Energizing the Machinery of Storage Lipid Synthesis in Plant Vegetative Tissues

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Project Goals: As part of the CABBI Feedstock Production theme, the overall goal of our research program is to enhance the production of vegetative lipids in bioenergy grass stems for industrial purposes.

Abstract text.

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 growing demand for fossil oil. Substantial progress has been made to enhance the production of vegetative lipids in bioenergy grass stems for industrial purposes by engineering factors directly involved in TAG biosynthesis and degradation. To further enhance TAG biosynthesis in vegetative tissues, we hypothesized that supplying the lipid synthesis machinery with additional energy may help to overcome some intrinsic limits. Our study has identified three adenosine triphosphate (ATP)-related factors (PAP2, NTT1 and NTT2) that, when overexpressed, have positive effects on TAG accumulation in plant cells. Purple Acid Phosphatase 2 (PAP2) has been shown to elevate ATP content and vegetative biomass when overexpressed in Arabidopsis, probably by dephosphorylating proteins and affecting their transport into chloroplasts and mitochondria. Arabidopsis plants overexpressing PAP2 showed increased FA synthesis rates in both leaves and siliques. The TAG content was also increased in *Nicotiana benthamiana* leaves when co-expressing PAP2 with lipogenic proteins (WRI1, DGAT1 and OLE1). The other two factors tested are plastid envelope-localized nucleoside triphosphate transporters (NTT1 and NTT2) that transport ATP from the cytosol into plastids. Transient overexpression of NTT1 or NTT2 alone or in combination with WRI1 significantly increased TAG accumulation in *N. benth.* leaves. Our findings provide novel targets that can be stacked with lipogenic factors to enhance TAG accumulation in plant vegetative tissues and potentially overcome the yield drag associated with high levels of vegetative TAG accumulation.

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