Leaf anatomical traits which accommodate the facultative engagement of crassulacean acid metabolism

Funding: Genomic Science Program DE-SC0008834

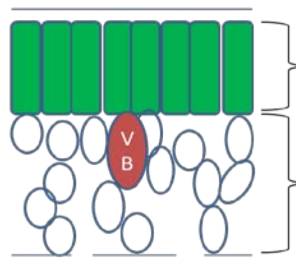
U.S. DEPARTMENT OF ENERGY
Office of Science

Background: The water-conserving mode of photosynthesis known as Crassulacean acid metabolism (CAM) is a strategic target for synthetic biology to engineer more productive C_3 crops for a warmer and drier world. Optimal operation of CAM requires a succulent leaf anatomy that can present diffusional limitations to CO_2 uptake. The aim of this work was to establish the optimal leaf anatomy that could accommodate bioengineered CAM in C_3 crops without incurring detrimental consequences for direct C_3-mediated photosynthesis

Approach:

- Leaf anatomical traits were measured across 9 species of *Clusia*, tropical trees that possess diverse modes of photosynthesis (e.g. CAM, inducible/facultative CAM and C_3 photosynthesis).
- Measurements of leaf thickness, depth of palisade and spongy mesophyll, cell size, intercellular air space, and localization of photosynthetic proteins (PEPC, Rubisco) in different leaf cell types were compared with magnitude of nocturnal CO_2 uptake (CAM).

Optimal leaf anatomy for bioengineered CAM



Modelled on anatomical data from *Clusia* (Zambrano et al, 2014)

Significance:

- The well-aerated spongy mesophyll of *Clusia* optimizes direct C_3-mediated CO_2 fixation, whilst enlarged and densely packed palisade cells with concentrated amounts of PEPC protein accommodate CAM and nocturnal accumulation of organic acids as a C store.
- The work indicates that traits likely to enhance the efficacy of engineered CAM in genotypes of C_3 target crops include; 1) increased ploidy (which is correlated with increased leaf cell size and biomass productivity) and 2) leaves with well-developed, large-celled palisade mesophyll.

Citation: Zambrano VA.B., Lawson T., Olmos E., Fernández-Garcia N. & Borland A.M. (2014) Leaf anatomical traits which accomodate the facultative engagement of crassulacean acid metabolism in tropical trees of the genus *Clusia*. *J.Exp.Bot.* First published online Feb 10, 2014. doi:10.1093/jxb/eru022

Contact: Anne Borland, anne.borland@ncl.ac.uk; borlandam@ornl.gov

UNIVERSITY OF LIVERPOOL | University of Nevada, Reno | Newcastle University | OAK RIDGE National Laboratory | THE UNIVERSITY of TENNESSEE KNOXVILLE | CAM cambiodesign.org

Journal of Experimental Botany (2014) doi:10.1093/jxb/eru022

U.S. DEPARTMENT OF ENERGY
Office of Science

Leaf anatomical traits which accommodate the facultative engagement of crassulacean acid metabolism in tropical trees of the genus *Clusia*

V. Andrea Barrera Zambrano[1], Tracy Lawson[2], Enrique Olmos[3], Nieves Fernández-García[3] and Anne M. Borland[1,4,*]

[1] School of Biology, Newcastle University, Newcastle upon Tyne NE17RU, UK, [2] School of Biological Sciences, University of Essex, Colchester CO4 3SQ, UK, [3] CEBAS–CSIC Campus Universitario de Espinardo, Department of Abiotic Stress and Plant Pathology, 30100 Murcia, Spain [4] Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6407, USA

Abstract

Succulence and leaf thickness are important anatomical traits in CAM plants, resulting from the presence of large vacuoles to store organic acids accumulated overnight. A higher degree of succulence can result in a reduction in intercellular air space which constrains internal conductance to CO_2. Thus, succulence presents a trade-off between the optimal anatomy for CAM and the internal structure ideal for direct C_3 photosynthesis. This study examined how plasticity for the reversible engagement of CAM in the genus *Clusia* could be accommodated by leaf anatomical traits that could facilitate high nocturnal PEPC activity without compromising the direct day-time uptake of CO_2 via Rubisco. Nine species of *Clusia* ranging from constitutive C_3 through C_3/CAM intermediates to constitutive CAM were compared in terms of leaf gas exchange, succulence, specific leaf area, and a range of leaf anatomical traits (% intercellular air space (IAS), length of mesophyll surface exposed to IAS per unit area, cell size, stomatal density/size). Relative abundances of PEPC and Rubisco proteins in different leaf tissues of a C_3 and a CAM-performing species of *Clusia* were determined using immunogold labelling. The results indicate that the relatively well-aerated spongy mesophyll of *Clusia* helps to optimize direct C_3-mediated CO_2 fixation, whilst enlarged palisade cells accommodate the potential for C_4 carboxylation and nocturnal storage of organic acids. The findings provide insight on the optimal leaf anatomy that could accommodate the bioengineering of inducible CAM into C_3 crops as a means of improving water use efficiency without incurring detrimental consequences for direct C_3-mediated photosynthesis.

UNIVERSITY OF LIVERPOOL · University of Nevada, Reno · Newcastle University · Oak Ridge National Laboratory · The University of Tennessee Knoxville · CAM

cambiodesign.org