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The Expression of Genes Encoding Novel Sesame Oleosin Variants Facilitates Enhanced Triacylglycerol Accumulation in Arabidopsis Leaves and Seeds

Background/Objective

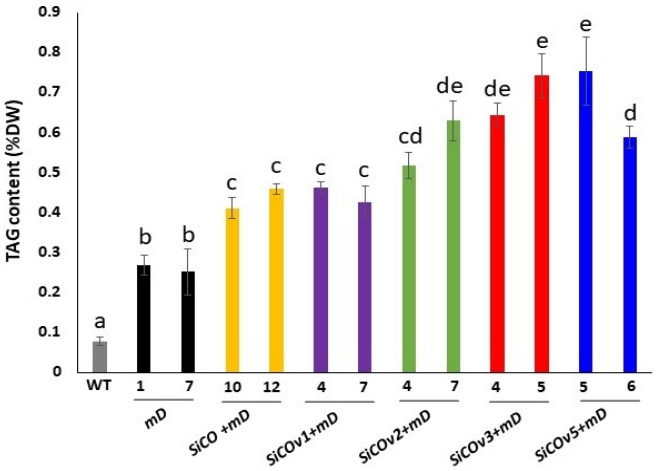
Vegetable oils are key feedstocks for biodiesel production, which is chemically derived from triacylglycerols (TAG). These accumulate within lipid droplets, predominantly surrounded by oleosins (OLE), that protect TAG from hydrolysis. In this study, we tested the theory, in yeast and tobacco, that identifying and removing degradation signals from OLE would promote its abundance, preventing TAG degradation and enhancing TAG accumulation.

Approach

We tested whether mutating potential ubiquitin conjugation sites in a previously reported improved *Sesame indicum* OLE (SiO) variant, o3-3 Cys-OLE (SiCO herein), would stabilize it and increase its lipogenic potential. SiCOv1 was created by replacing all five lysines in SiCO with arginines. Separately, six cysteine residues within SiCO were deleted to create SiCOv2. SiCOv1 and SiCOv2 mutations were combined to create SiCOv3.

Results

Transient expression of SiCOv3 in *Nicotiana benthamiana* increased TAG by two-fold relative to SiCO. Constitutive expression of SiCOv3 or SiCOv5, containing the five predominant TAG-increasing mutations from SiCOv3, in Arabidopsis along with mouse DGAT2 (mD), increased TAG accumulation by 54% in leaves and 13% in seeds compared to control lines co-expressing SiCO and mD.



Co-expression of SiCOv3 or SiCOv5 and mD resulted in the most significant increases in TAG content in Arabidopsis leaves of transgenic lines.

Significance/Impacts

This work identifies a series of key locations within OLE that, when mutated, led to increased TAG accumulation upon their transient expression in *Nicotiana benthamiana* leaves. These OLE variants represent novel factors for potentially increasing TAG accumulation in a variety of oil crops. Such renewable plant TAG crops represent feedstocks that can contribute to meeting the rapidly increasing demand for sustainable biodiesel and aviation fuels with reduced net carbon emissions relative to fossil sources.

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