

Development of Resources and Tools to Improve Oil Content and Quality in Pennycress

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Project Goals: The main objective of this research is to apply a biodesign strategy for improving oil content in a promising alternative source of jet-fuel, pennycress. To advance towards this goal, we are: 1) Investigating pennycress natural variation to identify candidate genes and biomarkers associated with oil accumulation and fatty acid composition; 2) Identifying targets to improve oil content and composition, and 3) Establishing metabolic engineering targets and develop community resources.

Bioenergy crops, which have potential for jet fuel production and do not compete with food crops, are urgently needed. Our strategy to address this fundamental challenge is to develop pennycress (*Thlaspi arvense*), a member of the Brassicaceae, as a bioenergy crop, taking advantage of its ability to produce seed oil that is ideally suited as a renewable source of biodiesel and aviation fuel¹. Moreover, pennycress performs well on marginal land, has a short maturity time and grows off-season, serving as a winter cover crop, and complementing the production of commodity food crops. Essential pennycress molecular and genetic resources can be rapidly developed by leveraging the fully sequenced genome and research tools of its close relative *Arabidopsis thaliana*. Furthermore, a draft genome² for *Thlaspi arvense*, as well as transcriptome³ and metabolome⁴ information have been made recently available. However, for this plant to become an economically viable and sustainable source of jet fuel, molecular and genetic resources need to be developed, and integrated with multi-variable techno-economical analyses to guide strategies for increase oil production through breeding and/or genetic manipulation. These are the gaps that this project intends to fill.

Specifically, we are: **1) Investigating pennycress natural variation to identify candidate genes and biomarkers associated with oil accumulation and fatty acid composition.** For this purpose, we are currently determining variation in genome-wide gene expression (derived from RNA-Seq) and intracellular metabolites (derived from metabolomics) in seeds from pennycress natural accessions. **2) Identifying targets to improve oil content and composition.** Towards this goal, we developed new mass spectrometric methods to follow the ¹³C-labeling in intracellular compounds⁵, and we are now generating a flux map of carbon partitioning in developing pennycress embryos. We will overlay metabolic maps with levels of transcripts and intracellular compounds to identify metabolic bottlenecks in oil accumulation. Finally, we will use ¹³C-based metabolic flux analysis to validate bottlenecks in two accessions with contrasting oil contents. **3) Establishing metabolic engineering targets and develop community resources.** For this purpose, we are analyzing techno-economics of pennycress based agronomic and supply systems that will provide targets for future efforts aimed at increasing seed oil production through rational metabolic

engineering and breeding. We are working with the Arabidopsis Biological Resource Center to develop a public seed collection of pennycress mutants and transgenic lines, facilitating community synergy and accelerating research towards the established production goals.

Taken together, the knowledge and resources generated through this interdisciplinary project will facilitate rational breeding and metabolic engineering of pennycress and related alternative bioenergy crops.

References

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