

Investigating the Natural Variation of Pennycress Metabolome, an Emerging Crop for Aviation Biofuel.

Cintia Arias¹, Tyler Swanson¹, Fan Yang², Erich Grotewold³, Ajay Shah², and **Ana Paula Alonso**^{1*} (Anapaula.Alonso@unt.edu),

¹University of North Texas, Denton, TX; ²The Ohio State University, Columbus, OH; ³Michigan State University, East Lansing, MI

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.

The goal of this part of the project is to find biomarkers positively or negatively correlating with oil content to boost the breeding and/or metabolic engineering process. For this purpose, we studied the natural variation in the metabolome of 11 pennycress accessions from around the world. Biomass components—oil, protein, starch and cell wall—were sequentially extracted and quantified from two different stages of embryos development. The quantification of intracellular metabolites such as sugars, amino acids, organic acids and phosphorylated compounds was achieved by liquid chromatography tandem mass spectrometry as previously described⁴⁻⁶. The present poster focuses on integrating the results of these analyses and attempts to highlight the correlations between certain intracellular metabolites and oil composition and accumulation. Ongoing experiments are expanding this study to a total of 20 pennycress accessions. The long-term goal of this research is to use the biomarkers that we identified to enhance oil production in pennycress using rational breeding and/or metabolic engineering.

References

1. Fan, J.Q., Shonnard, D.R., Kalnes, T.N., Johnsen, P.B. & Rao, S. A life cycle assessment of pennycress (*Thlaspi arvense* L.) -derived jet fuel and diesel. *Biomass & Bioenergy* **55**, 87-100 (2013).
2. Dorn, K.M., Fankhauser, J.D., Wyse, D.L. & Marks, M.D. A draft genome of field pennycress (*Thlaspi arvense*) provides tools for the domestication of a new winter biofuel crop. *DNA Res* **22**, 121-31 (2015).
3. Dorn, K.M., Fankhauser, J.D., Wyse, D.L. & Marks, M.D. De novo assembly of the pennycress (*Thlaspi arvense*) transcriptome provides tools for the development of a winter cover crop and biodiesel feedstock. *Plant Journal* **75**, 1028-1038 (2013).
4. Tsogtbaatar, E., Cocuron, J.C., Sonera, M.C. & Alonso, A.P. Metabolite fingerprinting of pennycress (*Thlaspi arvense* L.) embryos to assess active pathways during oil synthesis. *Journal of Experimental Botany* **66**, 4267-4277 (2015).
5. Cocuron, J.C., Anderson, B., Boyd, A. & Alonso, A.P. Targeted metabolomics of *Physaria fendleri*, an industrial crop producing hydroxy fatty acids. *Plant and Cell Physiology* **55**, 620-633 (2014).
6. Cocuron, J.C., Tsogtbaatar, E. & Alonso, A.P. High-throughput quantification of the levels and labeling abundance of free amino acids by liquid chromatography tandem mass spectrometry. *Journal of Chromatography A* **1490**, 148-155 (2017).

This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0019233.