Identification of Pennycress (*Thlaspi arvense* L.) Proteins that Influence Lipid Droplet Formation and Modulate Neutral Lipid Accumulation

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Project Goals: To investigate the function of pennycress candidate genes involved in lipid storage and stability using transient expression in *Nicotiana benthamiana*, and to generate stable transgenic pennycress lines for improved seed oil content and composition.

The finite nature of crude oil-derived fuels coupled with their adverse effects on the environment requires the search for alternative, renewable sources of energy that are more environmentally friendly. Pennycress (*Thlaspi arvense* L.) has been identified as a promising alternative crop for aviation fuel production. It is an annual winter *Brassicaceae* growing in most temperate regions including North America, and produces seeds with high oil content (26-39%). The average yield of pennycress seeds is 1,500 kg ha^{-1}, corresponding to 600–1200 L ha^{-1} of oil, which is higher than that of common oil crops such as soybean and camelina. While pennycress 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 dedicated bioenergy oilseed crop. Transcriptional analysis of 22 pennycress accessions resulted in the identification of 35 potential gene candidates whose expression levels were correlated with seed oil yield (DE-SC0019233). After transient over-expression of the coding sequences of these 35 pennycress genes, 15 enhanced the number of cytoplasmic lipid droplets, increased the total neutral lipid content, and altered the fatty acid composition in the neutral lipid fraction of *Nicotiana benthamiana* leaves. Confocal microscopy also showed that protein products of two of these ORFs - a lipid transfer protein (LTP6) and a lipid droplet associated protein (LDAP3) - mainly localized to lipid droplets. These two ORFs were cloned into expression constructs behind the *CaMV 35S* constitutive promoter or the pNAP seed specific promoter and transformed into pennycress plants through the floral-dip method. Future work is aimed at characterizing seeds of these transgenic lines to examine the impact of LTP6 and LDAP3 overexpression on seed lipid content and composition. These results may lead to new targets to effectively improve oil content and composition in pennycress seeds, and may ultimately contribute to the production of sustainable aviation fuel.

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