

# Elucidation of Triacylglycerol Overproduction in the C<sub>4</sub> Bioenergy Crop *Sorghum bicolor* by Constraint-Based Analysis

## Background/objective

There is growing interest in increasing the energy density in bioenergy crops by improving the capacity to accumulate triacylglycerols (TAG) in above-ground vegetative tissues. For such metabolic engineering efforts, a theoretical benchmark for the capacity of photosynthetic tissues to accumulate oils is needed. In this study, a genome-scale metabolic model of sorghum (*Sorghum bicolor*) was developed. Based on metabolic pathway modeling, the theoretical capacity of a leaf to accumulate TAG at economically viable levels was explored.

## Approach

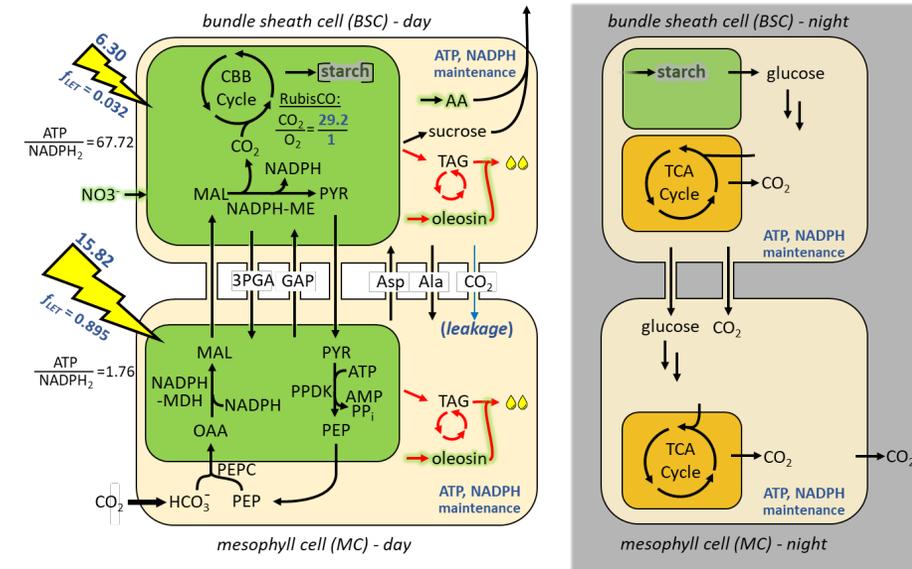
- ❖ A metabolic model was constructed and parametrized to represent photosynthetic CO<sub>2</sub> assimilation in a mature sorghum leaf.
- ❖ Daily photosynthetic rates were simulated for varying carbon allocation ratios (assimilate export vs. TAG accumulation) and in dependence of increasing impact of metabolic futile cycles.
- ❖ The relative demands in energy cofactors (ATP, NADPH) for sucrose and oil synthesis were compared with the supply by the photosynthesis light reactions (supply/demand balance).

## Results

- ❖ Energy demands in NADPH and ATP are ~1.3 to 1.4 times higher for TAG synthesis than for sucrose (on a per carbon basis).
- ❖ Based on empirical daily net CO<sub>2</sub> assimilation rates, a leaf TAG level of 20% (w/dw) is achievable within one month if only 5% of the photosynthetic net assimilation is re-allocated into oil droplets and if fatty acid futile cycling processes can be largely suppressed.
- ❖ Complete re-allocation of fixed carbon into TAG might be impeded by a supply/demand imbalance in energy cofactors (ratio ATP/NADPH).

## Significance

Relative to carbohydrates, the biosynthetic energy demands to photo-assimilate CO<sub>2</sub> into oils are not as high as often assumed. Since high TAG levels should be achievable within a month, engineering efforts could aim to limit the activation of genetic factors of TAG accumulation to the final phase of a plant's life cycle. This could mitigate yield drag effects that are often observable for transgenics where expression of TAG factors is active in all tissues and development stages.



**Diel model of NADP-ME subtype of C<sub>4</sub> photosynthesis in Sorghum:** Shown are major metabolic processes that take place across two cell types: photo-assimilation of CO<sub>2</sub> (day) and respiration of starch (night). Photosynthetically fixed CO<sub>2</sub> is exported (sucrose, amino acids). In engineered plants, carbon is re-allocated into oil droplets (red).