Pushing carbon into fatty acid synthesis to produce higher-oil plants

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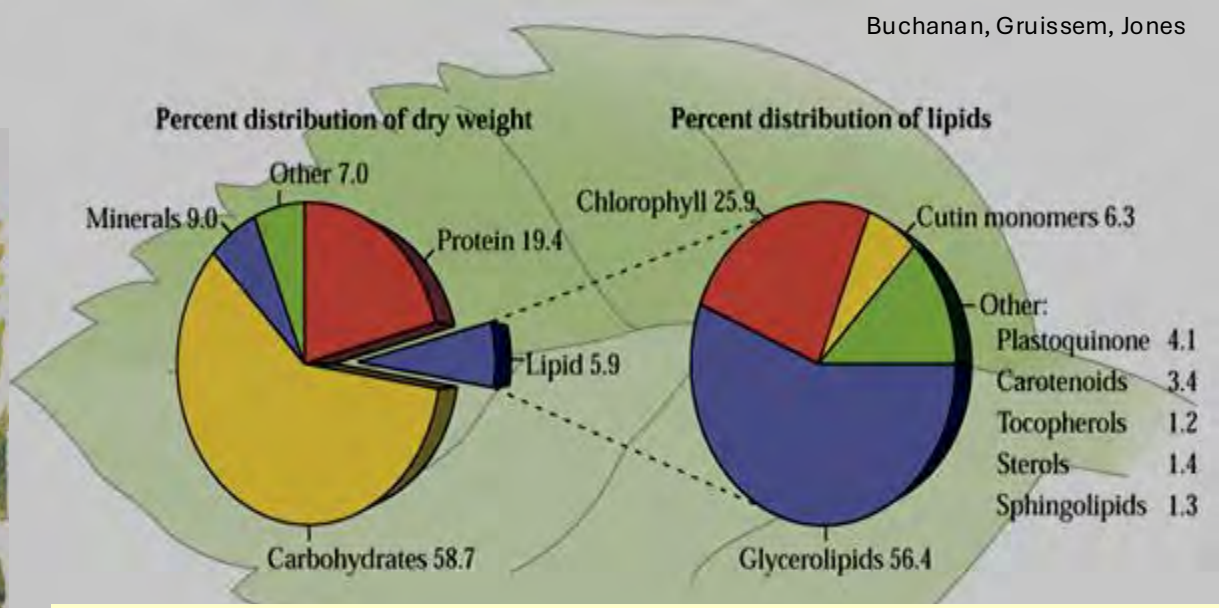


Lipids are a minor (6%) part of leaf dry matter, but a major part of many seeds

sunflower
45%

soybean
20%

canola (rapeseed)
50%

sources of vegetable oils

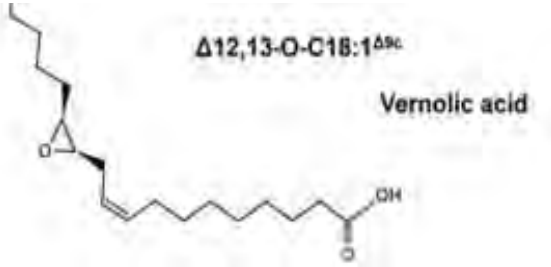
Impact: oilseeds and vegetable oils

Agriculture: Oilseeds represent $280B in world trade for 2023

Health: As much as 25% of human caloric intake is derived from vegetable oils

Environment: Cheap ($1.20/kg), renewable, alternative to petroleum for combustible fuel

Industrial: Plants are a reservoir of fatty acid diversity - synthesize over 200 different fatty acids. Diverse chemical and physical properties offer unlimited potential as cheap feedstocks for chemical & fuel industries

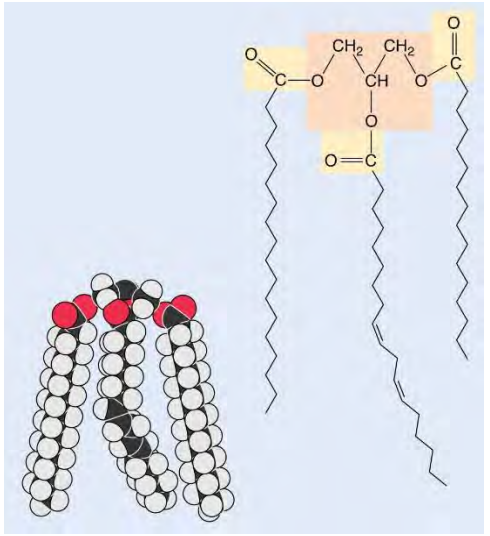


Outlook: oilseeds and vegetable oils

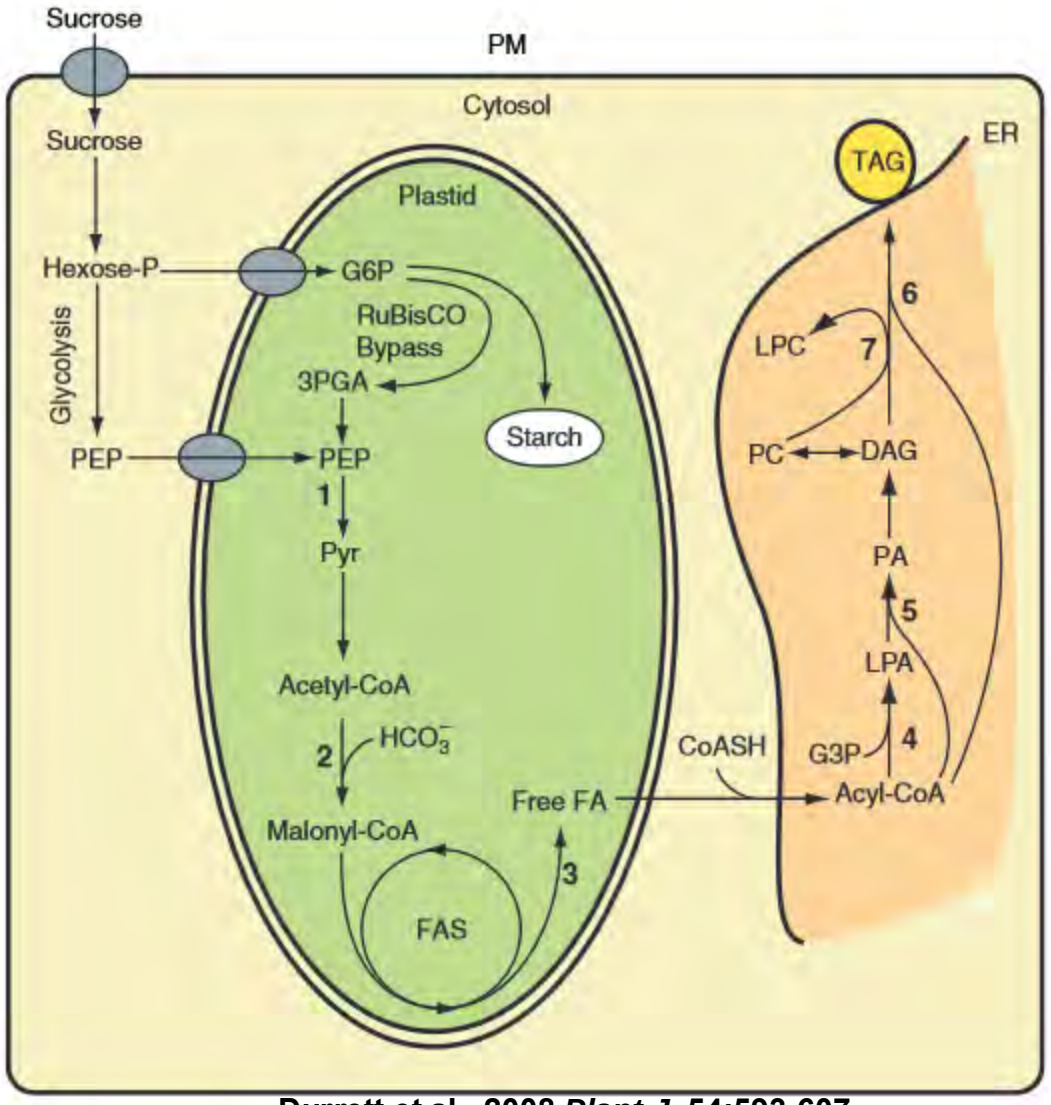
- Plant seeds are renewable sources of oil for food, chemical feedstocks, and combustible fuel – CO_2 and sun as input, pressed oil output used directly
- Demand for vegetable oils is strong and projected to double by 2050
- Sustaining all three growing industries requires the development of “cover” oilseed crops and higher-yielding varieties

Biotechnology goals for oilseeds

- **Increase healthful, decrease unhealthy fatty acids … a moving target**
- **Engineer "designer oils" for industrial & bioenergy applications, e.g. medium chain oils for SAF (DOE)**
- **Improve oil yield (NSF, USDA)**

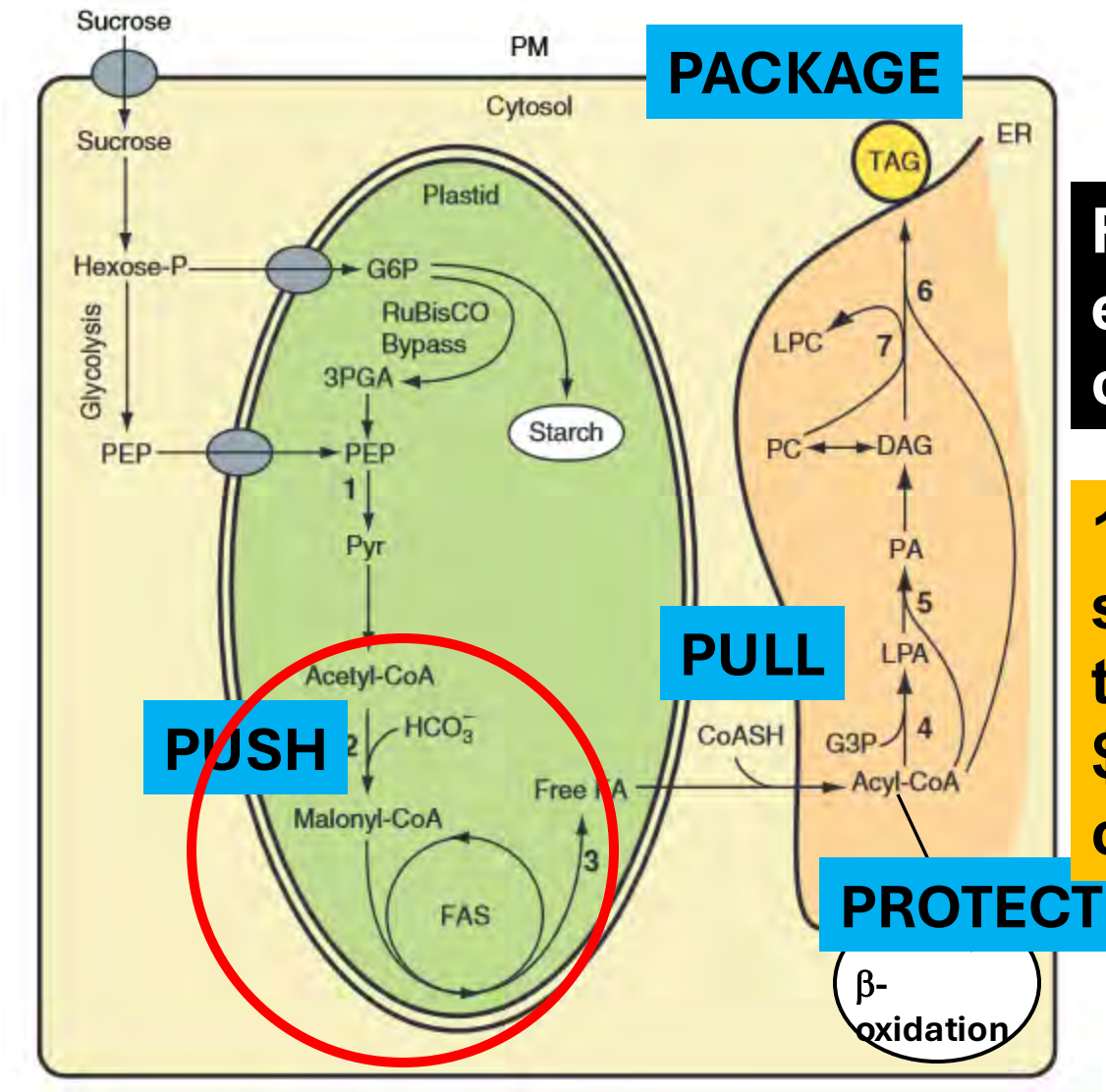


Oil (triacylglycerol) biosynthesis requires multiple pathways residing in different plant organelles



Durrett et al., 2008 *Plant J.* 54:593-607

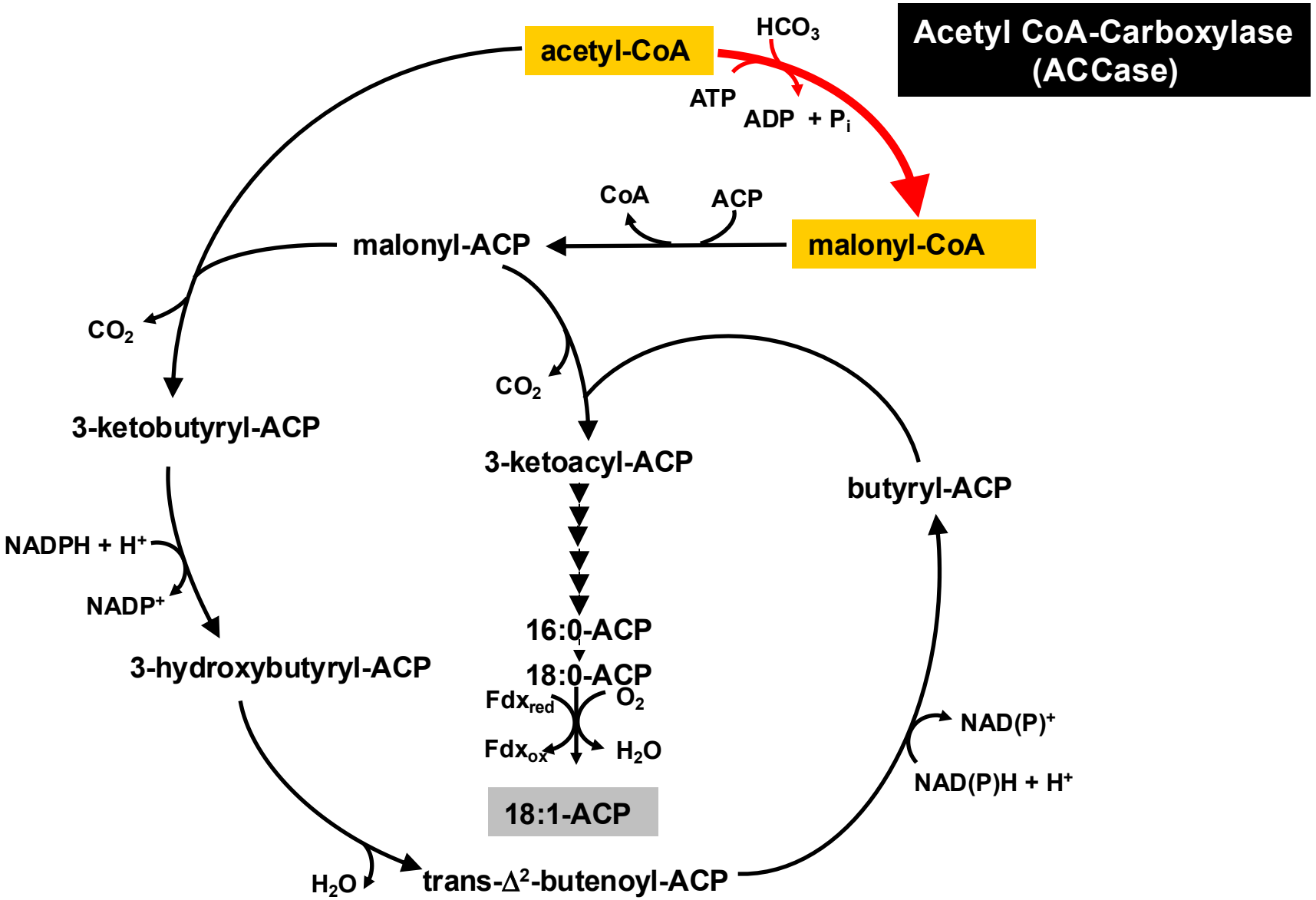
Engineering seed oil content



Four "P"s for engineering oil content

1% increase in soybean oil translates to $200M added crop value

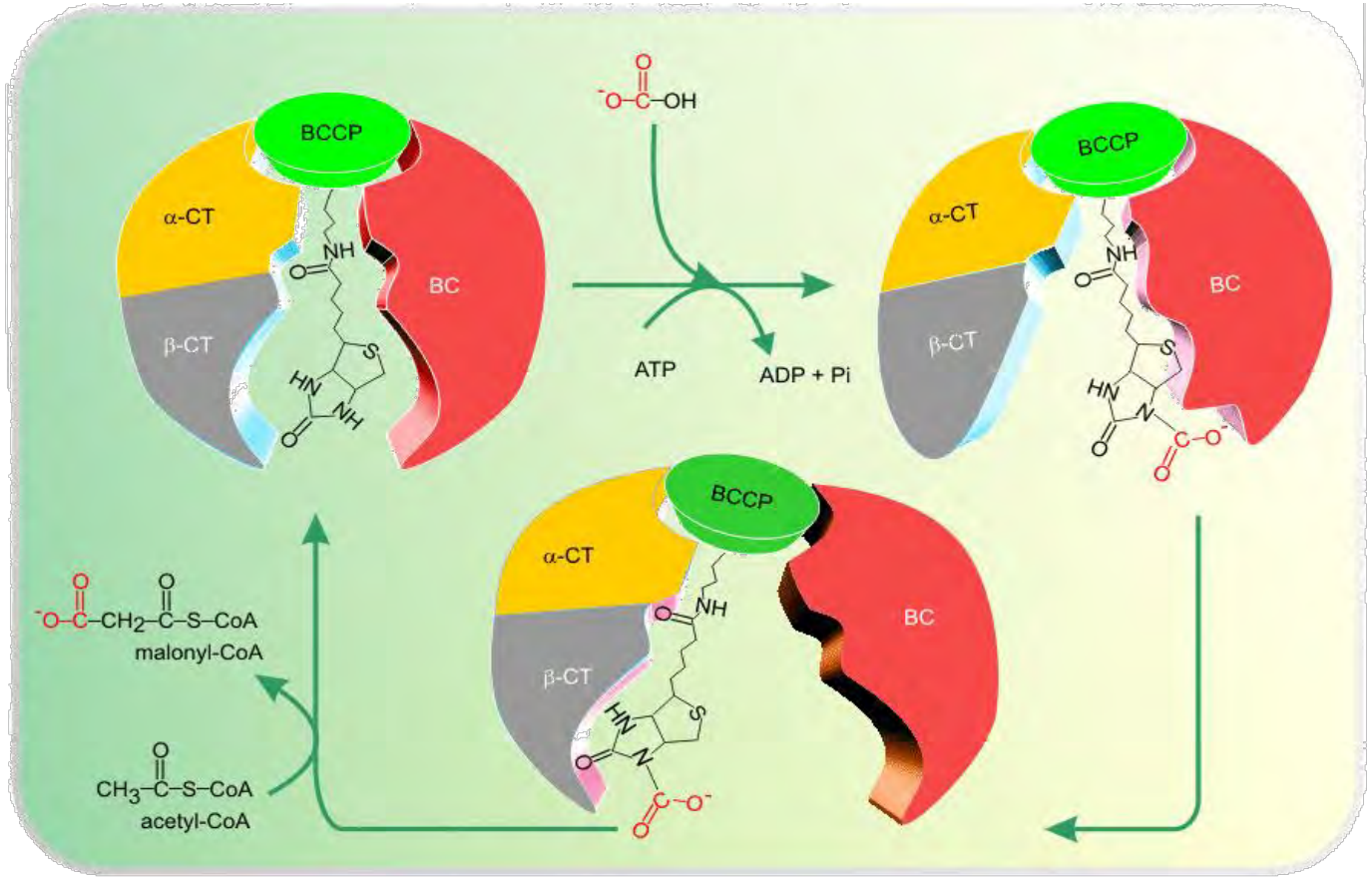
Acetyl-coenzyme A carboxylase is the "gate-keeper" for *de novo* fatty acid synthesis



ACCase is comprised of four components that catalyze a two-step reaction

BCCP: biotin carboxyl carrier protein
BC: biotin carboxylase

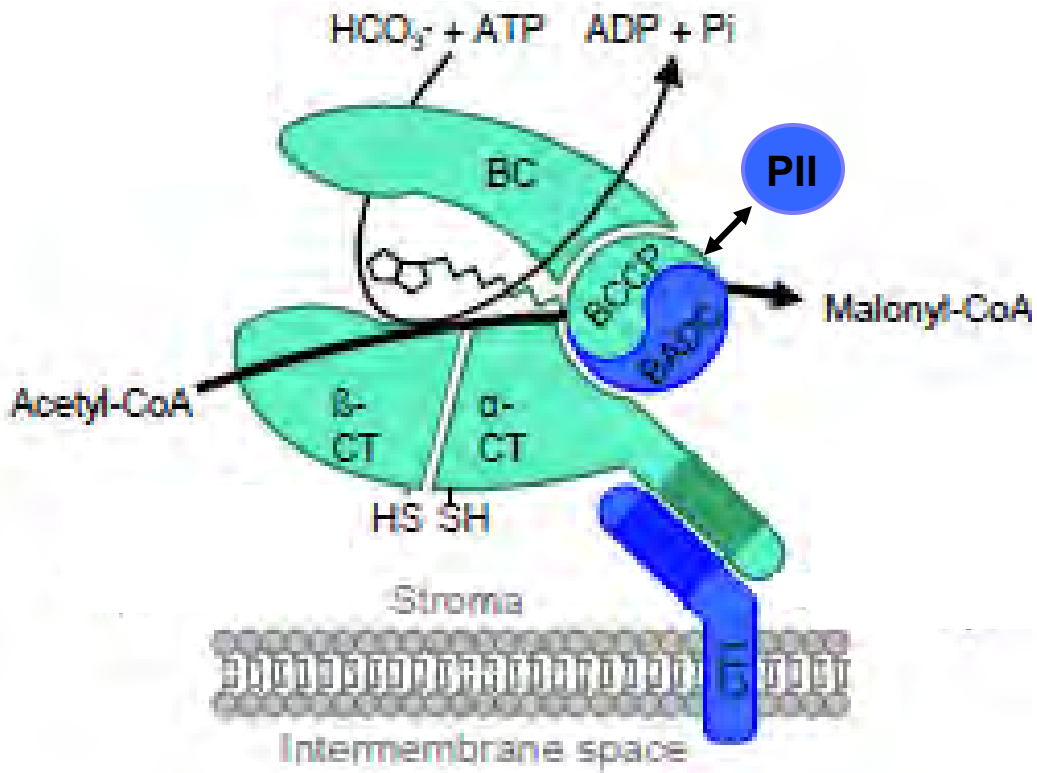
α-CT: carboxyltransferase
β-CT: carboxyltransferase



http://aralip.plantbiology.msu.edu

Li-Beisson Y et al., (2013) Acyl-lipid metabolism. Arabidopsis Book 11: e0161

Plant ACCase is regulated by three negative regulatory proteins



Ye et al (2020). *Nature Comm.*

Research areas for ACCase

1) Discovery proteomics – identifies a novel regulatory subunit (BADC)

2) Targeted proteomics – reveals a limiting catalytic subunit (α-CT)

3) Y2H – a new membrane-anchoring protein (CTI)

Arabidopsis ACCase organization

5 catalytic subunits (1 plastid encoded) and 7 regulatory subunits…

What is the *in vivo* subunit stoichiometry?

Absolute Quantitation by Selected or Multiple Reaction Monitoring (SRM, MRM) with AQUA peptides

Difference in molecular mass of AQUA and native peptides is key to resolving the two via mass spectrometry

Add known amount of AQUA peptide to total protein sample

↓

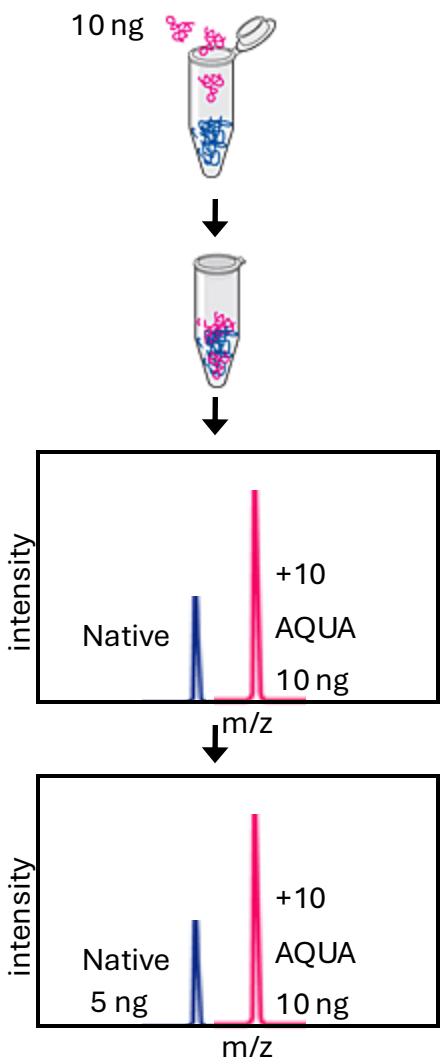
Digest mix with trypsin

↓

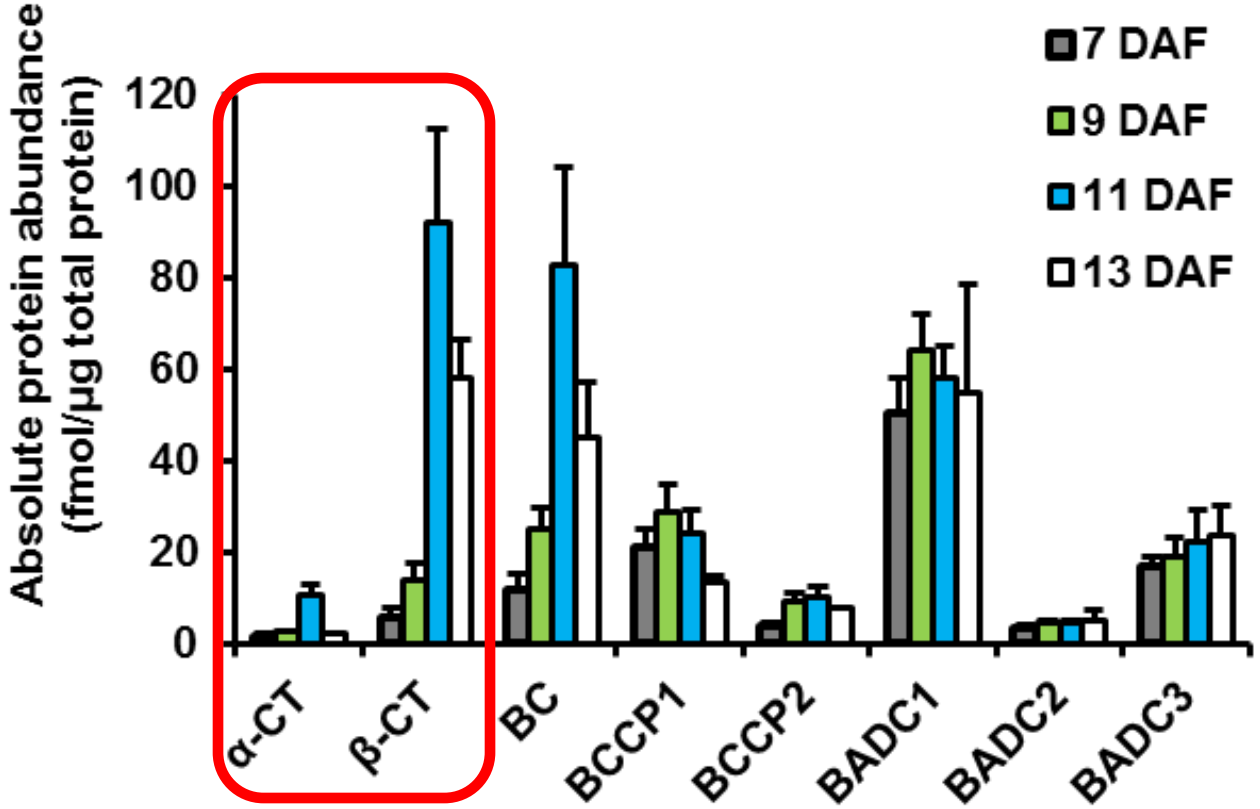
Monitor native and AQUA peptides simultaneously

↓

Quantify native protein using AQUA peptide as internal standard

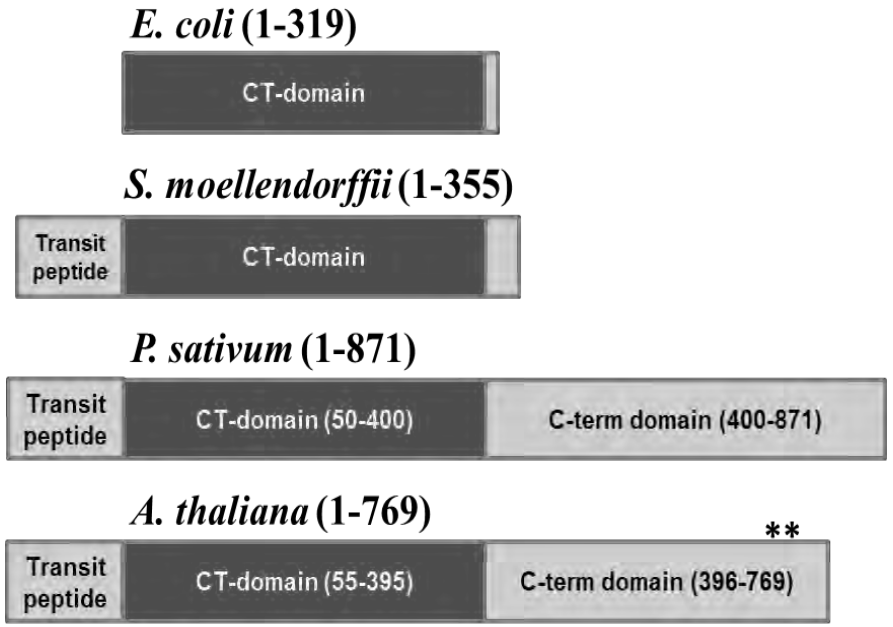


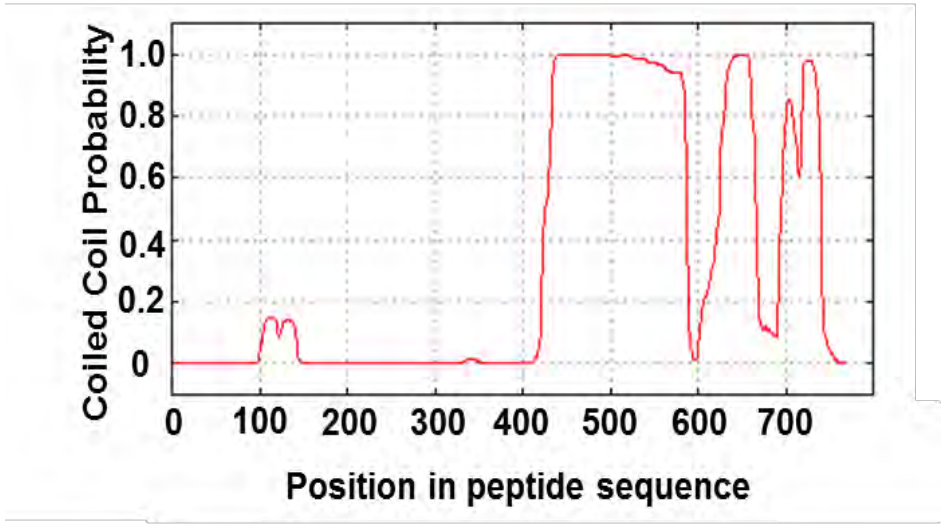
In vivo quantities of ACCase subunits in developing siliques determined by AQUA-MRM. The α-CT subunit is not expressed at a 1:1 stoichiometry with partner subunit β-CT



Wilson RS, Thelen JJ. (2018) J Proteome Res. 17:1773-

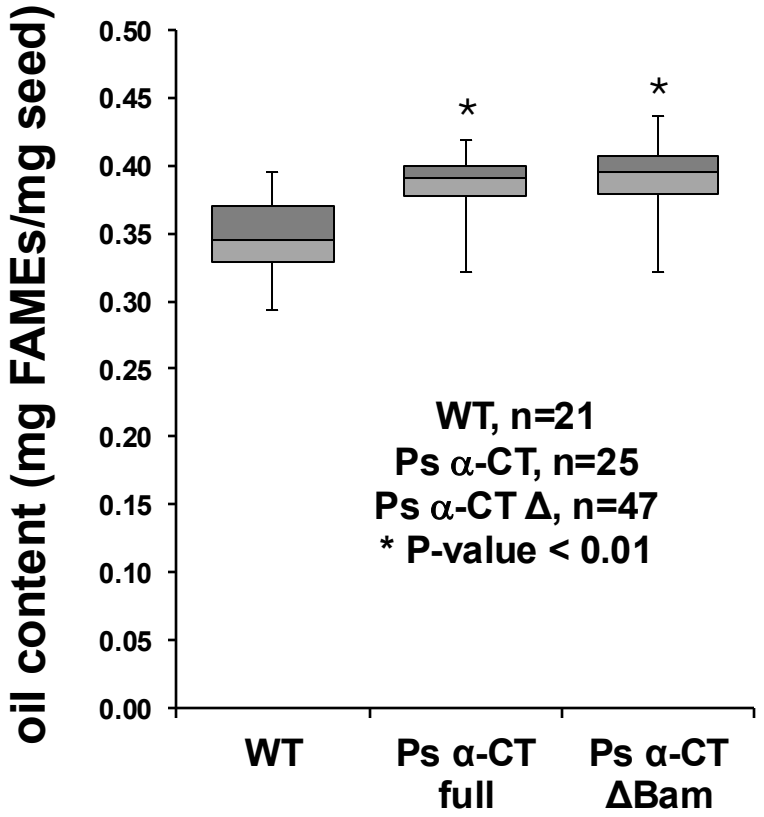
The plant α-CT subunit has a long non-catalytic domain of unknown function





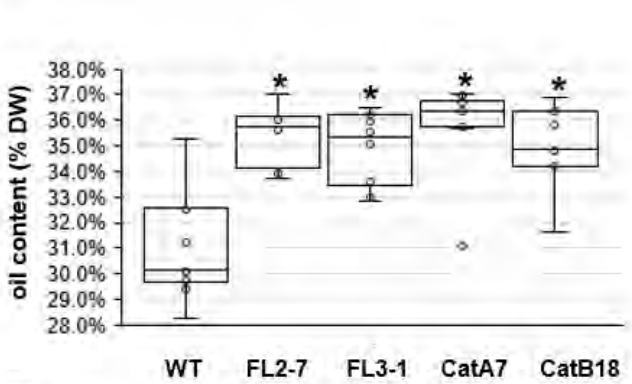
Expressed both full-length and catalytic domain of *Pisum sativum* α-CT

Overexpression of pea α-CT, either full length or catalytic region produced an average 14% increase in seed oil in T1 plants

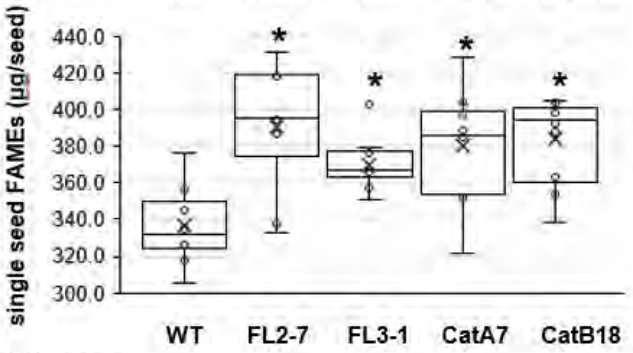


Overexpression of α-CT in camelina (either full length or catalytic region) increases seed oil content

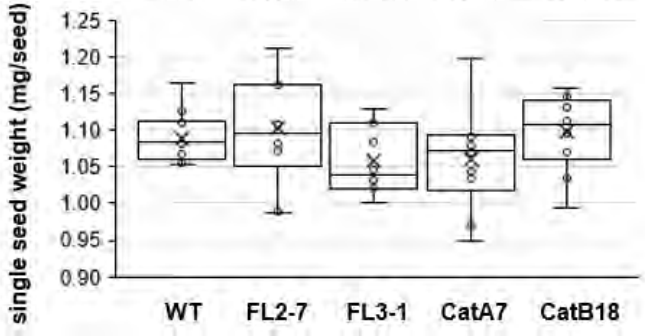
Oil (DW)



Oil (single seed)



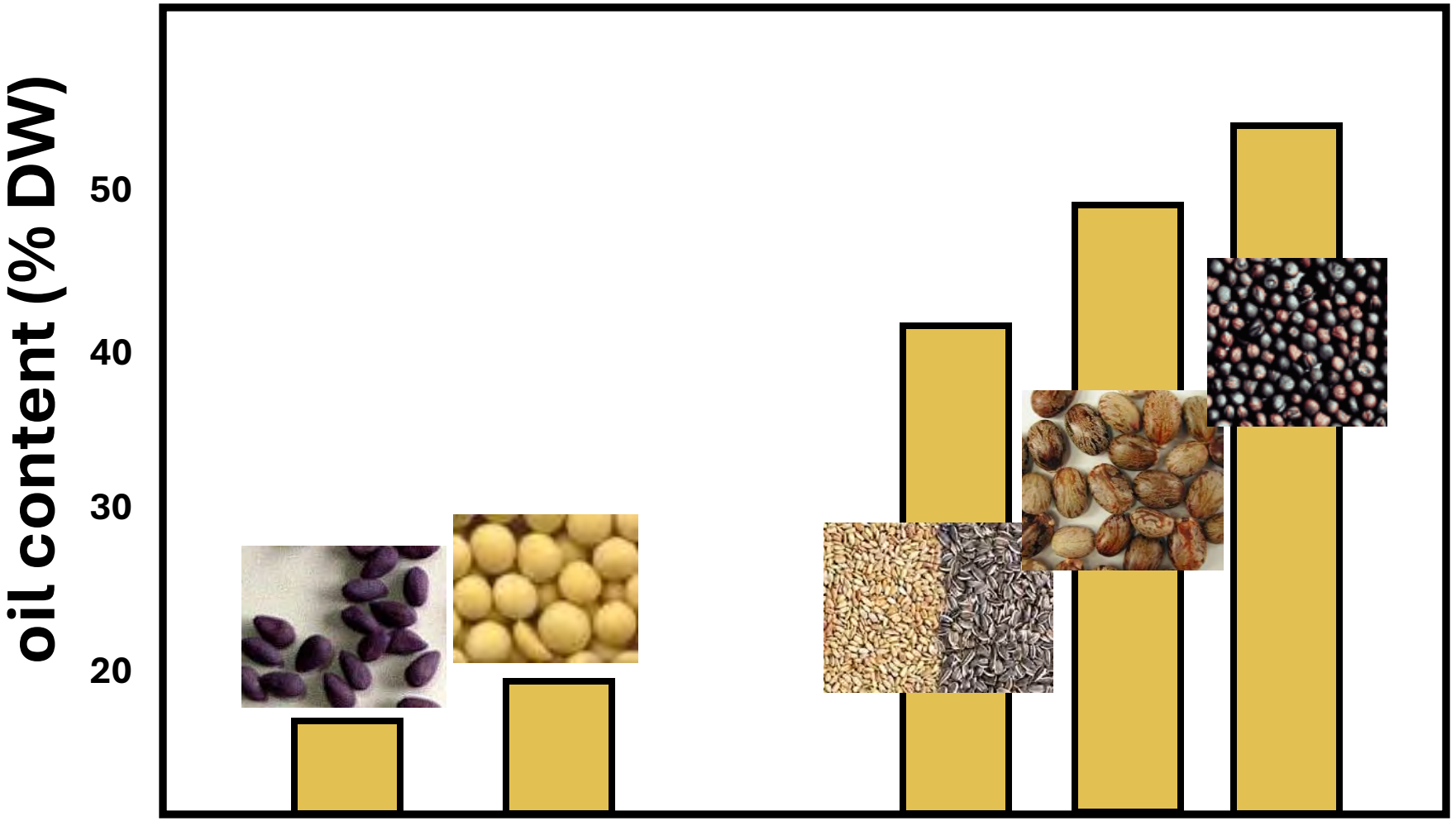
Seed mass



T4 seed, n=10

Abe Koo

Seed oil content varies considerably in nature…



Acknowledgements

Thelen Lab

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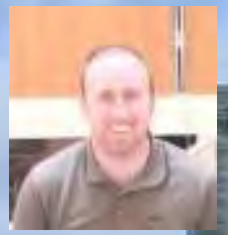

Collaborators

Sebastien Baud (INRAE, France)

Phil Bates (WSU)

Abe Koo (Mizzou)

Steve VanDoren (Mizzou)

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NSF-IOS1339385

NSF-MCB1716688

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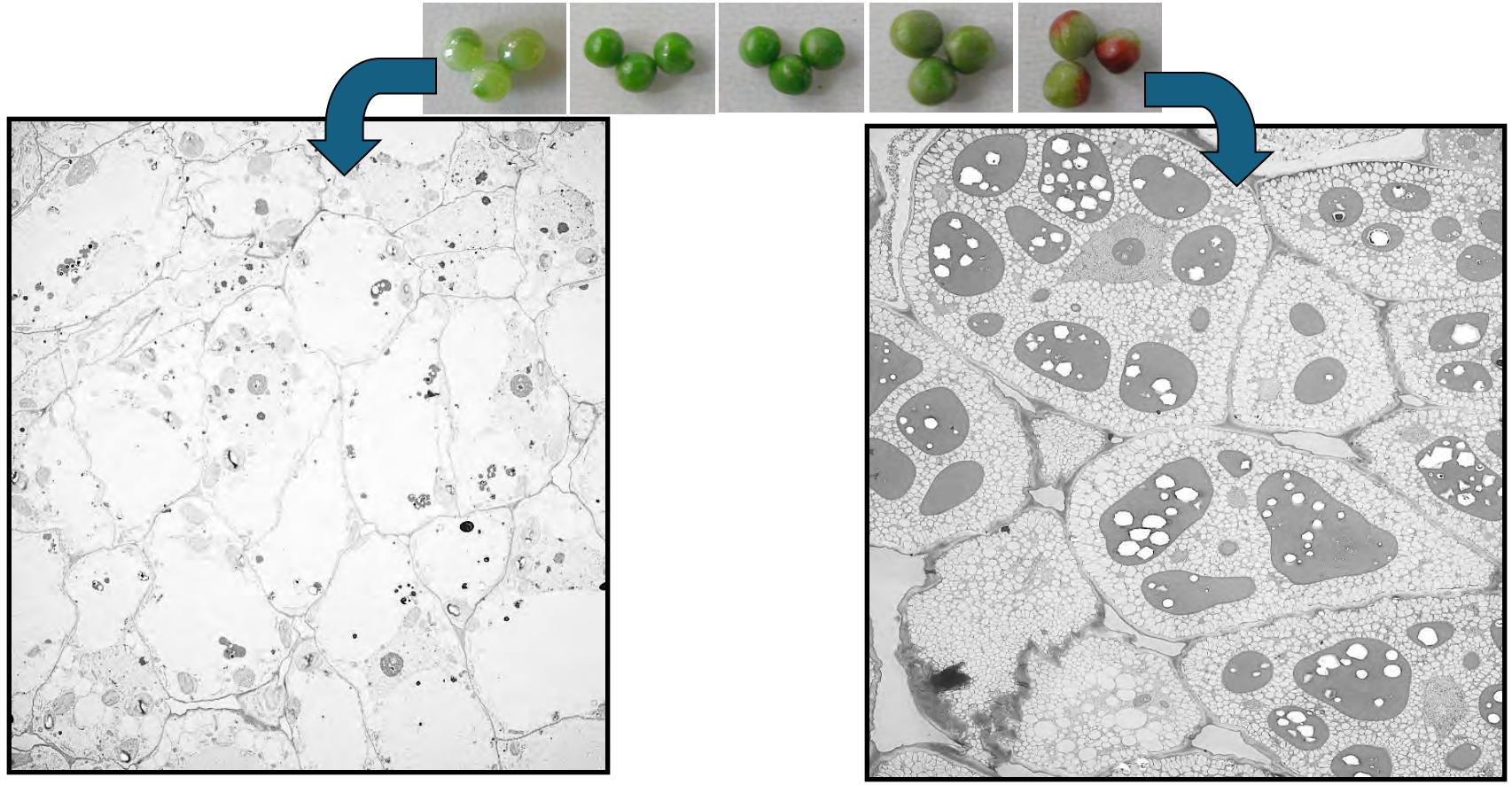
DOE-BER-SC0023142

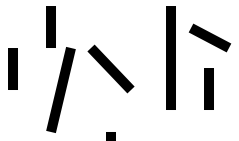
Plant embryonic cells are metabolic factories – primed for oil production



Relative protein quantitation by “bottom-up” mass spectrometry

intact protein

protease digestion
(e.g. trypsin cleaves after K,R residues)

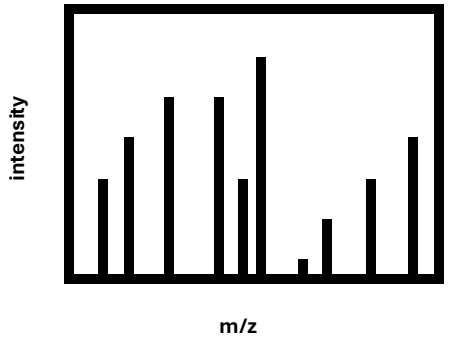


peptides

Peptides are quantified as surrogates for the cognate protein

mass spectrometry

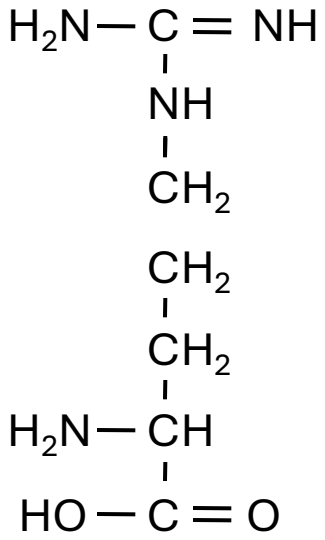
collision fragmentation



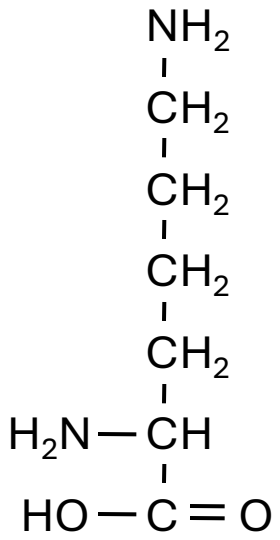
mass
952.0984
1895.9057
1345.6342
899.8743
2794.9761

227.13	AGV
383.23	AGVR
530.30	AGVRF
627.35	AGVRFP
756.39	AGVRFPE
912.49	AGVRFPER

AQUA peptides – either contain a labeled Arg or Lys (tryptic peptide mimics)



Arginine (R) – 174.2



Lysine (K) – 146.19

Isotope labeled	$^{13}C_6H_{14}{}^{15}N_4O_2$ – 184.2	$^{13}C_6H_{14}{}^{15}N_2O_2$ – 154.19
Native peptide	LSAEFGSLR – 978.51	VSDDEFNNYK – 1229.52
AQUA peptide	LSAEFGSLR – 988.51	VSDDEFNNYK – 1237.52