Manipulating Carbon Flux between Mesophyll and Bundle Sheath Cells to Optimize Photosynthetic Performance

Jennifer Arp[1]* (jarp@danforthcenter.org), Doug K. Allen,[1,2] Todd Mockler,[1] and **Ivan Baxter**[1]

[1] Donald Danforth Plant Science Center, St. Louis, MO
[2]United States Department of Agriculture, Agricultural Research Service, St. Louis, MO

www.foxmillet.org

Project Goals: This project aims to leverage Setaria viridis as a model system to develop novel technologies and methodologies to redesign the bioenergy feedstock Sorghum bicolor to enhance water use and photosynthetic efficiencies.

C_4 photosynthesis evolved to be more efficient than C_3 photosynthesis in hot and dry environments by utilizing specialized anatomy and biochemistry. C_4 is a highly convergent phenotype, having evolved 22-24 times in the grasses and over 60 times in plants and co-opting a variety of enzymes[1]. Previously, C_4 plants have been classified into three subtypes based on the decarboxylation enzyme used, but evidence now suggests plants may use a mixture of pathways. An untested hypothesis is that the mixed pathway allows smaller pools of transfer metabolites and flexibility in changing environments[2]. Maize uses both the NADP-malic enzyme (NADP-ME) and phosphoenolpyruvate carboxykinase (PEPCK) pathways, with aspartate and malate both serving as transfer metabolites into bundle sheath cells[3]; however, there is no evidence for mixed pathway use in the related grasses setaria and sorghum. The goal of this project is to install a supplemental PEPCK pathway into the NADP-ME type C_4 plants *Setaria viridis* and *Sorghum bicolor* using genetic elements from maize. Constructs are in progress to generate transgenic plants with the maize PEPCK gene driven by the native maize promoter or separately by a bundle sheath-preferred promoter such as RbcS. In addition, guide RNAs targeted to NADP-ME pathway genes have been designed to generate weak alleles and decrease relative flux through that pathway. Last, constructs are underway to modulate asparagine cycling genes to improve availability of aspartate as a transfer molecule in the mesophyll. In parallel, phenotyping tools are being developed to confirm flux through a PEPCK pathway which will be used to characterize the transgenic plants as they become available. The main approach will use isotopic labeling from $^{13}CO_2$ to measure relative flux through aspartate and malate and additionally will enable quantification of flux through the Calvin cycle. Gas exchange measurements will also be performed to measure enhancement of photosynthesis. The transgenic plants will demonstrate increased flux through the photosynthetic carbon concentrating mechanism which can be used to improve the performance of sorghum and related grasses in marginal and variable environments.

References

1. Sage, R. F., Christin, P.-A. & Edwards, E. J. The C(4) plant lineages of planet Earth. *J. Exp. Bot.* **62**, 3155–3169 (2011).
2. Wang, Y., Bräutigam, A., Weber, A. P. M. & Zhu, X.-G. Three distinct biochemical subtypes of C4 photosynthesis? A modelling analysis. *J. Exp. Bot.* **65**, 3567–3578 (2014).
3. Wingler, A., Walker, R. P., Chen, Z. H. & Leegood, R. C. Phosphoenolpyruvate carboxykinase is involved in the decarboxylation of aspartate in the bundle sheath of maize. *Plant Physiol.* **120**, 539–546 (1999).

This project is funded by grant DE-SC0018277 from The DOE Department of Biological and Environmental Research.