Optimizing Lipogenic Factors for Vegetative Oil Accumulation

Sanket Anaokar[1*] (anaokars@bnl.gov), Yuanheng Cai[2], Yingqi Cai[1] and **John Shanklin[1, 2]**

[1]Brookhaven National Laboratory, Upton, NY; [2]Stony Brook University, Stony Brook, NY

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Project Goals

1. Increasing oil accumulation and in mature stems of energycane and *Miscanthus*

2. Identifying overexpression/downregulation gene combinations to optimize TAG accumulation

3. Dissecting mechanisms underlying the strength of lipogenic factors

Abstract text

Plant vegetative tissues account for a most of plant biomass, so it is of great interest to engineer plants with high oil content in vegetative tissues. However, plants normally only accumulate high levels of oils in the seeds. Our work therefore focuses on translating knowledge gained from seed oil accumulation to vegetative tissues. Oil accumulation in plants generally falls into a 'push-pull-protect model in which push factors increase fatty acid synthesis, pull factors efficiently channel fatty acids into TAG, and protect factors minimize TAG degradation. Increased oil accumulation requires the up-regulation of genes involved in lipid synthesis and down-regulation of those involved in lipid degradation. We are investigating modifications to well-described lipogenic factors in addition to additional potential lipogenic factors to boost TAG accumulation.

The Push factor is acetyl Co-A carboxylase (ACCase), the initial and commonly rate-limiting step in FA synthesis, that produces malonyl-CoA for fatty acid biosynthesis. In plants, there are two types of ACCase, namely heteromeric ACCase (HetAcc) and homomeric ACCase (HoACC). HetACC is composed of four subunits and located in the plastid, whereas HoAcc is a cytosolic protein comprising a single large polypeptide. Although HetACC is predominantly involved in *de novo* fatty acid synthesis in the plastid, its activity is strongly feed-back inhibited by 18:1-ACP, PII and BADC. Therefore, we re-targeted the cytosolic HoACC into the plastid by fusing the N-terminal signal peptide of Rubisco small subunit fused to GFP with HoAcc (RGHoACC). Confocal image analyses showed that RGHoACC is correctly targeted into the plastids. Further analysis showed that RGHoACC transient over-expression in tobacco leaves resulted in more than 20% total fatty acid accumulation and a > 7-fold increase in TAG accumulation relative to EV control.

The Pull factor is diacylglycerol acyl transferase (DGAT), that catalyzes the final step of DAG to TAG in the ER. This can efficiently channel fatty acyl chains towards the synthesis of neutral lipid for storage. Overexpression of plant DGATs have been shown to increase TAG production. We identified a mammalian DGAT2 which was very efficient in producing TAG in tobacco transient overexpression assays. Tobacco leaves with DGAT2 overexpression driven by a senescence inducible promoter accumulated more than a 6-fold increase in TAG relative to the control leaves.

For the Protection of TAG in vegetative tissue we engineered an improved version of the *sesame* oleosin. Based on published reports, it has been suggested that oleosin is marked for degradation via conjugation with ubiquitin. Lysine, cysteine, serine, threonine or the N-terminus a target protein are the most common target residues for Ubiquitin ligation. In an attempt to stabilize the *sesame* oleosin, we choose to convert all six-lysine residue to arginine residues (KR) to maintain the positive charge distribution. Dr. N. Roberts previously reported a variant of *sesame* oleosin, in which he replaced six positions in the amino acid sequence for cysteines. In our current study, we choose to eliminate the amino acids at those six locations (CysDel) from the oleosin peptide creating a shorter version of Ole1. Our results indicate that both lysine-to-arginine conversions (KR) and amino acid deletions (CysDel) improved their ability to protect TAG in leaves of *N. benthamiana.* Upon combining the Ole1_CysDel and KR mutations, we created an oleosin that supported a 2-fold improvement of TAG accumulation in *N. Benthamiana* leaves. As the K-to-R replacements (KR) were applied to all the lysines, and amino acid deletions (CysDel) were applied to all six locations, we predicted some of the modifications would have a negative effect on TAG accumulation. By analyzing dropout mutations at each position, we determined the contribution of each mutation. We combined this information to create Ole1_5 Mod, containing mutations at five of the 11 locations. The Ole1_5 Mod variant further improved TAG accumulation in leaves of *N. Benthamiana* relative to all other *sesame* oleosin variants.

In summary, our strategy was first to individually optimize the Push, Pull and Protect factors described above. We subsequently combined the optimized factors with respect to promoting plant vegetative oil accumulation. Our results show that the effects of each optimized factor are additive. When the three factors are transiently expressed in *N. benthamiana* leaves, TAG accumulation increased more than 18-fold relative to empty vector controls, reaching a final TAG content of ~4% (w/w) over a three-day period.

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