

# CRISPR/Cas9-mediated Targeted Mutagenesis for Functional Genomics Research of Crassulacean Acid Metabolism Plants

## Background

Crassulacean acid metabolism (CAM) is a CO<sub>2</sub>-concentrating mechanism that reduces photorespiration, enhancing plant water-use efficiency and drought tolerance. Over the past seven years, research interest in the CAM pathway has increased markedly due to public concerns about the decreasing supply of clean water and adverse effects of increasing heat and drought stress on plant growth. However, the progress in CAM functional genomics research is much slower than the progress made in functional genomics studies in plants that rely on C<sub>3</sub> and C<sub>4</sub> photosynthetic pathways.

## Approach

To explore the potential of gene-editing for functional genomics research in CAM plants, we tested the capability of CRISPR/Cas9 gene-editing system to characterize the function of a CAM-related gene (*PHOT2*) in *Kalanchoë fedtschenkoi*.

## Highlights

- We demonstrated for the first time that CRISPR/Cas9 technology allowed for efficient targeted indel mutagenesis in the model obligate CAM species *K. fedtschenkoi*, resulting in the successful generation of desired knockout mutants.
- Our results showed that the *PHOT2* gene was involved in CO<sub>2</sub> fixation and stomatal regulation in *Kalanchoë* plants, providing new insight into the role of *PHOT2* in the CAM pathway.

## Significance

In this study, we established a new gene-editing platform for linking genes to traits in CAM plants. The simplicity, robustness, and flexibility of this platform will accelerate CAM functional genomics research, facilitating genetic improvement efforts of food and bioenergy crops through CAM-engineering.

Liu D, Chen M, Mendoza B, Cheng H, Hu R, Li L, Trinh CT, Tuskan GA, Yang X. 2019. CRISPR/Cas9-mediated targeted mutagenesis for functional genomics research of crassulacean acid metabolism plants. *J Experiment Botany*. doi: 10.1093/jxb/erz415

