

The *Kalanchoe* genome -- An important model for systems biology and synthetic biology of crassulacean acid metabolism

Xiaohan Yang¹(yangx@ornl.gov), John C. Cushman², Rongbin Hu¹, Jerry Jenkins³, Jeremy Schmutz^{3,4}, Gerald A. Tuskan¹, Hengfu Yin¹, Shengqiang Shu⁴, Daniel Rokhsar⁴, David Goodstein⁴, Uffe Hellsten⁴, Anne M. Borland^{1,5}, Ray Ming^{6,7}, Robert Van Buren⁸, Ching Man Wai⁶, Haibao Tang⁷, Paul Abraham⁹, Hao-Bo Guo¹⁰, Hong Guo¹⁰, Rebecca L. Albion², Travis Garcia², Jungmin Ha², Sung Don Lim², Jesse A. Mayer², Bernard W. M. Wone², Won Cheol Yim², Daniel A. Jacobson¹, Deborah A. Weighill¹, James Hartwell¹¹, Xueqiong Li¹², Yanbin Yin¹²

¹Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN; ²Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV; ³HudsonAlpha Institute for Biotechnology, Huntsville, AL; ⁴US Department of Energy Joint Genome Institute, Walnut Creek, CA; ⁵School of Biology, Newcastle University, Newcastle, UK; ⁶Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, IL; ⁷FAFU and UIUC-SIB Joint Center for Genomics and Biotechnology, Fujian Agriculture and Forestry University, Fuzhou, China; ⁸Donald Danforth Plant Science Center, St. Louis, Missouri; ⁹Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN; ¹⁰Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville, TN; ¹¹Department of Plant Sciences, University of Liverpool, Liverpool, UK; ¹²Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois

<http://cambiodesign.org/>

Project Goals: Crassulacean acid metabolism (CAM) is a specialized mode of photosynthesis that features a temporal CO₂ pump with nocturnal CO₂ uptake, facilitates increased water-use efficiency (WUE), and enables CAM plants to inhabit water-limited semi-arid or seasonally dry environments. CAM provides an excellent opportunity for engineering both enhanced WUE and photosynthetic performance into bioenergy crops. This project has two main goals: 1) to identify the CAM-associated genes and gene networks using systems biology approaches and 2) to engineer CAM gene modules into C₃ species using synthetic biology approaches. The success of the project could allow biomass production on semi-arid, abandoned, or marginal agricultural lands.

Kalanchoe laxiflora is an important model species for systems biology research to understand the molecular basis of the CAM pathway (Yang *et al.* 2015) and identify the key genes necessary for engineering CAM into C₃ species. *K. laxiflora* features a relatively small genome (~256 Mb), an established transformation system and a short life cycle. Recently, we constructed a high-

quality *K. laxiflora* genome assembly consisting of 1,324 scaffolds, with a total length of ~256 Mb and an N50 of 2.45 Mb. A total of 30,964 genes were annotated in this genome assembly. Our comparative analysis of the *K. laxiflora* genome and 24 other plant genomes, including two CAM species, three C₄ photosynthesis species and 19 C₃ photosynthesis species, revealed CAM-specific orthologous gene groups that were shared among the three CAM species [*K. laxiflora*, *Ananas comosus* (pineapple) and *Phalaenopsis equestris* (orchid)] but absent in C₃ or C₄ photosynthesis species. We analyzed the synteny between the *K. laxiflora* genome and several other plant genomes and found that the *K. laxiflora* genome has a distinctive whole-genome duplication history. Based on the comparative analysis of day-night time-courses of gene expression between CAM and C₃ photosynthesis genes, we identified several genes that are involved in the CAM pathway in *Kalanchoe*. We also performed an in-depth analysis of selected gene families relevant to CAM physiology and determined that positive selection has contributed to the molecular evolution of CAM plants. The results highlight the potential of the *K. laxiflora* genome as a model for CAM genomics research. The CAM-related genes identified in the *K. laxiflora* genome provide a solid foundation for the ongoing effort to engineer CAM into C₃ photosynthesis bioenergy crops.

References

Yang X, Cushman JC, Borland AM, Edwards EJ, Wullschleger SD, Tuskan GA, *et al.* (2015) A roadmap for research on crassulacean acid metabolism (CAM) to enhance sustainable food and bioenergy production in a hotter, drier world. *New Phytologist* 207: 491-504.

This material is based upon work supported by the Department of Energy, Office of Science, Genomic Science Program (under award number DE-SC0008834). Oak Ridge National Laboratory is managed by UT-Battelle, LLC for the US Department of Energy (under contract number DE-AC05-00OR22725).