Elucidating the Temporal and Spatial Organization of Storage Lipids using ^{13}C-labeling in Developing Embryos of pennycress, a Promising Source for Aviation Fuel

Umesh Prasad Yadav[1]* (umeshprasad.yadav@unt.edu), Trevor B. Romsdahl[1], Emmanuel Ortiz[1], Erich Grotewold[2], Kent D Chapman[1], **Ana Paula Alonso[1]**

[1]Biodiscovery Institute, University of North Texas, Denton, TX USA; [2]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI USA

Project Goal: Assessing temporal and spatial regulation of storage lipid accumulations in developing Pennycress seeds using ^{13}C-labeling and mass spectrometry imaging.

The US military and commercial aviation industry consume nearly 20 billion gallons of jet fuel per year. With unpredictable prices, finite fossil fuel sources, and concerns over environmental impact, the discovery of sustainable alternatives to fossil-derived jet fuels is critical. In the last few years, pennycress (*Thlaspi arvense* L.) emerged as a promising oilseed crop especially suited for aviation fuel production due to its moderate oil content and fatty acid composition. In addition to its excellent biofuel potential, pennycress requires low agricultural inputs and can serve as a cover crop when grown in a summer/winter rotation cycle with other conventional commodity crops, such as corn and soybean. Improvements to the understanding of regulatory factors that limit oil yields in pennycress seeds will be instrumental to advance the goal of developing bio-based aviation fuels for the future. Culture conditions were optimized to provide ^{13}C-labeled glucose to pennycress siliques to follow the temporal and spatial organization of storage lipid metabolism in pennycress seeds. This approach allows for the tracing of metabolism from carbohydrate sources to storage oils in near in vivo conditions. The fatty acid composition and growth rate of embryos harvested from these silique cultures were similar to *in planta*, which validates this method. Using this procedure, 100% [U-^{13}C]-glucose was supplied to 16 days after pollination (DAP) siliques for 120 h to track the incorporation of ^{13}C-labeled acetyl fragments into *de novo* synthesized fatty acids in seed plastids and in elongated fatty acids in the cytoplasm. The results showed that the percentage of labeling in plastidic and cytosolic fragments was found to be significantly lower in the axis of the embryos in comparison to the cotyledons. A time course-experiment was conducted to monitor the temporal incorporation of ^{13}C-acetyl fragment in embryos from 14 DAP siliques incubated for 24 h, 48 h, 72 h, and 96 h; the labeling in acetyl fragments gradually increased up to 19%. Further, to evaluate the spatial organization of lipid metabolites in developing seeds, ^{13}C-isotopic labeling and mass spectrometry imaging (MSI) were coupled to analyze metabolic flux *in situ*. Combining isotopic labeling and MSI presents technical challenges ranging from sample preparation, label incorporation, data collection, and analysis. Using currently available software and techniques, ^{13}C-labeled isotopomers were analysed for the membrane lipid and storage oil lipid intermediate phosphatidylcholine (PC). Consistent with ^{13}C-isotopic labeling of fatty acids, MSI revealed greater ^{13}C-isotopic labeling of PC molecular species in the cotyledons than the embryonic axis of developing embryos. Moreover, greater isotopic enrichment in PC molecular species with more saturated and longer chain fatty acids suggest differences in flux related to fatty acid desaturation and elongation. Expanding the combination of ^{13}C-isotopic labeling and MSI to additional time points and to additional lipid intermediates will provide an opportunity to visualize the spatial differences in lipid metabolism during seed development. These data-intensive,

comprehensive approaches will offer greater insights into the internal organization and regulation of oil synthesis in pennycress seeds, which will ultimately support metabolic engineering efforts to produce higher yield of biofuels.

This research was supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomic Science Program grant no. DE-SC0020325.