

## Engineering Vegetative Lipids in a Fast-Growing and High-Biomass Arabidopsis Line

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**Project Goals: The overarching goal of our research is to enhance the production of storage lipids in plant vegetative tissues whilst minimizing negative effects on plant growth and development.**

Triacylglycerols (TAGs) are storage lipids commonly found in plant seeds. As one of the most energy-rich compounds found in nature, TAGs have become an important target for renewable biofuel feedstocks. Seed-based TAGs are mostly dedicated for food and animal feed uses. Vegetative biomass, because of its high capacity for fatty acid (FA) synthesis, represents a potential renewable, sustainable, and economical platform for TAG accumulation to offset some of the increasing demand for fossil oil. While TAGs are barely detectable in plant vegetative tissues, bioengineering strategies have been developed to enhance the accumulation of storage lipids by directing carbon flux toward lipid synthesis. However, the high level of vegetative TAG accumulation in bioengineered crops is often associated with plant growth deficits (Zale et al., 2016). Previous studies identified a purple acid phosphatase (*PAP2*) that could increase plant growth rate, vegetative biomass, and seed yield when overexpressed in plants (Sun et al., 2012). *PAP2* targets both chloroplasts and mitochondria and has been shown to elevate adenosine triphosphate (ATP) content and photosynthesis rate, most likely by dephosphorylating proteins thereby facilitating their transport into chloroplasts and mitochondria. Here, we analyzed the influence of *PAP2* overexpression on lipid metabolism and designed strategies to enhance TAG accumulation in a fast-growing and high-biomass *PAP2*-overexpression line. Lipid analysis revealed that overexpression of *PAP2* in Arabidopsis increased FA synthesis rates in siliques, leading to elevated seed oil content. On the other hand, the levels of total lipids and TAG in leaves were not altered by *PAP2* overexpression, as both FA synthesis and turnover rates were increased in leaves overexpressing *PAP2*. To enhance the accumulation of TAG in vegetative tissues of the *PAP2*-overexpression line, we crossed it with a TAG lipase mutant (*sdp1*) and a phospholipid:diacylglycerol acyltransferase (*PDAT1*) overexpression line to impede lipid turnover and convert membrane lipids into TAG. Our results show that these combinations produced significantly higher amounts of TAG in vegetative tissues relative to the parental lines, suggesting that *PAP2* can be successfully stacked with other lipogenic factors to enhance TAG accumulation in vegetative tissues. Therefore, our work provides novel strategies to engineer storage lipids in plant vegetative tissues and potentially offsets yield drag associated with high levels of vegetative TAG accumulation.

## References

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