The impact of LIP36 seed-specific expression on seed and oil yields in *Camelina sativa* and its associated transcriptome and metabolome changes

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Project Goals:

Our research aims to sustainably increase oilseed yields in the non-food oilseed crop plant, *Camelina sativa*, thereby making it a commercially viable alternative for biofuels and bioproducts production. Camelina has shown considerable promise as a dedicated industrial oilseed crop because it requires low agronomic inputs, is naturally more resistant to both biotic and abiotic stress than other oilseed crops, and Camelina oil-based blends have been tested and approved as liquid transportation fuels[1,2]. This project was proposed to increase fixed carbon allocation by identifying metabolic bottlenecks and engineering metabolic pathways that limit or co-limit seed and oil production in Camelina. In the current project, we designed highly innovative metabolic engineering strategies to 1) increase carbon capture in photosynthetic source tissues and 2) redirect the carbon to seeds for seed oil production. We further investigated the roles of mitochondrial metabolism during seed development, seed germination, and seedling growth, through using transgenic plants.

Abstract

A member of the mitochondrial carrier protein family and a component of algal carbon concentrating mechanisms (CCMs) in *Chlamydomonas reinhardtii* (Chlamy), designated, low-CO_2 inducible protein (LIP36) was identified previously in our laboratory. LIP36 is localized to mitochondria in Camelina, and its suppression by RNA interference (RNAi) suggested its essential role in growth of Chlamy under low CO_2 conditions[3]. To gain insight into the role of LIP36 proteins, we constitutively expressed LIP36 into the oilseed crop *Camelina sativa*. LIP36 expression has resulted in higher photosynthetic CO_2 assimilation (30-46%), under limiting conditions, relative to the wildtype (WT) controls. LIP36 lines showed a more favorable redox status, higher water and nitrogen use efficiency, and increased seed and oil yields. The ^{13}C labeling experiments in Camelina leaves have also suggested that LIP36 improves the

capacity of anaplerotic pathways, thereby maintaining plant productivity under non-ideal growth conditions. Based on these preliminary results, we aim to confirm the impact of LIP36 on the flux through central carbon metabolism in both source and sink (seed) tissues through expressing it under seed-specific oleosin promoter, and to emphasize whether its increased activity in seeds could lead to enhanced carbon assimilation, and therefore further increased seed and oil yields. The transformation of Oleosin :: LIP36 construct into Camelina was successful and homozygous T4 seed lines were generated and screened/evaluated for seed attributes (seed size, seed weight, and % oil content). The elite lines expressing LIP36 were also subjected to RNA-Seq analysis to investigate the global changes in Camelina transcriptome in response to LIP36 expression in seeds, and to identify candidate genes/enzymes for further improving seed biomass and yield traits in Camelina. The steady-state metabolite profiling and the metabolic flux analysis (MFA) is currently ongoing in order to monitor the global metabolic changes in LIP36 transgenics in order to define the impact of LIP36 on the flux through central carbon metabolism, and to further integrate LIP36 with other yield traits to enhance water use efficiency and improve CO_2 assimilation under limiting environmental conditions.

References

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