

Tissue and environment-specific synthetic gene circuits to regulate crown root growth

Jennifer A. N. Brophy^{1*} (jbrophy@carnegiescience.edu), Katie J. Magallon¹, and José R. Dinneny^{1,2}

¹Carnegie Institution for Science, Stanford, CA; ²Stanford University, Palo Alto, CA

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.

Grass species develop the majority of their root system through the adventitious initiation of crown roots from the base of the shoot. Crown root growth regulation is important for modulating whole-plant water use and preserving soil water. We are engineering synthetic regulatory circuits that control the development and physiology of crown roots to enhance water use efficiency (WUE) and water acquisition in C4 grass crops. Our approach is grounded in evidence that C4 grasses tend to exhibit overly conservative crown root responses to drought due to their wild origins. By engineering the crown roots of the model C4 grass *Setaria viridis* to respond differently to water, we aim to improve water use efficiency (WUE) and enable the design of plants that are optimized for specific environments.

Three crown root drought response strategies are being engineered and tested for their effects on growth and physiology in the lab and field. First, we are engineering *Setaria* to constitutively produce crown roots, independent of changes in water availability. This “environment-independent strategy” is based on the observation that domesticated grasses tend to show reduced sensitivity to drought. In an agricultural context, such strategies may have been selected because crops are specifically planted in regions that historically have certain expected patterns of rainfall, whereas a wild species would need to be able to acclimate to a broad range of potential precipitation patterns depending on where its seed landed. Second, we are engineering *Setaria* to enhance the production of crown roots when water is available. This “enhanced response to water” strategy is based on the idea that plants should maximize the number of crown roots produced during a precipitation event. Finally, we are engineering *Setaria* to enhance the suppression of crown roots during drought. This “enhanced suppression” strategy could be used to shift domesticated crops into more conservative strategies similar to their wild ancestors. This last strategy could be useful for broadening the normal geographic range in which a crop is grown, particularly into regions with sporadic or limited rainfall.

The construction of synthetic regulatory circuits to control crown root growth in these conditions requires fine-scale control of gene expression. We generated an RNAseq data set of crown responses to drought in order to identify promoters active in *Setaria* crowns. These promoters were classified as either constitutive, induced by water deficit, or induced by re-watering of drought stressed plants. Promoters that drive expression specifically in crown roots or surrounding tissues will be used to express genes that alter hormone signaling to control root growth. To generate new spatial patterns of gene expression or combine environmental input signals that control root growth in new ways, we are constructing synthetic genetic circuits using a new library of synthetic transcriptional activators, repressors, and promoters. Through transient

expression assays in *Nicotiana benthamiana* and stably transformed *Arabidopsis thaliana*, we show that each of these parts can be used to regulate transcription in plants and to construct synthetic genetic circuits that implement Boolean logic functions. These model systems enable rapid design-build-test cycles for synthetic genetic regulation.

In order to implement changes in crown root development, we will manipulate signaling pathways associated with root growth under well-watered and water deficit stress: abscisic acid (ABA), auxin, and reactive oxygen species (ROS). In *Arabidopsis*, we show that targeted expression of mutant auxin response regulators (AUX/IAA proteins) can alter lateral root production. A similar approach will be implemented in *Setaria* to inhibit auxin signaling and suppress crown root growth. We used RNAseq datasets to identify auxin response regulators (AUX/IAA-type proteins) that are highly expressed in *Setaria* crowns and likely to be involved in crown root development. Dominant negative mutations are being introduced to these proteins to manipulate auxin signaling and root development in *Setaria*. Genes identified by RNAseq as being induced by re-watering after drought are being explored as potential positive regulators of crown root growth. By expressing these developmental regulators at varying levels in specific conditions, we will attempt to control crown root responses to the environment to generate more robust C4 crop plants.

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