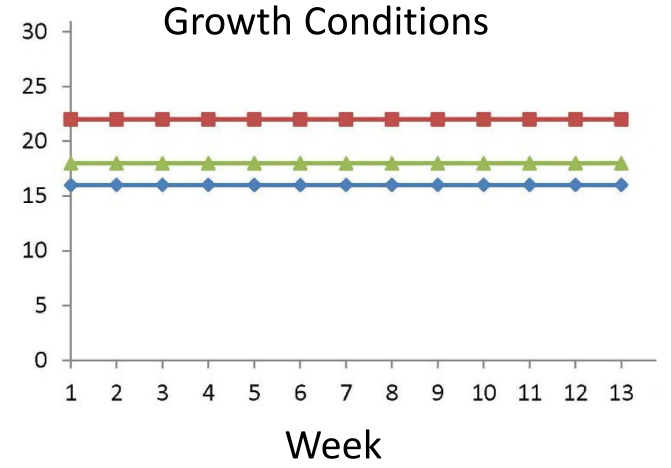
Only lines with ER targeted synthase pursued in later generations

ER Targeted Synthase: PHB Production in T_4 Seeds

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

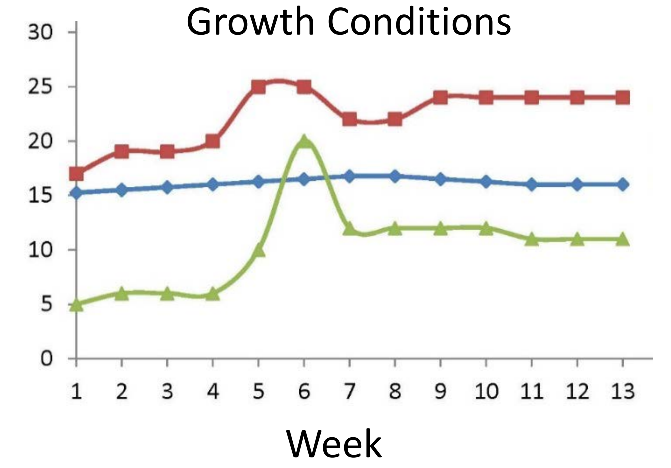
- Results for best line shown

Greenhouse



T_4 seed PHB range	3.9 - 7.1%
% survival T_3 seedlings	86
Avg T_4 seed yield (g)	8.2 $\pm$ 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 $\pm$ 3.9
Number of plants	4[2]

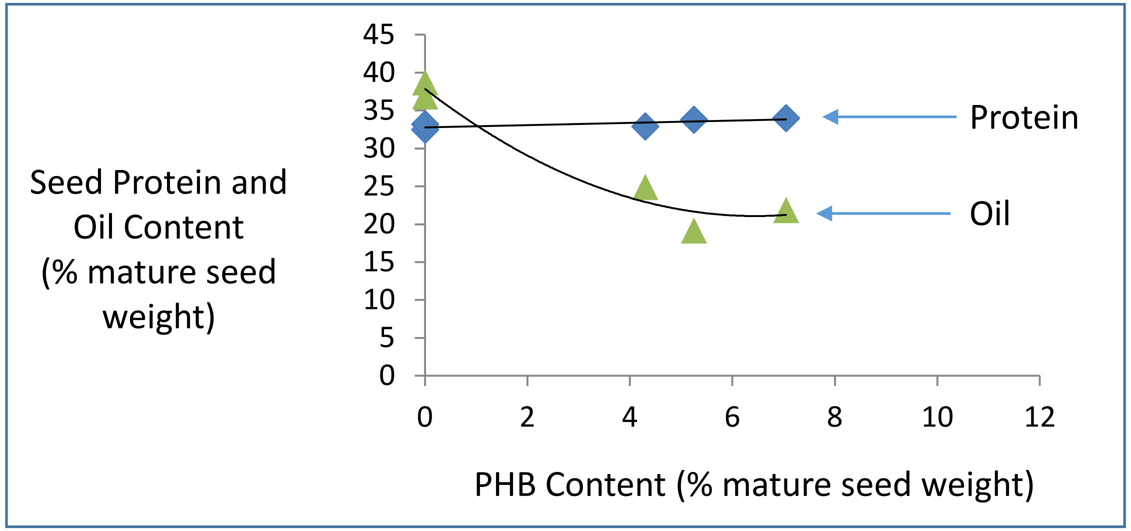
Up to 10.2% PHB obtained in T_4 seeds

[1]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. [2]Size of growth chamber limited number of replicates

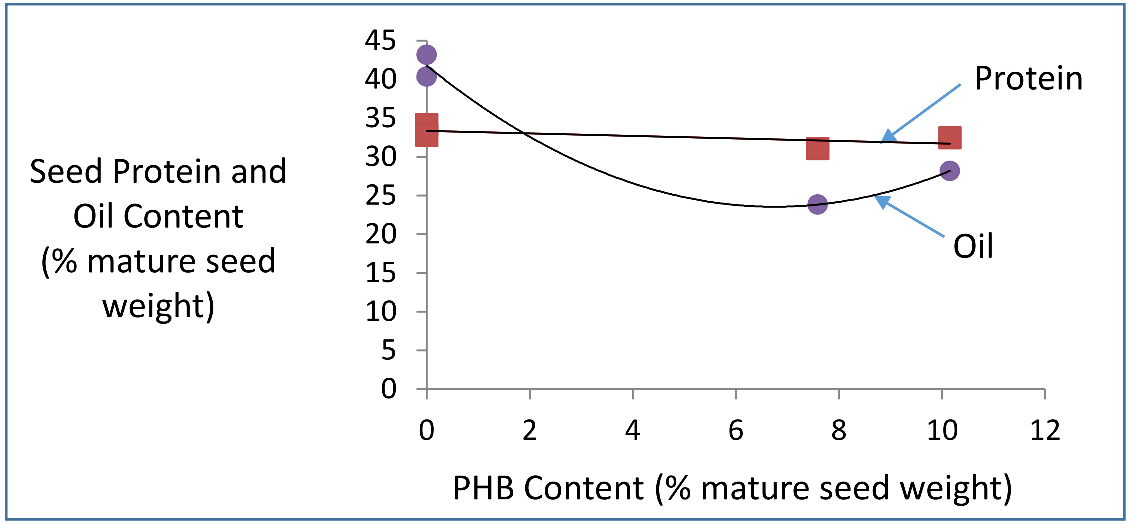
Partitioning of Carbon in PHB Producing Lines

Oil and protein content measured in top producing plants from greenhouse and controlled environmental chamber (simulated spring conditions) growth

Greenhouse



Controlled Environmental Chamber



- **Production of PHB reduces seed oil content**
  - *PHB has more value than oil*
  - Looking for genes to increase carbon to seed to boost oil using GRAIN modeling platform
- **Little difference in protein content observed with PHB production**
- **Higher levels of PHB and oil observed in plants grown under simulated spring conditions (chamber growth)**

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Conclusions and Future Directions

- Stable PHB production achieved in homozygous Camelina seeds by anchoring polymerization enzyme PHA synthase to endoplasmic reticulum (ER)
- Levels up to 10.2% of mature seed weight achieved when grown under controlled conditions simulating spring in Canadian prairies
- PHB produced at expense of oil but not protein
- Cotyledons healthy and narrower than wild-type controls
  - Significant improvement over plastid PHB producers that had chlorotic cotyledons with low survival[1]
- Field trials planned
- Using GRAIN modeling platform to identify genes to increase PHB and/or oil in ER targeted PHB lines
- Investigating use of PHB producing cover crop to address nutrient runoff
  - Cover crop mitigates nutrient runoff at source, product PHB used to treat contaminated water
  - Example of agricultural product to correct a serious negative environmental consequence of increased food production and consumption

[1]**Malik et al.**, 2015, Plant Biotechnol. J., 13, 675

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QUESTIONS?

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