The metabolic origins of non-photorespiratory CO_2 release during photosynthesis: A metabolic flux analysis

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Project Goals

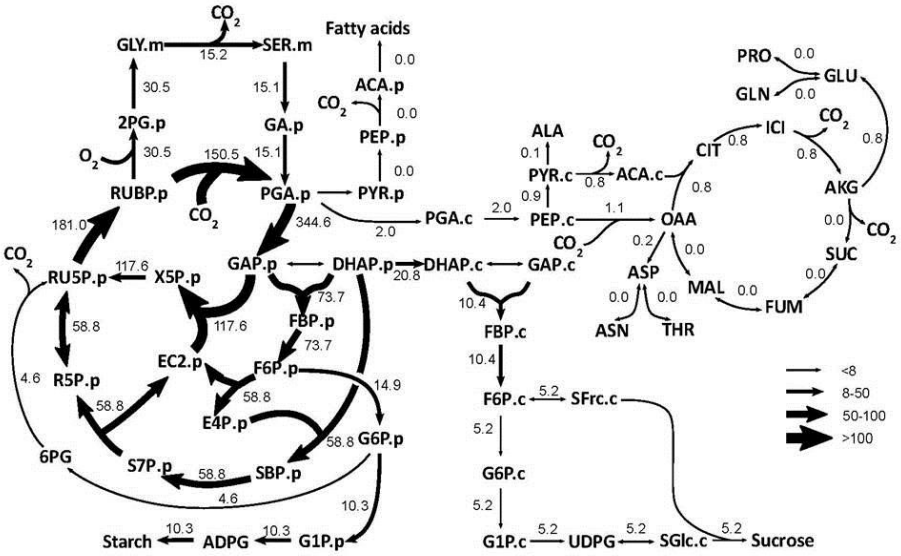
The overall goal of this project is to increase the triacylglycerol yield of the model oilseed crop plant, *Camelina sativa*, to increase its usefulness for producing fuels and chemical feedstocks. Camelina shows promise as a biofuel crop and is widely used as a model oilseed plant. A near relative of *Brassica napus* and *Arabidopsis thaliana* it is easily transformed, requires low agronomic inputs, and is naturally resistant to both biotic and abiotic stress; however its yields are lower than major oilseed crops. The aims of this sub-project were to establish and improve metabolic flux analysis tools to quantify fluxes through central metabolism in photosynthesizing Camelina leaves and to apply this approach to determine the source(s) of non-photorespiratory CO_2 release in the light, which lowers photosynthetic efficiency.

Abstract

Respiration in the light (R_L) releases CO_2 in photosynthesizing leaves and occurs independently from photorespiration. Since R_L lowers net carbon fixation, understanding it could help improve plant carbon-use efficiency and modeling of crop photosynthesis. Although R_L was identified more than 75 years ago, its biochemical mechanisms remain unclear. To identify reactions contributing to R_L, we mapped metabolic fluxes in photosynthesizing source leaves of the oilseed crop and model plant camelina (*Camelina sativa*). We performed a flux analysis using $^{13}CO_2$ isotopic labeling patterns of central metabolites during time course, gas exchange and carbohydrate production rate experiments. To quantify the contributions of multiple potential CO_2 sources with statistical and biological confidence, we increased the number of metabolites measured and reduced biological and technical heterogeneity by using single mature source leaves and quickly quenching metabolism by directly injecting liquid N_2; we then compared the goodness-of-fit between these data and data from models with alternative metabolic network structures and constraints. Our analysis predicted that R_L releases 5.2 μmol g^{-1} FW hr^{-1} of CO_2, which is consistent with a value of 9.3 μmol g^{-1} FW hr^{-1} estimated by CO_2 gas exchange. The flux analysis indicated that ≤10% of R_L results from TCA cycle reactions, which are widely considered to dominate R_L. Further analysis of the results indicated that oxidation of glucose-6-phosphate to pentose phosphate via 6-phosphogluconate (the G6P/OPP shunt) can account for >93% of CO_2 released by R_L.

The methods established in this study are being applied in the broader research on improving Camelina productivity to: (a) measuring changes in leaf central metabolism in transgenic Camelina plants with increased rates of CO_2 assimilation; (b) mapping leaf carbohydrate turnover during photosynthesis; and (c) to provide experimentally derived flux maps for improvement of predictive stoichiometric flux analysis by Flux Balance Analysis.

Central carbon assimilatory metabolic fluxes in photosynthetic *Camelina sativa* leaves.



Fluxes are shown in numbers and also depicted by the variable width of arrows. Fluxes were estimated by ^{13}C INST-MFA using the INCA software suite constrained by the metabolic network model and experimental inputs including mass isotopomer distributions of measured metabolites, net CO_2 assimilation, starch synthesis rate, sucrose synthesis rate and amino acid export rate. Fluxes were not constrained by measured R_L. Flux units are expressed as μmol metabolite g FW^{-1} hr^{-1}. The model network is compartmentalized into cytosol (".c"), which includes mitochondrial and peroxisomal reactions, plastid (".p"), and mitochondria (".m"). Metabolite pools (principally vacuolar) that do not become labeled on the time scale of the experiments are modeled but not shown here.

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