Systems Biology to Advance Sustainable Bioenergy Crop Development

Summary of projects awarded in 2020 under Funding Opportunity Announcement DE-FOA-0002214

Camelina

Populus

Brachypodium distachyon

The Genomic Science program (GSP) of the Office of Biological and Environmental Research (BER), within the U.S. Department of Energy (DOE) Office of Science, supports fundamental research to identify basic principles that drive biological systems. These principles govern translation of the genetic code into integrated networks of proteins, enzymes, regulatory elements, and metabolite pools underlying the functional processes of organisms. To address DOE's mission in bioenergy development, GSP aims to leverage omics-driven tools and systems biology approaches to meet key challenges facing the sustainable production of bioenergy crops as a viable alternative resource for fuels, chemicals, and products originating from fossil fuel resources.

Two areas vital to the nation's energy and environmental security motivate the BER research agenda: (1) developing cost-effective biofuels and bioproducts and (2) improving the ability to understand, predict, and mitigate the impacts of energy production amid a variable and changing climate. Toward this end, BER has invested in plant and plant-microbe interactions research with the goal of advancing biofuel and bioproduct production from domestic lignocellulosic biomass and oilseed crops. These efforts have increased understanding of the biological mechanisms underlying feedstock productivity and facilitated development of next-generation bioenergy crops that employ novel, efficient bioenergy strategies that can be replicated on a mass scale. However, several knowledge gaps and challenges remain in developing vigorous crops with superior growth and yield under varying environmental conditions. BER seeks a better understanding of the underlying genetic and physiological mechanisms influencing plant productivity, resource-use efficiency, and adaptation and resilience to abiotic stress. Also of interest is how beneficial plant-microbe associations may enhance requisite plant processes to enable manipulation of these associations for improving plant traits.

In fiscal year 2020, BER solicited applications to conduct systems biology–driven basic research on the fundamental principles of sustainable bioenergy feedstocks in relationship to the associated ecosystem. The funding announcement focused on environmental sustainability in the development of bioenergy feedstocks. Understanding the multiorganismal "biofuel crop + soil + microbe ecosystem" presents a unique challenge, which is further complicated by climate variability and change. The ability to predict the responses of plant and microbial species to a changing environment is critical for understanding potential environmental impacts and for optimizing feedstock production.

Species of interest included candidate bioenergy plants (e.g., sorghum, energy cane, *Miscanthus,* switchgrass, and *Populus*) and oilseed crops (e.g., *Camelina).* BER sought hypothesis-driven projects focused on understanding feedstock productivity and the effects of nutrient availability and abiotic stresses. Seven projects were awarded fiscal year 2020 funding in two specific research areas:

- **Systems-level research to improve understanding of the molecular mechanisms underlying bioenergy feedstock productivity under changing and at times suboptimal**

environmental conditions. Studies should provide better understanding of the molecular and physiological mechanisms that control bioenergy crop vigor, resource-use efficiency, and resilience or adaptability to abiotic stress, as well as interactions with the surrounding environment.

- **Systems biology–enabled investigations into the role(s) of microbes and microbial communities including rhizosphere consortia (e.g., bacteria, fungi, diazotrophs, endophytes, and viruses) in supporting plant productivity and vigor.** Studies should contribute to a better understanding of bioenergy feedstock plant performance, adaptation, and resilience in changing environmental conditions and abiotic stressors.

2020 Awards

A Systems Understanding of Nitrogen Fixation on the Aerial Roots of Sorghum

- **Principal Investigator:** Jean-Michel Ané (University of Wisconsin, Madison)
- **Co-Investigators:** Sushmita Roy and Ophelia Venturelli (University of Wisconsin, Madison); Wilfred Vermerris (University of Florida)

The goal of this project is to better understand the molecular and cellular networks controlling nitrogen-fixation traits in sorghum, using aerial roots as a system. The project plans to identify sorghum accessions that maximize nitrogen fixation via their aerial roots and use genome-wide association studies (GWAS) to map biological nitrogen fixation–related traits, characterize and develop bacterial communities involved in these nitrogen-fixation traits, generate models to predict the genetic networks involved in the plant-microbe interactions, and then validate these models through plant and microbial genetic studies. Knowledge and findings generated through this project will enable higher productivity of bioenergy sorghum on marginal lands, with a reduction in both inputs and environmental impacts.

Systems Analysis of the Beneficial Associations of Sorghum with Arbuscular Mycorrhizal Fungi Studied with Genetics, Genomics, Imaging, and Microbiomics

- **Principal Investigator:** Jeffrey Bennetzen (University of Georgia)
- **Co-Investigators:** Jonathan Arnold, Anny Chung, and Katrien M. Devos (University of Georgia); Nancy Johnson (University of Northern Arizona)

The goal of this project is to identify plant genes that attract specific arbuscular mycorrhizal fungi (AMF) interactions to determine the possible benefits or costs of these interactions to the biofuel crop sorghum under various field conditions. The project plans to accomplish this by (1) mapping sorghum genes that determine which families, genera, and species of AMF associate with sorghum roots under which field conditions (i.e., variations of water and nutrient conditions) and (2) determining what contributions these sorghum genes (and the AMF they attract) provide to various sorghum phenotypes, including biomass yield. Results from this project could generate a systems analysis of sorghum-AMF interactions and assist plant breeders and agronomists in using indigenous AMF to improve sorghum productivity.

Integration of Experimental and Modeling Approaches to Understand, Predict, and Modulate Rhizosphere Processes for Improved Bioenergy Crop Productivity

- **Principal Investigator:** Karsten Zengler (University of California, San Diego)
- **Co-Investigators:** Trent Northen and John Vogel (Lawrence Berkeley National Laboratory); Amélie Gaudin (University of California, Davis)

This project will couple lab and field studies to develop a predictive model of grass microbiomes based on new mechanistic insights into dynamic plant-microbe interactions in the grasses *Sorghum bicolor* and *Brachypodium distachyon.* The project plans to study root exudates from these plants through diurnal (light-dark) cycles and how they recruit and maintain beneficial microbes to improve nitrogen availability to *Sorghum* and *Brachypodium*. Studies will include labeling isotopes to trace nitrogen flow between plants and microbes; developing models to predict plant growth and exudate

Sorghum bicolor

metabolism to maximize nitrogen flux to the plant; and using these models to design nutrient and microbial amendments tailored to increase plant biomass production under nitrogen-limited conditions through optimized plant-microbe interactions.

ECON: Enhancing *Camelina* Oilseed Production with Minimum Nitrogen Fertilization in Sustainable Cropping Systems

- **Principal Investigator:** Chaofu Lu (Montana State University)
- **Co-Investigators:** Chengci Chen, Jed Eberly, Andreas Fischer, Jennifer Lachowiec, and Qing Yan (Montana State University); Luca Comai (University of California, Davis); Timothy Paulitz and William Schillinger (Washington State University); John Shanklin and Jörg Schwender (Brookhaven National Laboratory); Susannah Tringe and Trent Northen (Lawrence Berkeley National Laboratory and DOE Joint Genome Institute)

This project aims to address two challenges in *Camelina* biology that hinder its potential as a nonfood oilseed crop for biofuel production in the northwestern United States: enhancing nitrogen-use efficiency and boosting oil yield. Modern genomics and biochemical approaches will be applied to decipher the genetic and physiological mechanisms that determine these traits, providing a systems-level understanding of nitrogen uptake, assimilation, remobilization, seed development, and oil accumulation. This knowledge will facilitate development of next-generation high–oil yielding *Camelina* varieties with minimum nitrogen fertilization for sustainable bioenergy production.

Elucidation of the Roles of Diazotrophic Endophyte Communities in Promoting Productivity and Resilience of *Populus* Through Systems Biology Approaches

- **Principal Investigator:** Sharon Doty (University of Washington)
- **Co-Investigators:** Soo-Hyung Kim (University of Washington); Amir Ahkami and Christer Jansson (Pacific Northwest National Laboratory); Adam Deutschbauer (Lawrence Berkeley National Laboratory)

The goal of this work is to understand how, at a molecular level, microorganisms within the poplar tree microbiome can affect the host plant's health and stress tolerance. Using systems biology approaches at both lab and field scales, the project plans to identify the metabolic and physiological impacts of bioinoculants on the host plant under nutrient stress and water limitation. The project will then integrate plant physiology data and molecular plant-microbe interactions data to develop a systems-level understanding of the genetic and molecular basis for diazotrophic (nitrogen-fixing) endophytic mutualisms. This deeper level of understanding of plant responses will guide construction of microbial communities that best prime plant pathways for enduring abiotic stresses to optimize the impacts of bioinoculants for environmental sustainability and vigor of bioenergy crops.

Testing Predictions of Plant-Microbe-Environment Interactions to Optimize Climate Adaptation and Improve Sustainability in Switchgrass Feedstocks

- **Principal Investigator:** Tom Juenger (University of Texas, Austin)
- **Co-Investigators:** Ulrich Mueller (University of Texas); Jeremy Schmutz and Kankshita Swaminathan (HudsonAlpha Institute for Biotechnology); Felix Fritschi (University of Missouri); Julie Jastrow and Roser Matamala (Argonne National Laboratory); Laura Bartley (Washington State University); David Lowry and Carolyn Malmstrom (Michigan State University); Denise E. Costich (International Maize and Wheat Improvement Center, or CIMMYT, Mexico); Francis Rouquette (Texas A&M University); Philip Fay (U.S. Department of Agriculture, Agricultural Research Service, Temple, Tex.); Alina Zare (University of Florida); Arvid Boe (South Dakota State University)

This work will leverage a field-established genetic resource network (S-GENE) to deepen understanding of local adaptation and to identify beneficial traits, genes, and microbial associates that contribute to switchgrass productivity. Switchgrass performance will be assessed across a continental-scale latitudinal gradient, allowing identification of key genes and providing a better understanding of the genetic architecture of adaptation and the plant-microbe interactions that influence adaptation and sustainability traits. Ultimately, these studies will facilitate manipulation of critical plant-microbe-soil traits through breeding and agronomic management to improve the sustainability of biofuel feedstocks.

Switchgrass

Interrogating Pennycress Natural and Induced Variation to Improve Abiotic Stress Tolerance and Oilseed Bioenergy Crop Resilience

- **Principal Investigator:** John Sedbrook (Illinois State University)
- **Co-Investigators:** Nicholas Heller and Wondy Seyoum (Illinois State University); Seung Rhee and M. Exposito-Alonso (Carnegie Institute of Washington); Dmitri Nusinow and Christopher Topp (Donald Danforth Plant Science Center); M. David Marks, Ratan Chopra, and Katherine Frels (University of Minnesota); Karen Sanguinet and Tarah Sullivan (Washington State University); Andrea Gschwend and Alexander Lindsey (Ohio State University); Winthrop Phippen (Western Illinois University); Dan Jacobson, Mike Garvin, David Kainer, and Jared Streich (Oak Ridge National Laboratory); Pubudu Handakumbura (Pacific Northwest National Laboratory)


Pennycress

The goal of this project is to identify genetic variants that have enabled field pennycress, an oilseed bioenergy crop that can be used in a crop rotation cycle as well as a cover crop for soil maintenance, to locally adapt to and colonize all temperate regions of the world. Eco-evolutionary computational genomics combined with high-throughput growth analyses, in-field analytical methods, and CRISPR gene-editing strategies will be employed to identify key genes and develop pennycress varieties with superior stress tolerance. This project will deliver speed-breeding methods to facilitate the introduction of superior genetic changes into a wide range of commercial pennycress varieties precisely adapted to the desired local environment.

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DOE Office of Science

- energy.gov/science

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