Unravelling the Role of Pennycress (Thlaspi arvense L.) Proteins in the Modulation of Neutral Lipid Droplet Abundance

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Project Goals: To investigate the function of candidate genes involved in lipid storage and stability using transient expression in Nicotiana benthamiana.

The finite nature of crude oil-derived fuels coupled with their adverse effects on the environment means the search for alternative, renewable sources of energy that are more environmentally friendly is paramount. 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 parts of North America, and produces seeds with high oil content (26-39%). It can be grown in a summer/winter rotation cycle with other conventional commodity crops such as maize and soybean and requires low agricultural inputs. 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 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 bioenergy oilseed crop. Transcriptional analysis of 22 pennycress accessions resulted in the identification of potential gene candidates whose expression levels were correlated with seed oil yield (DE-SC0019233). Here, we show that protein products of six of these candidate genes- a lipid transfer protein homolog (LTP6), a lipid droplet associated protein homolog (LDAP3), an annotated lipase (α/β hydrolase), a long-chain acyl-coA synthase protein (LACS1), an endomembrane regulatory protein (RABA3), and a lipid storage and packaging protein (Oleosin)- mainly localize to lipid droplets when transiently expressed in Nicotiana benthamiana. The overexpression of coding sequences for these six proteins in N. benthamiana leaves resulted in a proliferation of cytoplasmic neutral lipids appearing as droplets under confocal microscopy. Analysis of the infiltrated leaves using GC-MS indicated that the overexpression of these proteins increased the total neutral fatty acid content and somewhat altered the fatty acid composition of N. benthamiana leaves. Our data point to possible roles of these six candidate proteins in the compartmentalization and/or stability of pennycress lipid droplets and represent interesting targets for genetic manipulation of pennycress seeds with increased oil content.

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