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STI Product Title	Final Technical Report for Modulation of Phytochrome Signaling Networks for Improved Biomass
STI Product Type and Reporting Period	Final Technical Report for Period August 2011-August 2015
Date of Issuance	November 7, 2016
Author	Todd C. Mockler
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Final Technical Report

Principal Investigator: Todd C. Mockler 314-587-1694

Institution: Donald Danforth Plant Science Center

Title: Modulation of Phytochrome Signaling Networks for Improved Biomass Accumulation Using a Bioenergy Crop Model

SC Division: SC-23.2

Program Manager: Catherine M. Ronning 301-903-9549

1. Summary of completed activities.

A. Developed and deployed an automated high-throughput yeast one-hybrid platform for *Brachypodium distachyon.*

We cloned and validated a total of 483 Brachypodium transcription factor (TF) ORFs. These included ~192 TFs specifically of interest in this project based on their predicted functions in phytochrome signaling networks. We cloned and integrated into yeast strains a collection of 768 promoters for use in yeast one-hybrid assays with Brachypodium TFs. We developed and deployed a Thermo Automation robotics system (**Fig. 1**) for automating high-throughput yeast one-hybrid screening. This platform has liquid handling, incubating, and plate reading capabilities and is being used to automate TF:promoter interaction screening for selected candidate Brachypodium TFs. This system was funded by private donations to the Center.

B. Developed and deployed a high-throughput plant phenotyping platform for imaging Brachypodium plants misexpressing Brachypodium transcription factors implicated in phytochrome signaling networks.

We developed and deployed a LemnaTec-based high-throughput plant phenotyping facility at Danforth (**Fig. 2**). The facility houses a Conviron walk-in growth chamber that contains a LemnaTec high throughput phenotyping system. The system has the capacity to image 1140 Brachypodium plants daily with visible, NIR and fluorescent imaging stations. The Conviron chamber enables precise control of environmental variables and weighing and watering stations allow for precise control and quantification of water availability and usage. This system was funded by private donations to the Center.

C. Developed and deployed a Brachypodium transformation pipeline and improved artificial microRNA (amiRNA) constructs optimized for grasses.

We developed and deployed a Brachypodium tissue culture and transformation pipeline at the Danforth Plant Science Center. To facilitate our study of Brachypodium transcription factors implicated in phytochrome signaling networks, in collaboration with Dr. James Carrington's group (Danforth Center) we developed new constructs and methods for amiRNA knockdown of target genes in Brachypodium (Carbonell et al., The Plant Journal. Volume 82, Issue 6, June 2015, Pages 1061–1075). These constructs were used in the aforementioned Brachypodium transformation pipeline to knock down target transcription factors.

D. Modeling of Brachypodium gene regulatory networks and implications for elucidation of subnetworks that mediate phytochrome signaling based modulation of plant growth and biomass productivity.

In Brachypodium we used whole-genome microarray based gene expression profiling datasets generated in a previous project (Title: A Universal Genome Array and Transcriptome Atlas for Brachypodium Distachyon; Project ID: 0014513; PI: Todd C. Mockler; Award Register#: ER64630) to generate a gene network model through co-expression relationships among Brachypodium genes (**Fig. 3**). This network model was intended to guide the prioritization of candidate genes that when manipulated would be likely to impact biomass yield under drought and high-density growth conditions. Upon further analysis we identified a network module strongly over-represented with genes implicated in or associated with photosynthesis (corrected p-value 3.50e-36). Closer examination of this module and identification of TFs having connections to the photosynthesis genes in this module revealed a subnetwork of putative photosynthesis regulating Brachypodium TFs and their targets (**Fig. 4**) that included a large number of the TFs we had originally prioritized for *in planta* perturbation in this project, including TFs likely to function in phytochrome signaling such as the Brachypodium homologs of Arabidopsis PIF1/PIL5, HY5/TED5, PAP3/PIF3/POC1, PIF5/PIL6, PIF4/SRL2, PIF6/PIL2, PIL1, and PIF7. To put this bioinformatics observation in context, the genesis of this project was our observation that biomass accumulation was greater in Brachypodium, Switchgrass, and rice plants grown in monochromatic red light (i.e. a very high red:far-red ratio) than in blue or white light, despite the levels of photosynthetically active radiation (PAR) being the same in all conditions. Taken together, our bioinformatics analysis and physiological studies suggested a hypothesis that the enhanced biomass productivity observed in monochromatic red light is a consequence of phytochrome signaling that modulates photosynthesis. To test this hypothesis, focused on interrogating the promoters of the identified subnetwork of Brachypodium photosynthesis genes with the identified putative photosynthesis regulating TFs. We also prioritized these TFs for in planta overexpression and knockdowns.

E. Transgenic misexpression of putative phytochrome signaling associated candidate genes.

We conducted a total of 294 Brachypodium transformations with 264 constructs. The resulting transgenic plants were verified to contain the expected constructs and then they were phenotyped for putative phytochrome signaling associated phenotypes and obvious defects in growth and development. Most lines phenotyped did not exhibit obvious growth phenotypes, but a few did. Three examples are shown in **Fig. 5**.

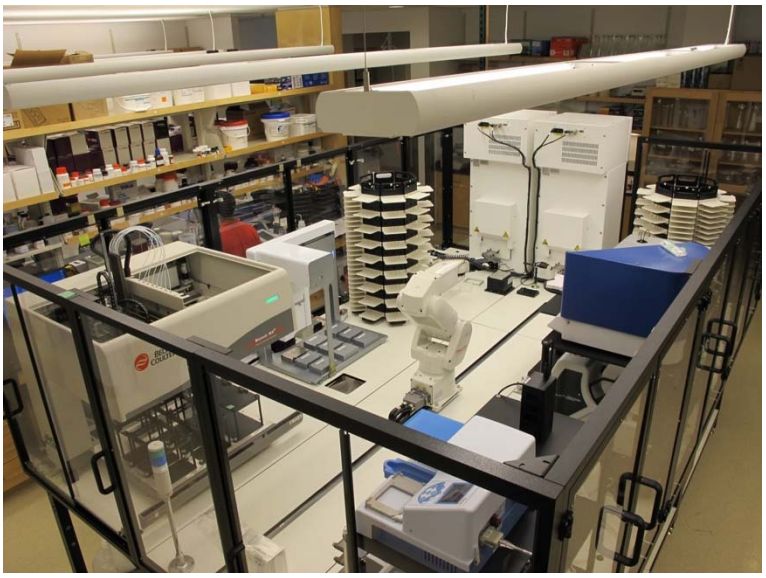

Figure 1. The Thermo Automation robotic yeast one-hybrid platform.

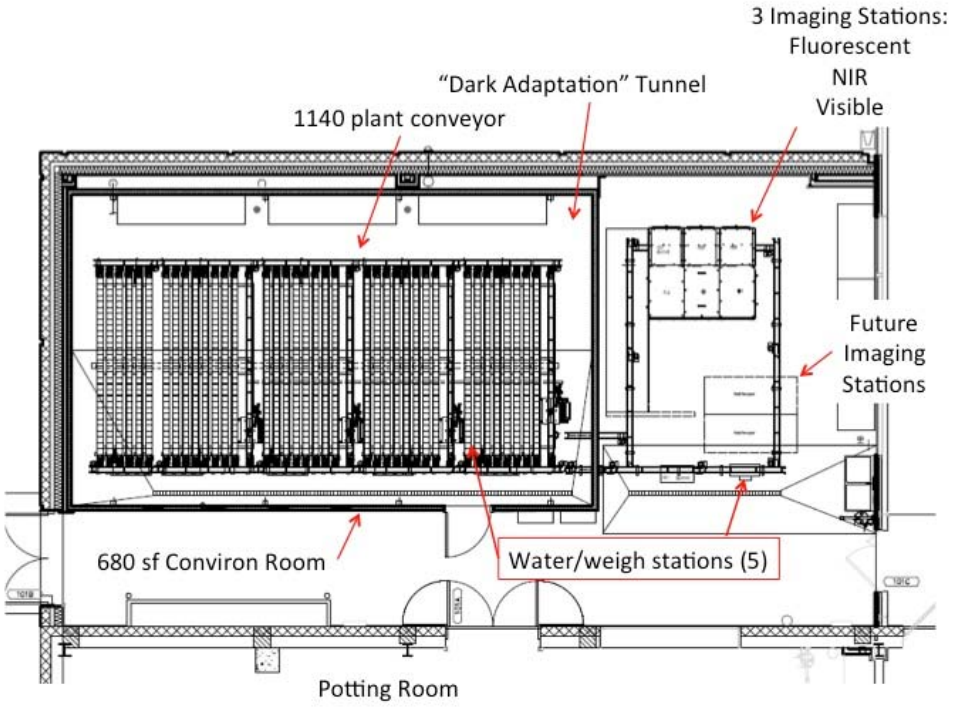

Figure 2. The LemnaTec/Conviron controlled environment high-throughput phenotyping facility at the Danforth Plant Science Center.

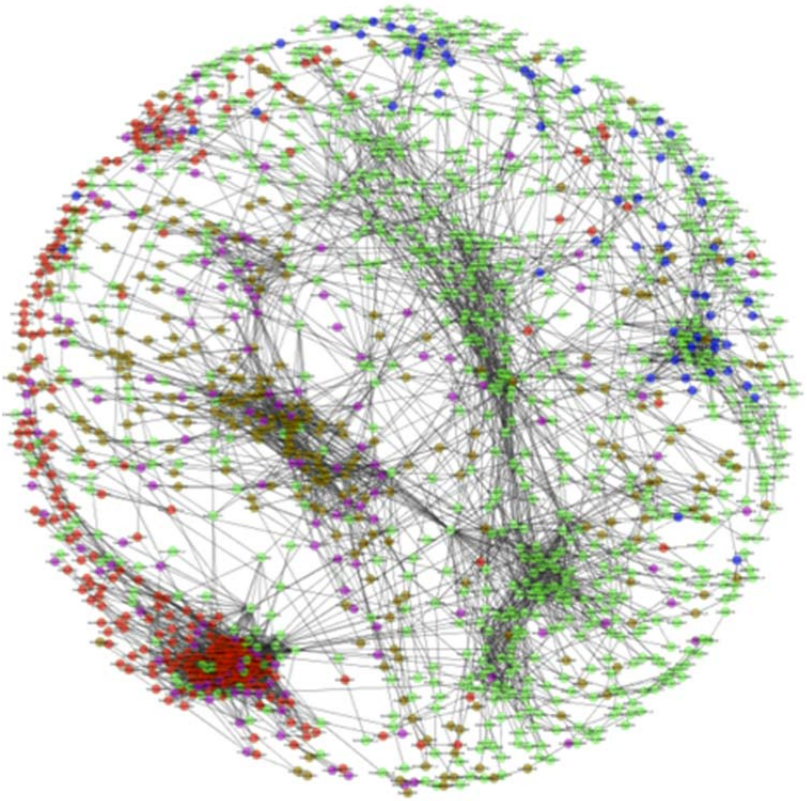

Figure 3. Network of co-expression relationships among Brachypodium genes.

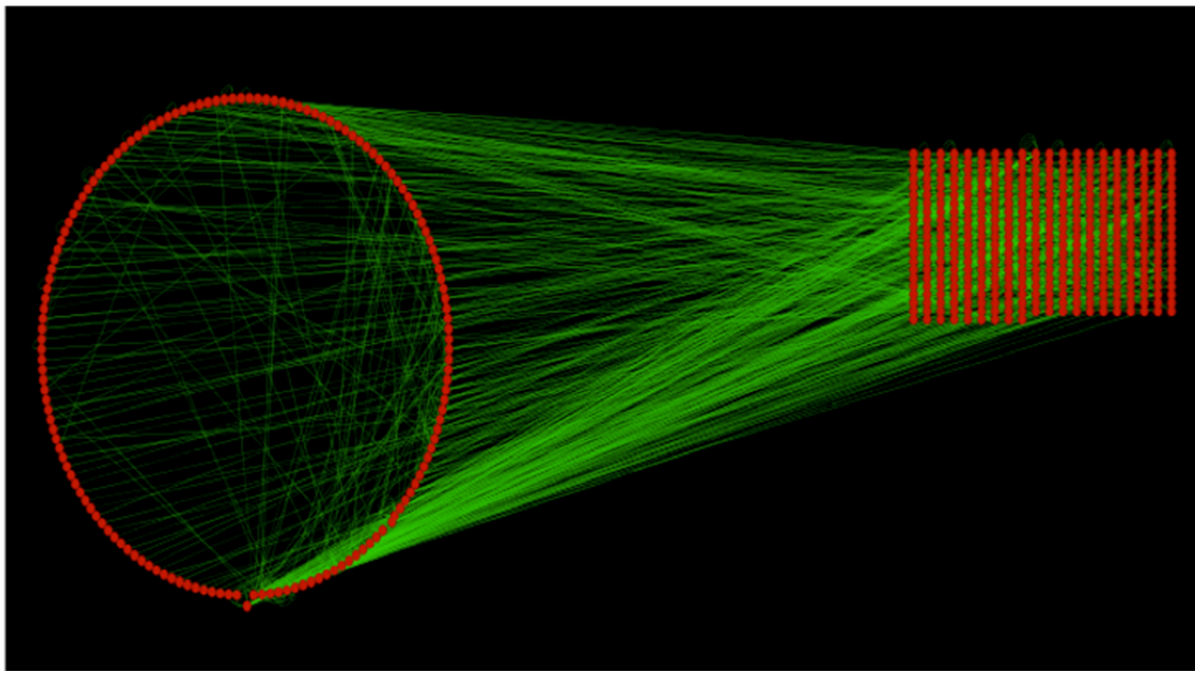

Figure 4. Subnetwork of Brachypodium photosynthesis genes (grid on the right) and putative photosynthesis regulating TFs (circle on left).

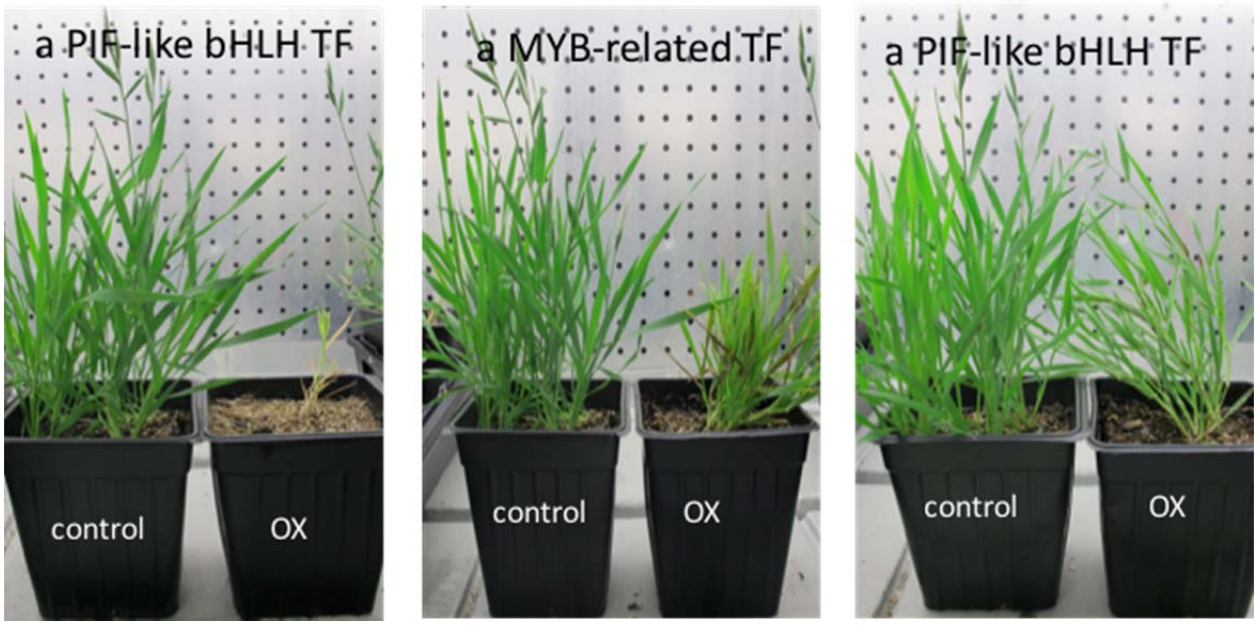

Figure 5. Examples of interesting transgenic Brachypodium overexpression (OX) phenotypes.

2. Papers and other products delivered:

Carbonell A, Fahlgren N, Mitchell S, Cox KL Jr, Reilly KC, Mockler TC, Carrington JC. Highly specific gene silencing in a monocot species by artificial microRNAs derived from chimeric miRNA precursors. Plant J. 2015 Jun;82(6):1061-75. doi: 10.1111/tpj.12835. PMID: 25809382

Gordon SP, Priest H, Des Marais DL, Schackwitz W, Figueroa M, Martin J, Bragg JN, Tyler L, Lee CR, Bryant D, Wang W, Messing J, Manzaneda AJ, Barry K, Garvin DF, Budak H, Tuna M, Mitchell-Olds T, Pfender WF, Juenger TE, Mockler TC, Vogel JP. Genome diversity in Brachypodium distachyon: deep sequencing of highly diverse inbred lines. Plant J. 2014 Aug;79(3):361-74. doi: 10.1111/tpj.12569. PMID: 24888695

Priest HD, Fox SE, Rowley ER, Murray JR, Michael TP, Mockler TC. Analysis of global gene expression in Brachypodium distachyon reveals extensive network plasticity in response to abiotic stress. PLoS One. 2014 Jan 29;9(1):e87499. doi: 10.1371/journal.pone.0087499. PMID: 24489928

Jeong DH, Schmidt SA, Rymarquis LA, Park S, Ganssmann M, German MA, Accerbi M, Zhai J, Fahlgren N, Fox SE, Garvin DF, Mockler TC, Carrington JC, Meyers BC, Green PJ. Parallel analysis of RNA ends enhances global investigation of microRNAs and target RNAs of Brachypodium distachyon. Genome Biol. 2013 Dec 24;14(12):R145. doi: 10.1186/gb-2013-14-12-r145. PMID: 24367943

Trabucco GM, Matos DA, Lee SJ, Saathoff AJ, Priest HD, Mockler TC, Sarath G, Hazen SP. Functional characterization of cinnamyl alcohol dehydrogenase and caffeic acid O-methyltransferase in Brachypodium distachyon. BMC Biotechnol. 2013 Jul 31;13:61. doi: 10.1186/1472-6750-13-61. PMID: 23902793

Accomplishments and Products Delivered:

Brachypodium TF collection: We cloned and validated a total of 483 Brachypodium transcription factor (TF) ORFs.

Synthetic promoter collection: We designed, synthesized, verified, and integrated into yeast strains a collection of 768 synthetic promoters for use in yeast one hybrid assays with Brachypodium transcription factors.

Yeast one-hybrid (Y1H) platform: We developed and deployed a custom integrated Thermo Automation robotics system for automating high-throughput yeast one-hybrid screening. This platform has liquid handling, incubating, and plate reading capabilities and is being used to automate TF:promoter interaction screening for selected candidate Brachypodium TFs.

Phenotyping platform: We developed and deployed a LemnaTec-based high-throughput plant phenotyping facility at Danforth Plant Science Center.

Brachypodium transformation pipeline: We developed and deployed a Brachypodium tissue culture and transformation pipeline at the Danforth Plant Science Center.

Improved artificial microRNA (amiRNA) constructs: In collaboration with Dr. James Carrington's group (Danforth Plant Science Center) we developed new constructs and methods for amiRNA knockdown of target genes in Brachypodium.

Invention disclosures

This project led to two invention disclosures that were submitted to the Danforth Plant Science Center and subsequently licensed by an early stage biotechnology company, Benson Hill Biosystems, Inc.

1. Disclosure Title: Temporal, spatial, and conditional promoters and promotor motifs for regulation of plant gene expression. This invention disclosure involved promoters from groups of co-expressed and co-regulated genes in the model grass Brachypodium distachyon and the associated regulatory DNA motifs occurring in those promoters. The gene expression datasets included the light treatment datasets (red, far-red light) underlying this project. Gene expression data were clustered and regulatory DNA analysis was conducted. This analysis identified promoters and regulatory DNA motifs and modules capable of driving specific gene expression patterns in plants. For example, we identified Brachypodium promoters and regulatory motifs capable of causing adjacent genes to be expressed under specific (and

multiple) light conditions. The purposes of this invention included 1) enabling in vivo control of gene expression in plants including expression of genes using natural plant promoters; 2) enabling discovery of DNA binding transcription factor proteins that regulate gene expression in plants; 3) enabling modification of gene expression patterns in plants, including generation of novel expression patterns via synthetic promoter sequences; and 4) enabling deduction of plant transcriptional regulatory networks. The characteristics of the invention included 1) Brachypodium regulatory DNA sequences (promoters) that confer specific expression characteristics to adjacent genes; 2) individual Brachypodium regulatory DNA motifs (cis-regulatory elements) and modules that confer specific expression characteristics to adjacent genes; and 3) synthetic promoter sequences and regulatory modules that confer specific expression characteristics to adjacent genes.

2. Disclosure Title: Conditionally, environmentally, and stress-associated TFs. This invention disclosure involved transcription factor proteins co-expressed with groups of co-expressed and co-regulated genes in the model grass Brachypodium distachyon. The gene expression datasets included the light treatment datasets (red, far-red light) underlying this project. Gene expression data were clustered and co-expressed transcription factors were identified. This analysis identified transcription factors that drive specific gene expression patterns in plants. For example, we identified Brachypodium transcription factors that regulate expression of target genes in response to specific (and multiple) light conditions.

3. Notes concerning the project:

Changes in Approach:

During the course of the project we implemented one substantial change to the approach, which was clearly far superior to our original proposed approach. In the original project plan we proposed to use what was essentially a makeshift digital phenotyping approach that involved use of consumer-grade digital cameras coupled with open-source software for image analysis. Instead of that approach we used the far superior LemnaTec phenotyping platform available at Danforth Center.