

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

Julius Ver Sagun^{1*} (juliusver.sagun@unt.edu), Athanas Guzha¹, Cintia Arias¹, Tatiana Garcia², Allison Barbaglia², Erich Grotewold², Kent D. Chapman¹, and **Ana Paula Alonso**¹

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

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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