

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

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-GFP)*-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

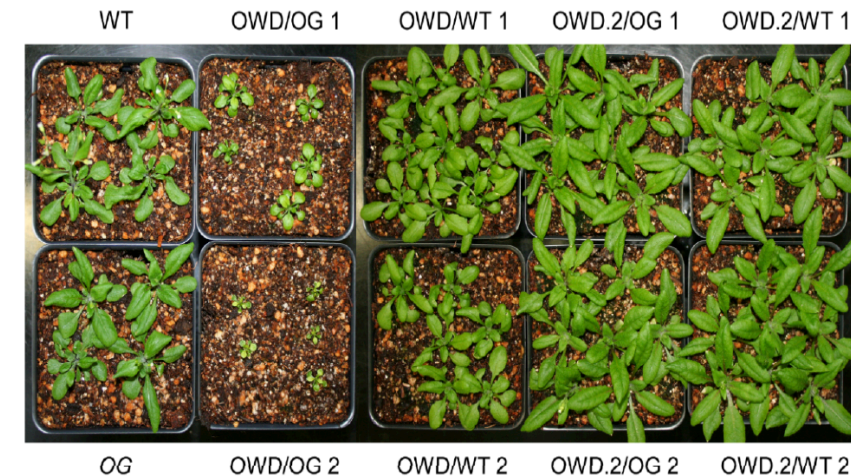
- ❖ A T-DNA containing *OLE1-GFP* T-DNA line (*OG*) was investigated to understand why it had larger than expected effect on TAG accumulation by sequencing the DNA bordering its insertion site.
- ❖ Lipogenic factors *WRINKLED1 (WRI1)*, *Diacylglycerol O-Acyltransferase (DGAT1)*, and *Cys-OLEOSIN1* (engineered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



Representative phenotype of constitutive or inducible OWD transgenic plants. Two representative 8-week-old soil grown transgenic lines for constitutive (OWD) or inducible expression (OWD.2) of OWD in WT or OG background were shown.