

Functional Analysis of Candidate Genes Involved in Oil Storage and Stability in Pennycress

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Project Goals: 1) To investigate the function of candidate genes involved in lipid storage and stability using transient expression in *Nicotiana benthamiana*. 2) To use stable transformation to evaluate the impact of these candidate genes on lipid droplets, biomass content, lipid spatial distribution and composition in pennycress plants. 3) To characterize the effect that co-expression of these genes has on central metabolism and the temporal organization of storage lipids in developing pennycress seeds.

The US military and commercial aviation industry together consume 20 billion gallons of jet fuel per year, the cost of which has more than tripled since 2000. In order to reduce the reliance on fossil fuels, and our environmental footprint as well as costs, it is imperative to develop renewable sources of aviation fuel. In recent years, pennycress (*Thlaspi arvense* L.) has been identified as a promising alternative oilseed crop suitable for aviation fuel production due to its high oil content and fatty acid composition. Biodiesel produced from pennycress exhibits excellent characteristics such as low cloud point temperature, pour point, and cold filter plugging point, with 50% less greenhouse gas emissions compared to petroleum-based fuel. Pennycress requires low agricultural inputs and can serve as a cover crop to utilize excess nitrogen, slow soil erosion, suppress weeds, and attract pollinators when grown in a summer/winter rotation cycle with other conventional commodity crops such as corn and soybean. Its yields reach 2,000 Lbs/acre, producing up to 100 gallons of oil per acre, which is twice more than soybean or camelina (another bioenergy crop under consideration). Given that this crop could be grown in the 90 million-acre US Midwest Corn Belt without displacing commodity crops, pennycress has the potential to produce 9 billion gallons of aviation fuel per year, nearly meeting half of the national demand.

Our group has been developing tools and resources to improve oil content and quality in pennycress (DE-SC0019233). Technoeconomic analyses of the costs associated with production and logistic operations revealed that pennycress would be more competitive than other crops considered for renewable jet fuel production, such as camelina, carinata, and canola. Also, the preliminary findings from our analyses of pennycress natural variation have identified candidate genes and metabolites associated with increased oil accumulation. These biomarkers are involved in lipid synthesis/degradation, and in primary metabolism. Of particular interest is the correlation between transcript levels of candidate genes involved in fatty acid storage and stability with total oilseed content at maturity. This finding underlines the central role of the packaging and storage of triacylglycerols in our quest to enhance oil production in plant organs.

While pennycress, as a member of family Brassicaceae, benefits from the fully sequenced genome and research tools of the closely related model plant *Arabidopsis thaliana*, there are still significant challenges associated with establishing gene function that would make pennycress much more valuable as a bioenergy oilseed crop. A detailed functional genomics study of these genes will be the focus of this project. To achieve this goal, we will first evaluate the function and subcellular localization of 43 priority candidates in *N. benthamiana*. In parallel, we will screen for changes in lipid droplet morphology and organization as well as variations in overall biomass and

lipid composition. We will then test up to 15 single/combined candidates on pennycress plants using stable transformation. Seeds from homozygous mutant plants and wild type will be examined to evaluate the impact of the mutation(s) on lipid droplets, biomass content, lipid spatial distribution and composition. Finally, we will analyze the effect that co-expression of these genes has on the pathways of central metabolism. We will also assess the impact on the temporal organization of storage lipids in developing seeds using ^{13}C -labeling and MALDI-MS imaging.

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