Ectopic Expression of OLEOSIN 1 and Inactivation of GBSS1 Have a Synergistic Effect on Oil Accumulation in Plant Leaves

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Background/objective
Oil, i.e., triacylglycerol (TAG), is an energy-dense feedstock molecule for biofuel and bioproduct production. TAG mainly accumulates in plant seeds, whereas the major portion of plant vegetative biomass has very low levels. Genetic and metabolic engineering attempts to accumulate TAG in leaves of crop plants have been somewhat successful, but those attempts often lead to significant reductions in plant growth. This study aimed to characterize a high-leaf-oil OLEOSIN-GREEN FLUORESCENT PROTEIN (OLE1-GSP)-expressing transgenic line with elevated TAG, and to use this information to develop a new Push-Pull-Protect strategy for enhancing vegetative oil accumulation.

Approach
- A T-DNA containing OLE1-GFP T-DNA line (OG) was investigated to understand why it had larger than expected effect on TAG accumulation by sequencing the DNA bordering its insertion site.
- Lipogenic factors WRINKLED1 (WRI1), Diacylglycerol O-Acyltransferase (DGAT1), and Cys-OLEOSIN1 (engineered sesame OLE1) were stacked into the OLE1-GFP line to increase leaf TAG accumulation.

Results
- Sequencing the borders of the T-DNA in the OG line showed it had inserted into a gene encoding the GRANULAR BOUND STARCH SYNTHASE (GBSS), a key gene in starch biosynthesis. Inactivation of GBSS resulted in a significant reduction in amylose and an increase in leaf oil content.
- Further stacking WRI1, DGAT1, and Cys-OLE1, under the control of an ethanol-inducible promoter in OG, significantly boosted oil content to 2.3% of dry weight in mature leaves, which is 15 times greater than levels found in WT Arabidopsis.

Significance
- We found that simultaneously overexpressing OLE1-GFP and knocking out GBSS1 significantly increases TAG accumulation.
- Our study provides a new proof-of-concept for elevated production of TAG in vegetative tissues with minimal effects on plant growth, development, and yield by overexpressing lipogenic factors in an OG background.