Using ^{13}C-labeling to Unravel the Temporal and Spatial Production of Seed Oil in Developing Embryos of Pennycress, a Promising Source for Sustainable Aviation Fuel

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

[1]Biodiscovery Institute, University of North Texas, Denton, TX; [2]University of Texas Medical Branch, Galveston, TX

Project Goal: Assessing temporal and spatial regulation of storage lipid accumulation 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. Development of sustainable alternatives to fossil-derived jet fuels is critical due to unpredictable prices, finite fossil fuel sources, and concerns over environmental impact. To mitigate the impact of the growing demand for jet fuel, the US aviation industry is targeting an increase in the production of sustainable aviation fuel by 3 billion gallons per year by 2030. In the last few years, pennycress (*Thlaspi arvense* L.) emerged as a promising oilseed crop, especially suited for aviation fuel production, due to its 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 yield 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 incorporation of labeled carbon in intracellular metabolites and analyze lipid synthesis and storage in pennycress seeds. This approach allows tracing metabolism from carbohydrate sources to storage oils *in vivo* conditions. For this purpose, 100% [U-^{13}C]-glucose was supplied for 120 h to siliques 16 days after pollination (DAP) 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 percentage of labeling in plastidic and cytosolic acetyl fragments was found to be significantly lower in the axis of the embryos in comparison to the cotyledons. Moreover, mass spectrometry imaging (MSI) was performed in ^{13}C-labeled pennycress embryos to analyze the isotopologues of phosphatidylcholine (PC), an important membrane lipid and intermediate in storage oil biosynthesis. MSI displayed a greater ^{13}C-labeling of PC molecular species in the cotyledons than the embryonic axis[1]. Monitoring the temporal incorporation of ^{13}C-acetyl units into fatty acids of developing embryos showed that the ^{13}C-labeling in acetyl fragments slowly increased to 12%, whereas ^{13}C incorporation in sugars, amino acids, and organic acids occurred more quickly and reached a plateau. These results provide insights on the temporal and spatial production of oil in pennycress seeds, and will guide metabolic engineering efforts to produce higher oil yield for use as aviation fuel.

Reference:

1. Romsdahl, T.B.; Kambhampati, S.; Koley, S.; Yadav, U.P.; Alonso, A.P.; Allen, D.K.; Chapman, K.D. Analyzing Mass Spectrometry Imaging Data of 13C-Labeled Phospholipids in Camelina sativa and Thlaspi arvense (Pennycress) Embryos. Metabolites 2021, 11, 148.

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