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The biodesigner

Professor John C Cushman is dedicated to developing new techniques for the biodesign of economically and environmentally important plant species. Here, he discusses the importance of his research and why collaboration is key

When did you become interested in the biofuel potential of plants? How did your academic background lead to your role in this five-year project?

I became interested in this area over a decade ago, having worked on model crassulacean acid metabolism (CAM) plants for the last 25 years. Most of this work has focused on the common ice plant *Mesembryanthemum crystallinum*, which was one of the earliest facultative CAM plants described. CAM plants are six-fold and three-fold more water-use efficient than C_3 and C_4 plants, respectively, and our project followed the realisation that engineering this biochemical pathway could dramatically improve the water-use efficiency (WUE) of crops. Our research has gradually turned towards agronomically important CAM crops such as *Agave* and *Opuntia*, which only have 20 per cent of the crop water demands of C_3/C_4 crops, and offer great potential as low water-input feedstocks for bioenergy production due to their high biomass production potential.

Unlike most plants, which perform CO_2 uptake and fixation during the day, CAM plants take up CO_2 at night. What is the significance of this for your work?

More than 6 per cent of vascular plant species across more than 35 different families are estimated to engage in some form of CAM. CAM plants are distinguished from C_3 and C_4 plants by two major features – nocturnal CO_2 uptake and fixation leading to the formation of C_4 organic acids that are stored in the vacuole, and an inverse stomatal behaviour whereby stomata are open at night and closed during all or part of the day. These features mean that daytime evaporative water losses are curtailed, and daytime decarboxylation of stored C_4 organic acids creates an inorganic carbon-concentrating mechanism (CCM). In terms of our research, these features present an opportunity to introduce the improved WUE of CAM plants into C_3 or C_4 crops.

Can you offer an overview of your research into the biodesign of CAM? What are the main objectives?

The long-term goal of our CAM biodesign project is to improve the WUE of food and bioenergy crops. To achieve this, four major objectives will be pursued for enhancing photosynthetic performance and WUE in *Populus*. Firstly, we aim to define the basic genetic requirements of CAM in both eudicot and monocot species. Secondly, we want to characterise the regulation of the three major carboxylation, decarboxylation and inverse stomatal control functional modules of CAM. Thirdly, we hope to stably transfer fully functional carboxylation and decarboxylation modules into C_3 species and analyse the effects of these transgenic modules on WUE and biomass yield in *Arabidopsis* and *Populus*. Lastly, additional projects will expand the range of uses and products derived from CAM crops.

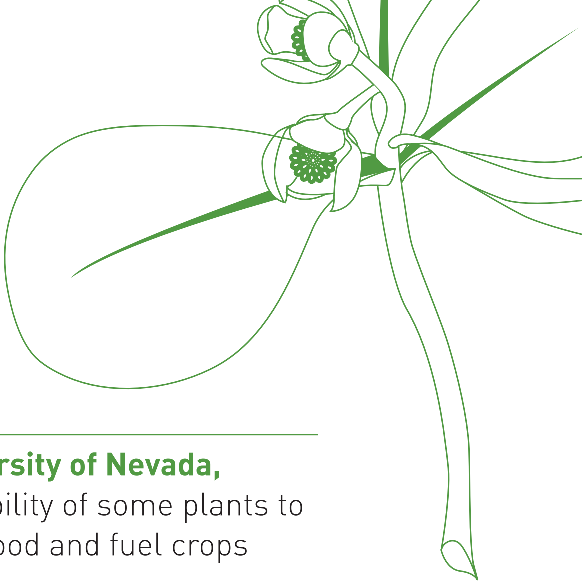
In the case of fast-growing, short-rotation forestry bioenergy crops such as poplar and willow, how does engineering CAM help improve WUE?

Here, engineered CAM is expected to improve WUE by shifting all or part of primary atmospheric CO_2 fixation to the nighttime, when the leaf-to-air vapour pressure deficit is lower due to cooler temperatures and higher relative humidity. This results in reduced stomatal conductance (and transpiration) and more conservative water use, avoiding reductions in leaf water potential and helping the plant to avoid hydraulic failure.

The utility of CAM in trees is exemplified by *Clusia* – a neotropical genus of woody eudicotyledonous tree that can colonise semi-arid habitats with nutrient-poor soils. Our evaluation of *Clusia* species and attempts to identify the anatomical and physiological traits that might best accommodate CAM in woody bioenergy species such as *Populus* are led by Professor Anne Borland at the University of Newcastle, UK, and colleagues at Oak Ridge National Laboratory, USA.

What have been the key advantages of the international collaborative nature of the project in furthering your studies?

Each laboratory brings its own unique expertise to the project, creating a great deal of synergy within the working group. The individual strengths of each lab are highly complementary, allowing us to divide up the workload in an efficient way and delegate specific tasks across the consortium accordingly. It also helps that most of our project partners have collaborated with one another at various times on past projects. A great deal of trust exists within the group, enabling us to maintain a dynamic and productive collaboration.



Spotlight on crassulacean acid metabolism

A groundbreaking international consortium led by the **University of Nevada, Reno**, USA, is making great strides towards exploiting the ability of some plants to assimilate CO_2 at night to boost the water-use efficiency of food and fuel crops

PROVIDING SUFFICIENT FOOD, feed and fuel for the Earth's growing population is one of the biggest challenges facing farmers, agroforesters and plant scientists in the 21st Century. At present, more than 40 per cent of the Earth's land is comprised of arid or semi-arid regions that are highly vulnerable to degradation. Due to the unsuitability of these regions for high-yield farming, they account for only a small proportion of the planet's primary production. If the resource needs of an expanding world population are to be met, such dry and hot growing regions will need to be utilised more effectively in order to improve their contribution to food, feed and fuel crop production.

In pursuit of this goal, a transatlantic consortium is attempting to discover and harness the molecular mechanisms of crassulacean acid metabolism (CAM), a carbon fixation pathway that has evolved in more than 35 families of plants in response to arid conditions. Combining the diverse specialties of a wide variety of plant science researchers, the consortium is focused on making plants for use in dryland agricultural systems more water-use efficient. In doing so, the team is expected to make a significant contribution to reclaiming dryland for a range of economically and environmentally important crops.

IDENTIFYING TARGET GENES

The primary aim of the consortium's research is to enhance the ability of plants to adapt to arid and semi-arid conditions, primarily through the introduction of CAM properties. The first stage of the work involves generating a comprehensive list of all the genes that might play a functional role in the performance of CAM. This endeavour identifies candidate genes by surveying gene coexpression and regulatory network patterns in both obligate and facultative CAM species. Dr James Hartwell and his colleagues at the University of Liverpool, UK, then systematically knock down candidate genes to ascertain whether their loss of function negatively affects CO_2 fixation, and indicate which genes play a role in CAM – one of the team's model systems.

COMPARATIVE GENOMICS AND BIODESIGN

Once functional roles of genes have been identified in model CAM species, Dr Xiaohan Yang and his colleagues at Oak Ridge National Laboratory, USA, use these results to perform comparative genomics analyses to identify related genes in other CAM species and understand the functional conservation of these genes across plant evolution. Dr John Cushman – a Professor in the Department of Biochemistry and Molecular Biology at the University of Nevada, Reno, and principal investigator of the project – underlines the importance of this element of the research: "These comparative analyses allow us to better understand the mechanisms by which CAM has evolved across at least 35 different plant families, with many independent evolutionary origins, and to look for variations in the biochemical pathway to better inform our biodesign strategies".

> If the resource needs of an expanding world population are to be met, dry and hot growing regions will need to be utilised in order to improve their contribution to crop production

Cushman and his colleagues then design appropriate expression cassettes for each gene, enabling them to ensure that the expression patterns are synchronised with CAM's specific temporal requirements. Lastly, the research team uses genera such as *Arabidopsis* and *Populus* to assess the impact of these genes on a range of attributes, including growth performance, water-use efficiency (WUE), drought tolerance and the role of tissue succulence in improving the performance of the CAM pathway. Professor Anne Borland from the University of Newcastle, UK, and Oak Ridge National Laboratory analyses the anatomical and physiological traits to identify which *Populus* species are best suited to serve as hosts for the introduced CAM pathway. This multidisciplinary, multi-stage project structure has enabled the researchers to make significant strides in elucidating the role of specific genes in CAM performance.

SUSTAINABLE AGROFORESTRY

The effects of global climate change are not limited to arid and semi-arid areas, as increasing heat, drought, surface evaporation and soil drying also pose large risks to tropical and temperate regions.

Forests account for almost a third of the planet's land surface and more than a quarter of global carbon sequestration. The scientific consensus is that as global temperatures rise and soils dry, tree mortality will significantly compromise the carbon sink capacity of forests. CAM could be the key to alleviating this risk: "It is possible that CAM could reduce tree mortality under drought conditions, which would help to retain forest carbon sink capacity during dry years," explains Cushman.

Cushman and his colleagues have identified *Populus* species as potential targets for temperate climates, and *Eucalyptus* for subtropical and tropical regions. These woody species – which are used in short-rotation forestry bioenergy crop production – have a high economic value, a plethora of genomic resources and established genetic transformation systems, making them ideal focus species for safeguarding the carbon sequestration potential of agroforests for decades to come.

UNIQUE SET OF CHALLENGES

While environmentally and economically significant, research on the CAM pathway has to overcome a number of complexities. Unlike C_3 or C_4 photosynthesis, CAM plant species conduct much or all CO_2 uptake at night when evaporative water loss is minimal. As such, their stomata operate in the exact opposite manner to those of C_3 and C_4 plants, which take up CO_2 mostly during the day, and this is

precisely the advantage of CAM in a dry, hot environment. To gain a better understanding of the mechanisms underpinning this behaviour, researchers within the consortium have been carrying out detailed, tissue-specific profiling of gene and metabolite expression in CAM species. Additionally, they are also performing thorough functional tests to elucidate the anatomical requirements for optimisation of CAM performance.

Furthermore, many CAM species possess leaf structures that are different from those typically found in C_3 and C_4 plants. Several of these species can vary their leaf succulence so as to reduce intercellular airspace and leaf internal CO_2 conductance, thereby minimising net CO_2 efflux from the leaf during the day. This form of leaf structure presents a diffusional constraint on both day- and nighttime CO_2 uptake. As a result, Borland, Cushman and colleagues are actively seeking a clearer understanding of the optimal leaf anatomy to identify and engineer, and investigating how best to encourage stomatal guard cell behaviour that allows for both C_3 and CAM photosynthesis.

AN INTEGRATED -OMICS APPROACH

Throughout their research to date, Cushman and his colleagues have been challenged and inspired by the sheer complexity of the CAM pathway. As Cushman elaborates: "Gaining a more complete understanding of the regulation of the circadian clock control of the temporal separation found in potentially competing primary and secondary carboxylation reactions has been a major challenge throughout this project".

By taking a cutting-edge, integrated -omics approach, the team has already generated a significant amount of new knowledge on how an apparently simple set of enzymatic reactions is temporally controlled and which other metabolic factors affect it. Cushman and his colleagues are optimistic that such breakthroughs at the molecular level could play a crucial role in securing food, feed and fuel stocks long into the future.

TYPES OF PHOTOSYNTHESIS

C_3 PHOTOSYNTHESIS

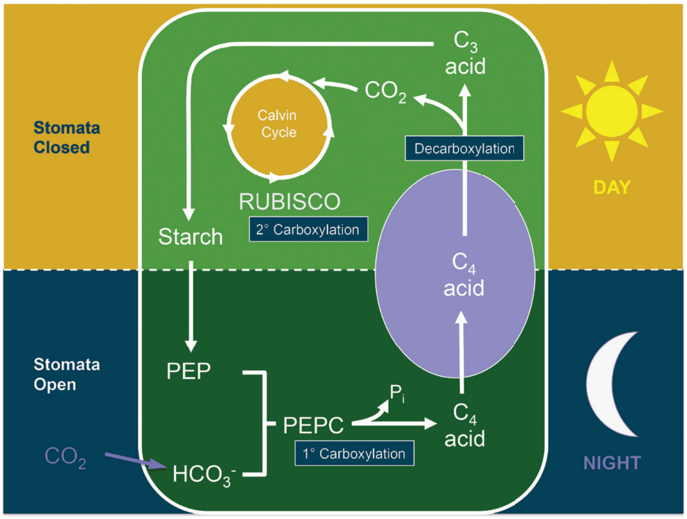
- Daytime process
- More efficient than C_4 or CAM plants under cool and moist conditions and under normal light
- More than 90 per cent of plant species perform C_3 photosynthesis

C_4 PHOTOSYNTHESIS

- Daytime process
- More efficient and better water-use efficiency than C_3 plants under high light intensity and high temperatures
- Roughly 4 per cent of all plant species, and half of all grass species use C_4

CAM

- Daytime and nighttime process
- Better WUE than C_3 and C_4 photosynthesis under hot and arid conditions; allows plants to survive dry spells and recover very quickly when water is available again
- More than 6 per cent of all plant species, including most cacti and succulents, are CAM



A simplified diagram summarising the major biochemical steps involved in crassulacean acid metabolism (CAM) including nocturnal uptake and fixation of CO_2 into C_4 acids, and subsequent daytime decarboxylation and refixation. Modified from Borland A M, *et al.*, 2014, Engineering crassulacean acid metabolism to improve water-use efficiency, *Trends in Plant Science*, **19**, 327-38 with permission from Cell Press, Elsevier Ltd.

INTELLIGENCE

BIODESIGN OF CRASSULACEAN ACID METABOLISM

OBJECTIVE

To improve the photosynthetic performance and water-use efficiency of food and bioenergy crop plant species by engineering crassulacean acid metabolism (CAM).

KEY COLLABORATORS

For a full list of collaborators, please visit:
www.cambiodesign.org/collaborators.jsp

FUNDING

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JOHN C CUSHMAN received his BSc in Biology from Ursinus College, Pennsylvania, USA, in 1982. Following this, he earned both his MSc and PhD in Microbiology at Rutgers University, New Brunswick, in 1984 and 1987, respectively. As a National Science Foundation (NSF) Plant Biology Fellow at the University of Arizona, Tucson, Cushman's postdoctoral research in plant biology focused on CAM induction by salinity and water-deficit stress. He is currently Foundation Professor in Biochemistry and Molecular Biology at the University of Nevada, Reno. Cushman has served as Associate Editor for *Plant Cell and Environment* and *The Plant Journal*, and as a member of the American Society of Plant Biologists (ASPB) education committee (2007–13). He is also an elected ASPB Fellow.

CAM