

# Diel rewiring and positive selection of ancient plant proteins enabled evolution of CAM photosynthesis in *Agave*

## Background

- Crassulacean acid metabolism (CAM) enhances plant water-use efficiency through an inverted day/night pattern of stomatal closure/opening. *Agave* species are model CAM crops for beverage, fiber, and biofuel production in semi-arid and arid regions. However, the genomic basis of evolutionary innovation of CAM in *Agave* is largely unknown.

## Approach

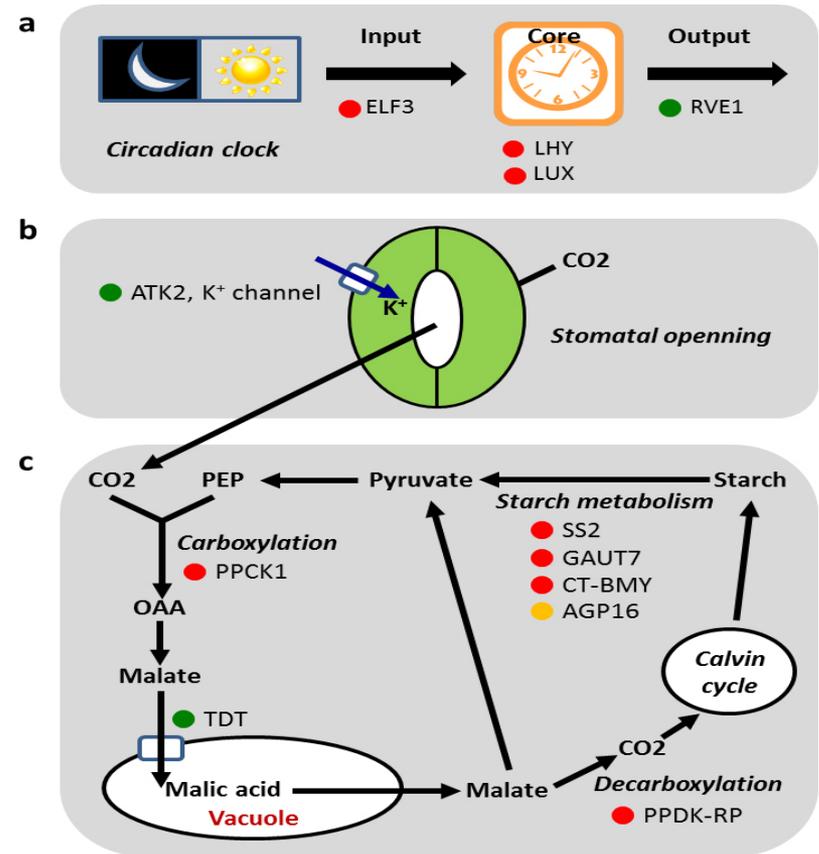
- Using an approach integrating genomics, gene co-expression network, comparative genomics and protein structure analysis, we investigated the molecular evolution of CAM in *Agave*.

## Outcomes

- The genetic components of core CAM machinery in *Agave* have an ancient origin traceable to non-vascular plant lineages.
- Regulatory proteins, which are shared between  $C_3$ , CAM and  $C_4$  species, were essential to the  $C_3$ -to-CAM transition.
- The evolution of CAM in *Agave* from  $C_3$  photosynthesis plants involved positive selection in protein sequences of enzymes and transporters implicated in metabolism and signaling associated with CAM, as well as diel re-programming of gene expression related to some key biological processes, such as circadian rhythms, redox homeostasis, and carbohydrate metabolism.

## Significance

- The results from this study provide unprecedented new insight into the molecular evolution of CAM pathway.
- This study generated new candidate genes for CAM-engineering to increase water-use efficiency in *Populus* and other bioenergy crops.



**Agave genes showing positive selection and rewiring of temporal expression pattern relative to  $C_3$  plants.** (a) Genes involved in circadian clock. (b) A gene involved in stomatal opening. (c) Genes involved in CAM pathway. Red circles indicate positive selection. Green circles indicate morning-to-night shift in peak gene expression. Yellow circles indicate afternoon-to-night shift in peak gene expression.