

Biochemical Genomics - Quizzing the Chemical Factories of Oilseeds



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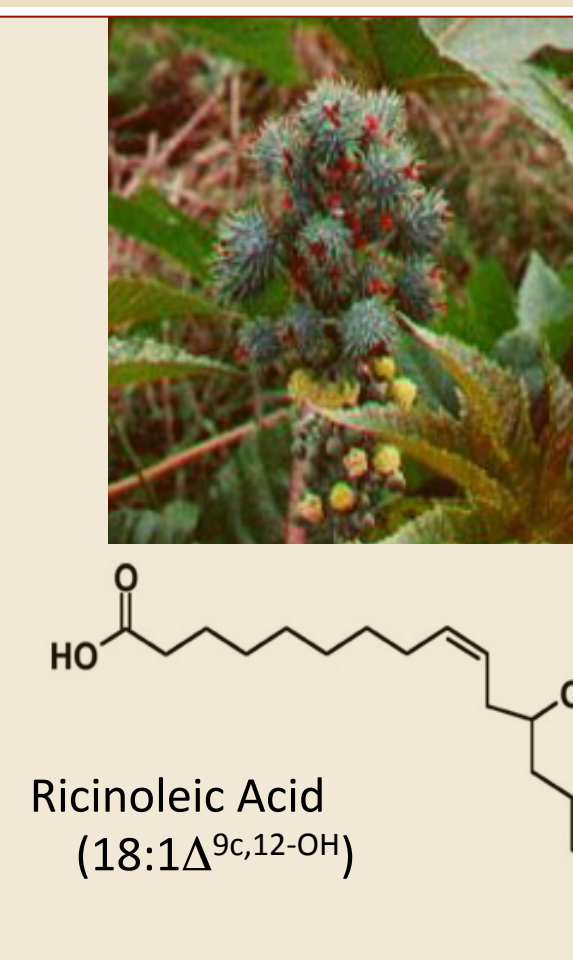
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PROJECT OVERVIEW

Plant oils are the major source of highly-reduced carbon available from nature. Mining genomes of natural source plants that accumulate high levels of unusual fatty acids has helped us to identify factors that facilitate their accumulation such as phosphatidylcholine : diacylglycerol choline phosphotransferase (PDCT) and translationally controlled tumor protein (TCTP). Our data provides supports for the hypothesis that multiple enzymes have evolved to channel unusual fatty acids into storage triacylglycerols. We have also discovered that fusion of membrane-integrating sequence for translation of IM protein constructs (MISTIC) polypeptide to fatty acid hydroxylase (FAH) improves germination of seeds containing high levels of unusual fatty acids. Improved accumulation is demonstrated for all three target fatty acids, with dramatic increases seen for cyclopropane and hydroxy fatty acids. The basic scientific knowledge gained in this work will be critical for future biotechnological efforts to control the chemical structure of stored carbon in production organisms that will constitute a new generation of specialty crops that will become the green factories of the future.

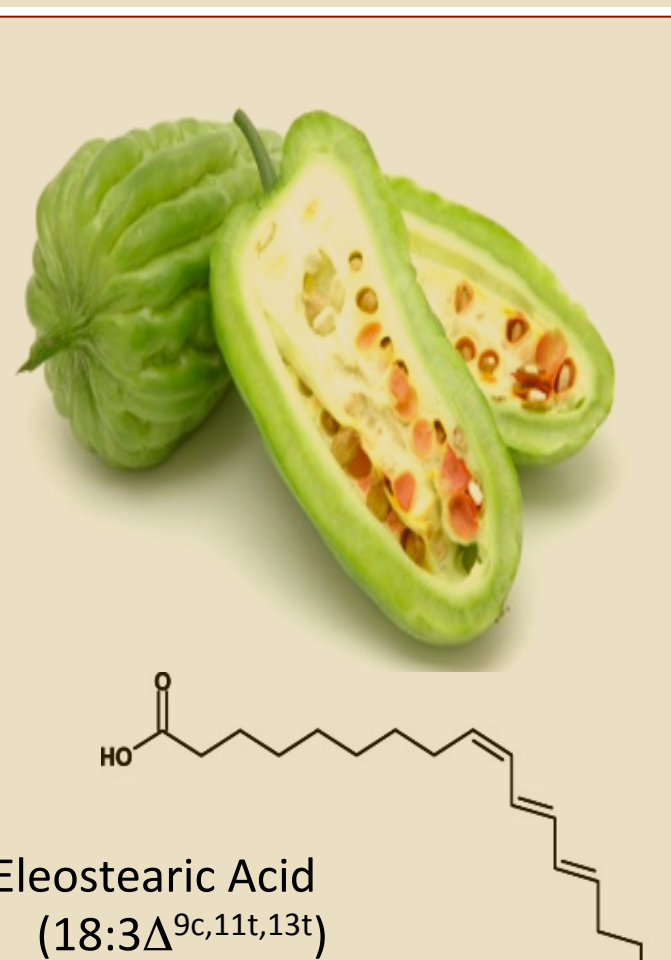
We focus on three unusual fatty acids produced by:

Castor



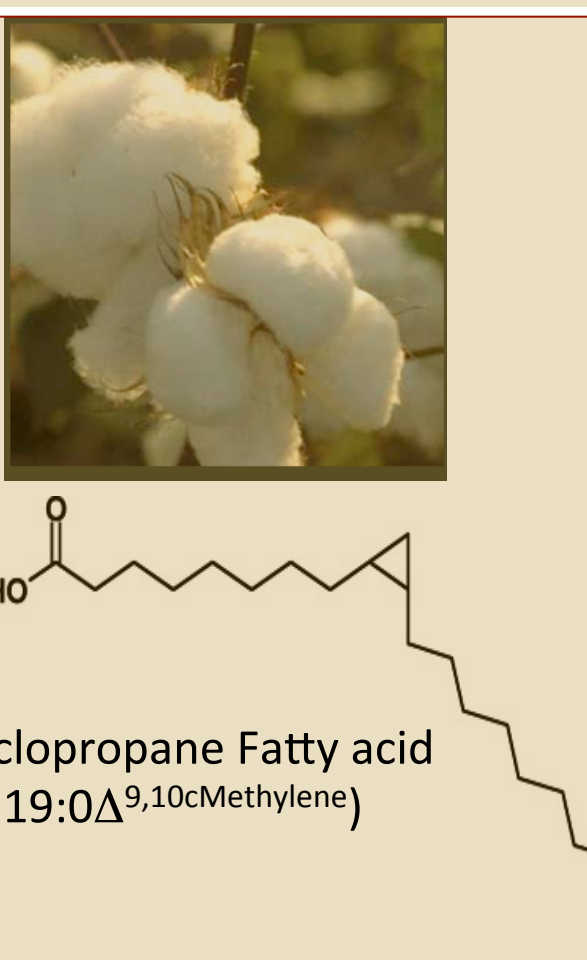
Castor seeds produce the hydroxy fatty acid (HFA) ricinoleic. HFA and derivatives are used in lubricants, nylon, soaps, and adhesives. Industrial processes consume 600-800 million lbs of HFA each year, and demand is growing rapidly.

Momordica



Momordica charantia produces conjugated FA (CFA). CFA are used in drying oils and can serve as replacements to petroleum additives to reduce VOC emissions from paints and other coating materials.

Cotton



Cotton seeds synthesize cyclopropane FA (CpFA). CpFA are prized for their combination of oxidative stability and low viscosity. These properties make them ideally suited for applications as lubricants and biodiesel.

Major Advances

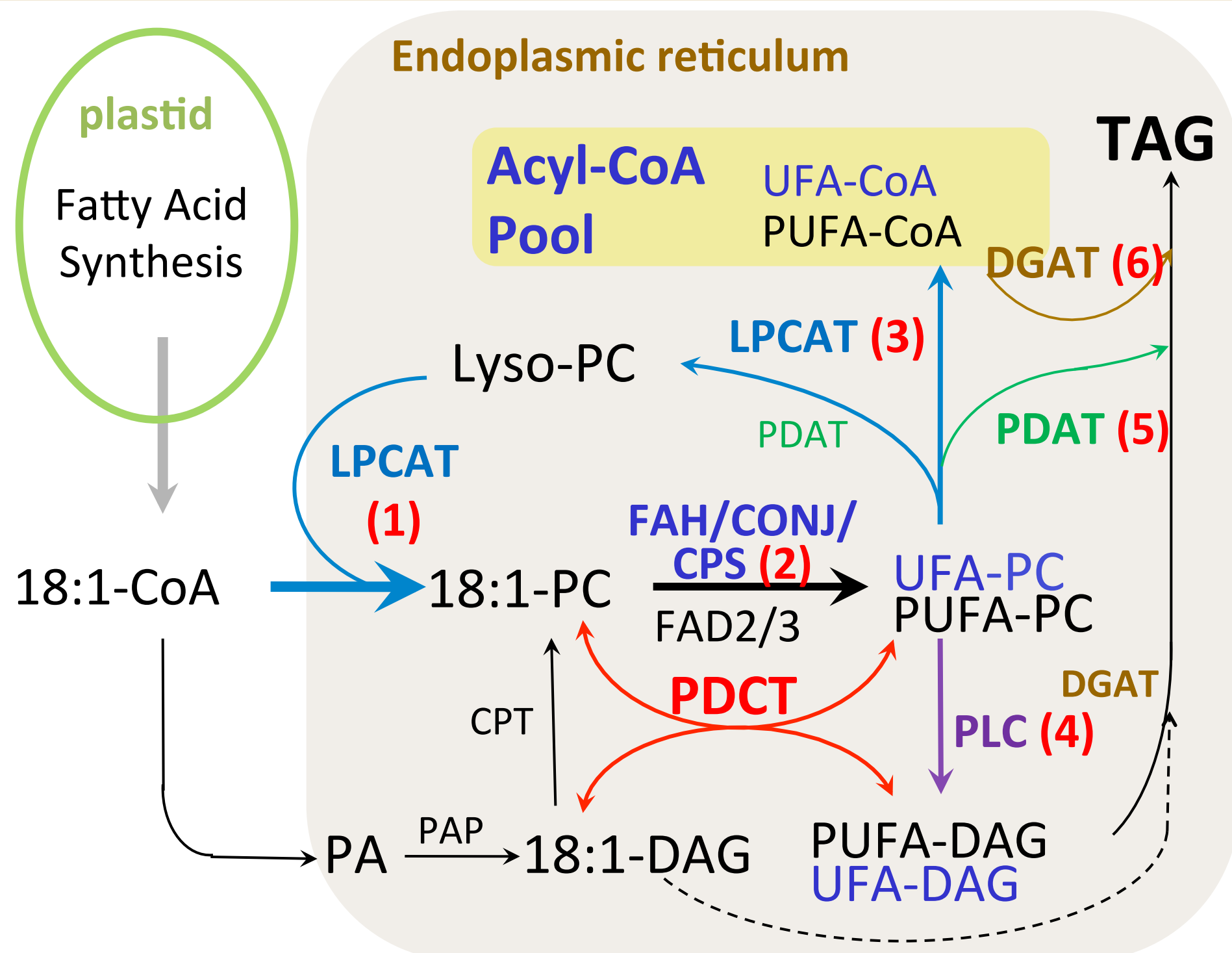
- Five discoveries that have advanced our knowledge of plant lipid metabolism: 1-PDCT, 2-TCTP, 3-the application of MISTIC peptide fusions to FAH12 enzymes, 4-identified feedback regulation in the synthesis of hydroxy and conjugated fatty acids, 5-that acyltransferases compete
- We have leveraged genomic information to produce high-value oils in transgenic plants by identifying species-specific isoforms of lipid metabolizing enzymes and expressing them in transgenic seeds
- We have identified and partially mitigated biochemical barriers to unusual fatty acid accumulation, by increasing gene dosage of the unusual fatty acid producing enzymes, and coexpression of specific acyltransferase isoforms to increase the flux of unusual fatty acids from membranes to storage lipids
- We determined the protein interaction network for lipid pathway enzymes

Future Directions

- Coexpression of genes that increase accumulation unusual fatty acids
- Understand the basis for feedback inhibition of unusual fatty acids and use that knowledge to defeat the feedback in engineered oilseeds
- Explore the role of PDCT enzymes in the accumulation of modified oils
- Utilize MISTIC sequences to enhance expression of key enzymes required for accumulation of high-value oils.
- Leveraging knowledge of protein-protein interactions

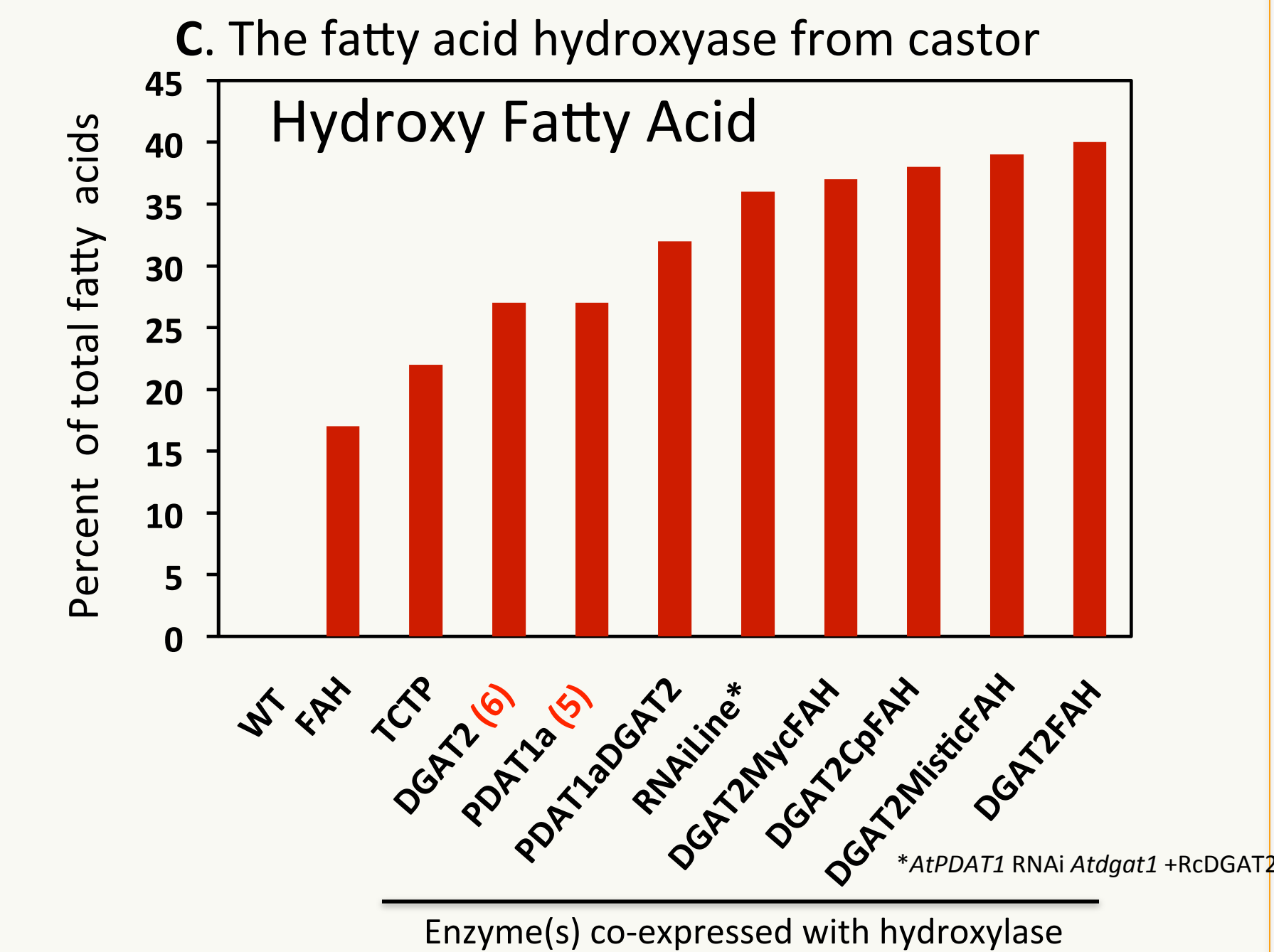
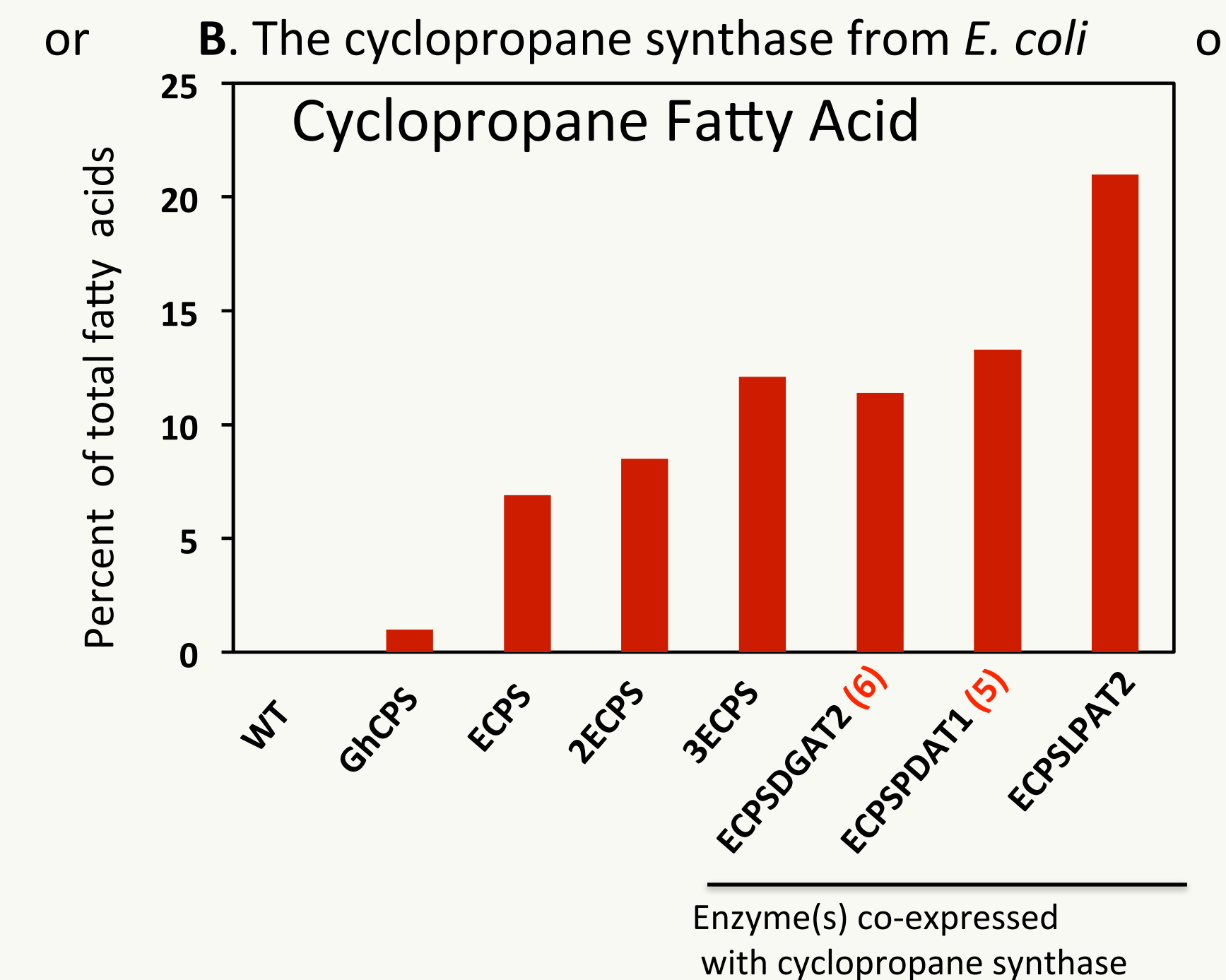
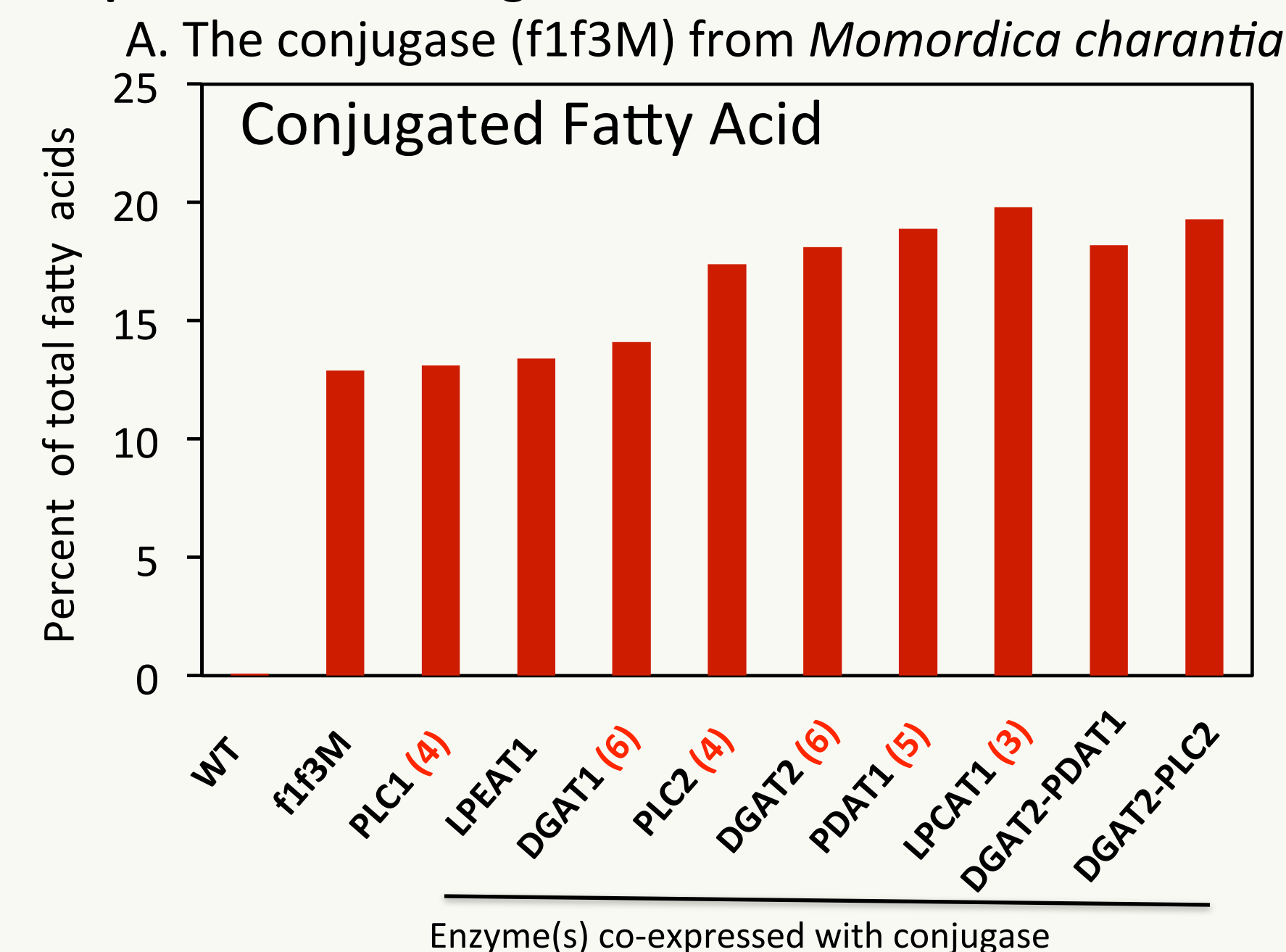
Discovery of a new enzyme regulating seed oil composition

Phosphatidylcholine : diacylglycerol choline phosphotransferase (PDCT)



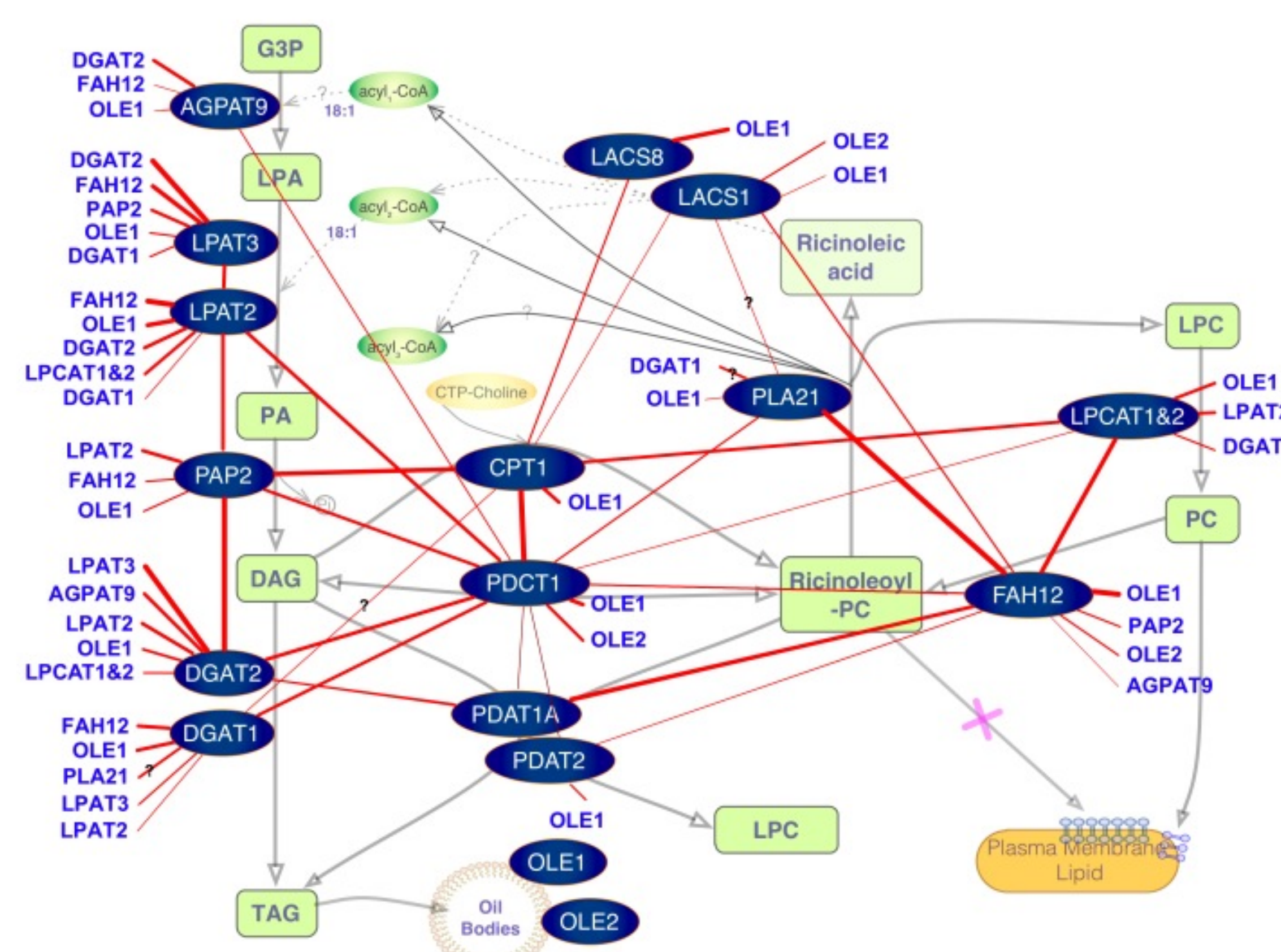
Co-Expressing Key Pathway Genes Enhances Hydroxy, Cyclopropane or Conjugated Fatty Acid Production in Arabidopsis Seeds

Arabidopsis lines were engineered with:



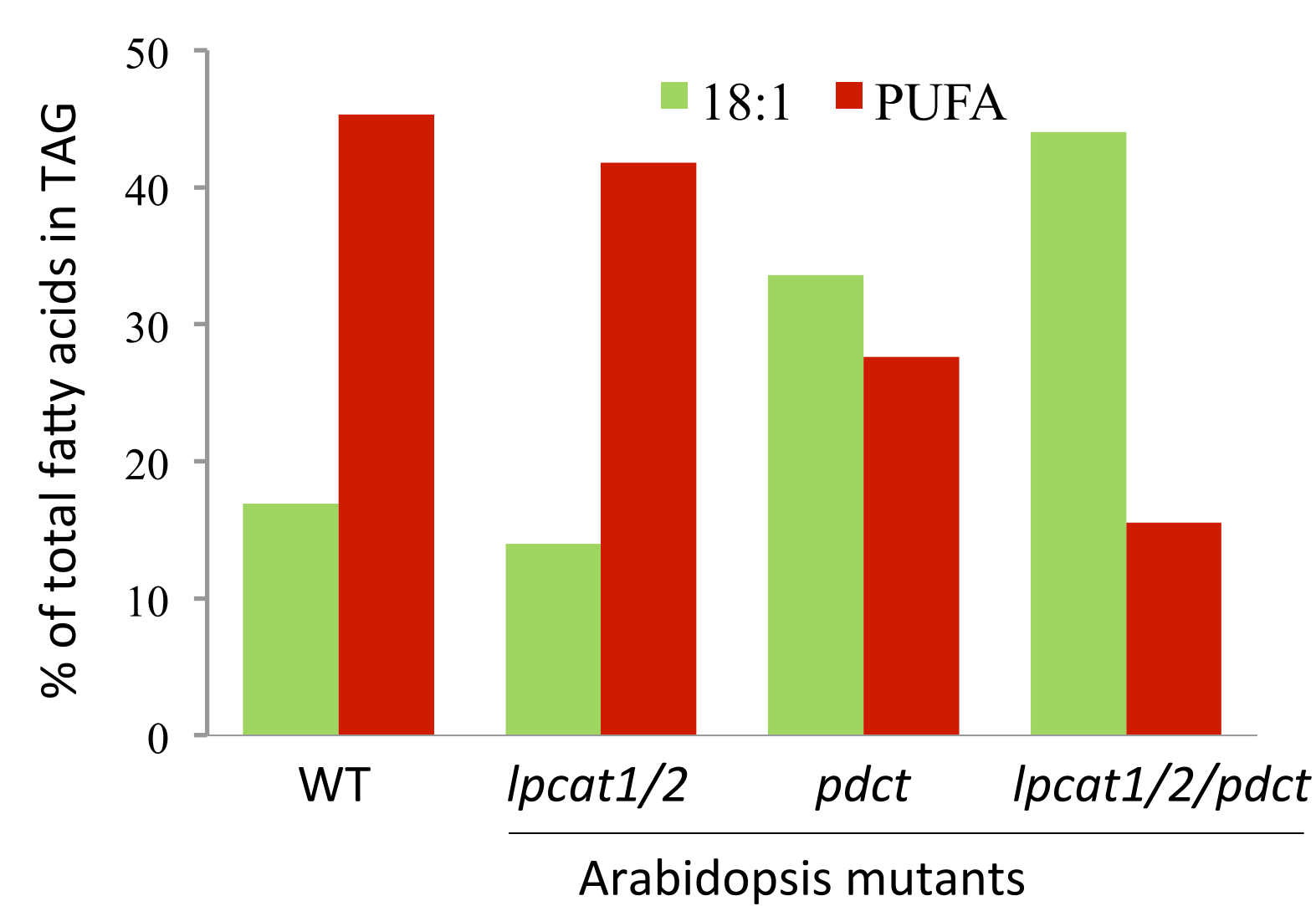
Red numbers refer to enzyme reactions shown in pathway at left

Mapping of Protein-Protein Interactions

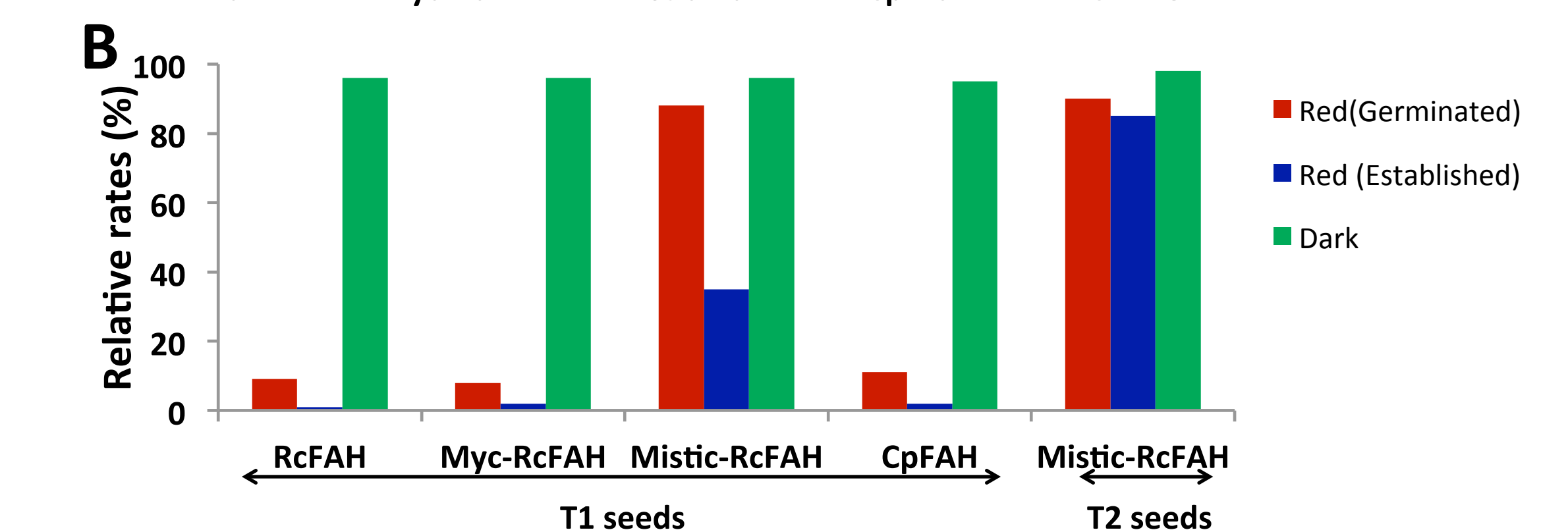
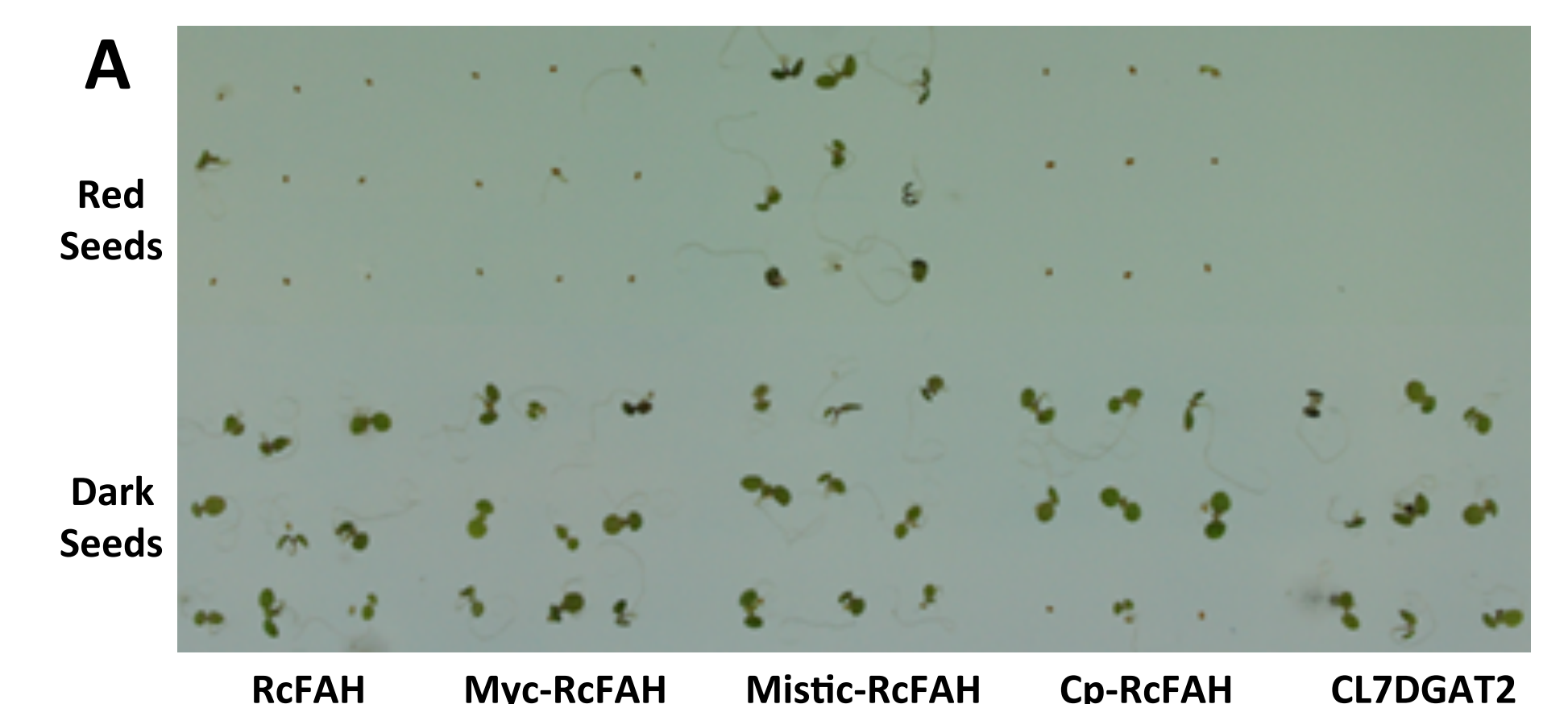


Protein-protein interaction map for lipid biosynthetic enzymes from castor bean (*Rc, Ricinus communis*), determined by the mating-based split-ubiquitin system (mbSUS) and overlaid on the castor lipid metabolic pathway. Proteins known to be involved in the synthesis of TAGs are highlighted with a blue background. The following bait and/or prey proteins were selected to generate a network: RcAGPAT9, RcLPAT2, 3, RcPAP2, RcDGAT1, 2, RcLACS1, 8, RcCPT1, RcPDCT1, RcPDAT1A, 2, RcOLE1, 2, RcPLA21, RcLPCAT1&2 and RcFAH12. Red lines symbolize interacting protein pairs. The strength of the interaction is indicated by the thickness of the line. Note that the fatty acid hydroxylase (FAH12) interacts with many of the lipid biosynthetic enzymes.

Increased FAH dosage resulted in increased UFA accumulation, but seeds exhibit reduced germination and seed size. Expression of an N-terminal MISTIC-FAH fusion improves germination and mitigates shriveling



PDCT and LPCATs (acyl-CoA lysoPC acyltransferases) control the majority of newly synthesized fatty acids (e.g., 18:1) entering phosphatidylcholine (PC) for modification, resulting in accumulation of polyunsaturated (PUFA) or unusual fatty acids (UFA) studied in this project. Arabidopsis mutants deficient in these enzymes accumulated dramatically reduced PUFA in seeds (above).



Representative germination rate pattern of FAH12 transformed CL7DGAT2 seeds. The untransformed CL7DGAT2 seeds were germinated as control. (A). For direct comparison the red transformed seeds and the dark untransformed seeds were sowed in a 3 X 3 matrix on MS plate containing 1% sucrose. (B). Germination and establishment rates of FAH12 transformed CL7DGAT2 seeds.