**Project Title**: Systems Level Regulation of Rhythmic Growth Rate and Biomass Accumulation in Grasses

**Final Project Report** - **Award No. DE-SC0016374**

**Principal Investigators**: Steve Kay, Samuel Hazen and John Mullet

**Original Objectives**

Several breakthroughs have been recently made in our understanding of plant growth and biomass accumulation. It was found that plant growth is rhythmically controlled throughout the day by the circadian clock throught a compelx interplay of light and phytohormone signaling pathways. While plants such as the C4 energy crop sorghum (*Sorghum bicolor* (L.) Moench) and possibly the C3 grass *Brachypodium distachyon* also exhibit daily rhythms in growth rate, the molecular details of its regulation remain to be explored. A better understanding of diurnally regulated growth behavior in grasses may lead to species-specific mechanisms highly relevant to future strategies to optimize energy crop biomass yield. We plan to devise a systems approach to identify, in parallel, regulatory hubs associated with rhythmic growth in C3 and C4 plants. We propose to use rhythmicity in daily growth patterns to drive the discovery of regulatory network modules controling biomass accumulation.

**Project Summary**:

Critical to the development of renewable energy sources from biofuels is the improvement of biomass from energy feedstocks, such as sorghum and maize. The specific goals of this project include 1) characterize the growth and gene expression patterns under diurnal and circadian conditions, 2) select transcription factors associated with growth and build a cis-regulatory network in yeast, and 3) perturb these transcription factors *in planta* using transgenic Brachypodium and sorghum, and characterize the phenotypic outcomes as they relate to biomass accumulation. A better understanding of diurnally regulated growth behavior in grasses may lead to species-specific mechanisms highly relevant to future strategies to optimize energy crop biomass yield.

**Accomplishments:**

We initiated a series of experiments designed to identify and manipulate molecular networks involved with diurnal growth regulation in sorghum and the emerging monocot model, Brachypodium distachyon. Successfully, we collected large datasets of gene expression form the model grass Brachypodium. We used and developed bioinformatics analysis tools to investigate the structure, dynamics and robustness of circadian regulated gene expression in Brachypodium. Moreover, we developed a method that utilizes the underlying concept of the yeast one hybrid assay (Y1H) but is modified further to study and validate protein complexes binding to their target DNA. Thus, it is referred to as the modified yeast one-hybrid (Y1.5H) assay. This assay will allow us to determine whether the interaction between two proteins is required for the activation of the target. The Y1.5H assay can facilitate researchers across the community to test and validate their hypothesis towards defining the role of multimeric protein complex binding to a common DNA target using a heterologous system.

**Relevant Discoveries:**

We were able to determine that the endogenous circadian clock appears to play a much more subdued role in growth regulation in Brachypodium, that has been demonstrated in either Arabidopsis, or crop plants like Rice, Corn and Soybean. This led to our conclusion that Brachypodium unfortunately is unlikely to serve as an informative model for understanding how growth regulation in plants is under the control of circadian network circuitry. However, we were able to leverage our datasets in Brachypodium to inform us and reinforce a large collaborative study on gene networks governing cell wall deposition in Arabidopsis. In addition, towards our efforts to characterize the candidate transcription factors in yeast we developed a new assay and named it modified yeast-one hybrid (Y1.5H). The goal of this assay is to express the protein of interest with an activation domain (pDEST22: TF) and evaluate the activation of the candidate DNA regions fused to a reporter in the presence and/or absence of the interacting protein partner (pDEST32$\Delta$DBD) in the yeast system. This assay will allow us to determine whether the interaction between these two proteins is required for the activation of the target. This method is very useful for studying plant genomics where *in vivo* validation could be challenging given the level of ploidy of the plant and time and labor intensive transformation procedures. Thus, the use of this method can accelerate the hypothesis testing at least in a heterologous system. This method could be a

valuable tool for the plant community to test and validate the heteromeric protein complex binding to their DNA target(s) for plant functional genomics.

**Publications:**

Matos DA, Whitney IP, Harrington MJ, Hazen SP (2013) Cell walls and the developmental anatomy of the brachypodium distachyon stem internode. PLoS ONE 8(11): e80640. doi:10.1371/journal.pone.0080640

Matos, D.A., Cole, B.J., Whitney, I.P., MacKinnon, K.J.M., Kay, S.A., Hazen, S.P. (2014) Daily changes in temperature, not the circadian clock, regulate growth rate in *Brachypodium distachyon*. *PloS one,* 9(6):e100072, PMID: 24927130

Da Costa, R.M.F., Lee, S.J., Allison, G.G., Hazen, S.P., Winters, A., Bosch, M. (2014) Genotype, development and tissue-derived variation of cell-wall properties in the lignocellulosic energy crop Miscanthus. *Annals of Botany* 114: 1265–1277, 2014 doi:10.1093/aob/mcu054

Tyler, L., Lee, S.J., Young, N.D., Delulio, G.A., Benavente, E., Reagon, M., Sysopha, J., Baldini, r.m., troia, a., hazen, s.p., caicedo, a.l. population structure in the model grass *brachypodium distachyon* is highly correlated with flowering differences across broad geographic areas. *Plant Genome* Volume 9. doi: 10.3835/plantgenome2015.08.0074;

Da Costa, R.M.F., Pattathil, S., Avci, U., Lee, S.J., Hazen, S.P., Winters, A., Hahn, M.G., Bosch, M. A cell wall reference profile for Miscanthus bioenergy cropshighlights compositional and structural variations associated with development and organ origin. New Phytologist (2017) 213: 1710–1725 doi: 10.1111/nph.14306

**Patents:** None

**Software Releases:** None

**Other Products:** None