Project Descriptions for RISE 2024

1. **Project:** Spatial modeling of stomatal patterning in diverse Sorghum varieties in the early and late canopy
   Mentor: John Hodge
   Faculty Member: Andrew Leakey
   Location: University of Illinois Urbana-Champaign
   Prerequisites: Basic algebra preferred but not essential; relevant biology will be taught over the course of the project

   Project Description: This project will initially focus on collecting images of stomata (small mouth-like pores plants use to regulate their water loss) from diverse Sorghum varieties in both lower and upper canopy leaves. Students will then utilize image analysis and spatial statistics workflows developed by the mentor for scoring how these pores are patterned across the leaves of genetically diverse Sorghum varieties.

   Skills Overview: Students will gain experience imaging live plant materials using microscopy, annotating them using the Biodock machine learning pipeline, and using spatial analysis methods to score stomatal patterning as a measurable trait.

2. **Project:** Oleaginous yeast (*Y. lipolytica*) engineering for improved tolerance to growth inhibitors and production of oleochemicals
   Mentor: Susan Hubbard
   Faculty Member: Brian Pfleger
   Location: University of Wisconsin-Madison
   Prerequisites: None

   Project Description: One of CABBI's goals is the conversion of plant biomass into higher value chemical products. Oleochemicals, lipids that are derived from biological rather than petrochemical means, are of interest as precursors to jet fuel (among other things) and oleaginous yeasts such as *Yarrowia lipolytica* make great hosts for production of these chemicals. Use of food-grade carbon sources (i.e. sugar) is not an effective use of resources, which is where the utilization of plant biomass comes in. Plant biomass is an abundant source of carbon but must undergo hydrolysis for the carbon to be consumable by *Y. lipolytica*. Because the process of hydrolysate production also results in the formation of various compounds which inhibit yeast growth, we are engineering strategies to overcome this toxicity effect. This project will focus on engineering *Y. lipolytica* for growth and/or lipid production on biomass-derived lignocellulosic hydrolysate.

   Skills Overview: The student will learn traditional metabolic engineering techniques including strategies to direct the flux of a substrate to the desired product while gaining experience working with *Y. lipolytica* (media prep, cell culturing, transformations) and making genetic modifications (building plasmids, minipreps, PCR, running gels).

3. **Project:** Role of metal ions in metabolism of CABBI yeasts and its effect on the conversion of feedstocks to bioproducts
   Mentor: Sujit Jagtap
   Faculty Member: Chris Rao
   Location: University of Illinois Urbana-Champaign
   Prerequisites: Basic biology

   Project Description: Metal concentrations can either stimulate or suppress growth of CABBI yeasts (*Rhodosporidium toruloides*, *Yarrowia lipolytica*, and *Issatchenkia orientalis*) and titers of CABBI bioproducts (fatty alcohol, triacetic acid lactone, citramalate, and 3-hydroxypropionic acid). We have found that metal concentrations in sorghum hydrolysate and sugarcane juice are either excessive or limiting when used as a growth media for yeast. This project aims to investigate the role of metals in CABBI yeast metabolism and its effects on cellular processes such as respiration, DNA synthesis, ribosome biogenesis, and stress response.

   Skills Overview: The student will have the opportunity to learn and contribute with experiments, data collection, data analysis, and maintaining accurate laboratory records including the following:

   Laboratory Techniques and Instrumentation may include: Electrophoresis (protein, plasmid, DNA, and RNA), qPCR, DNA assembly (Golden Gate, Gibson assembly etc.), genome editing (e.g., CRISPR-Cas9), analytical methods (HPLC, GCMS, LC-MS); high-throughput microbioreactors.

   Data Analysis: The student will gain experience interpreting genomics, transcriptomics, and metabolomics datasets related to substrate use, gene expression, and metabolic pathways.

   Literature Review: The student will be encouraged to read relevant scientific literature which will improve capacity to critically evaluate scientific articles and synthesize knowledge.

   Communication and collaboration: The student will collaborate and participate in regular meetings with other members of the research team, which includes graduate students and postdocs.

   Scientific Writing: There is the possibility of contributing to the authorship of a review or research paper with your mentor.

4. **Project:** Role of Rubisco activase in thermotolerance in sorghum
   Mentor: Nikita Bhatnagar
   Faculty Member: Don Ort

Location: University of Illinois Urbana-Champaign
Prerequisites: Basic knowledge of plant molecular biology and plant physiology helpful but not required
Project Description: This project will focus on analyzing sorghum rubisco activase α isoform (SbRca- α) CRISPR knockout plants under heat stress in the field. The study will comprise of molecular biology assays at the protein and RNA level and plant physiology assays using a portable photosynthesis measurement system thereby establishing the role of SbRca-α in thermotolerance in C4 crop species.

Skills Overview: The student will gain experience in molecular biology and plant physiology, primarily: protein extraction from plants and bacterial cells, transcript analysis using RNA isolation and qPCR assay, genomic DNA confirmation, CABP assay, whole plant physiology analysis using Li-Cor 6800, heat stress analysis in the field using the T-FACE facility.

5. **Project:** Understanding tissue-specific gene expression patterns in response to environmental cues in sorghum engineered for greater water use efficiency
Mentor: Sanbon Gosa
Faculty Member: Andrew Leakey
Location: University of Illinois Urbana-Champaign
Prerequisites: None

Project Description: The student will engage in a project aiming to understand tissue-specific gene expression patterns of stomatal developmental genes in response to environmental cues in sorghum engineered for greater water use efficiency which is among the main research foci of the Leakey lab.

Skills Overview: The student will use optical tomography to image and determine the number of stomata per unit area using machine learning, characterize gene expression, extract RNA, use qPCR machines, measure photosynthesis and stomatal conductance using a Li-Cor machine, and analyze data.

6. **Project:** Genome-wide characterization of the MYB gene family in Miscanthus
Mentor: Dessiree Zerpa Catanho
Faculty Member: Erik Sacks
Location: University of Illinois Urbana-Champaign
Prerequisites: Experience with laboratory bench work (e.g. pipetting or PCR), sequence alignment, use of databases like NCBI, and basic programming in R would be helpful background but are not required.

Project Description: MYB family genes are transcription factors involved in different biological processes in plants. They are important because they could be related to abiotic stress tolerance in Miscanthus since they have been reported to respond to abiotic stresses in other plant species. The research project would involve mostly bioinformatics analysis using available genomes and databases, with a small experimental pilot study at the end in which we will collect leaf or rhizome tissue to measure the expression of MYB genes with either transcriptome analysis or qPCR.

Skills Overview: The student can expect to learn bioinformatics skills for the classification and characterization of genes. Depending on the availability of plant material for qPCR or transcriptome data, the student will learn the skills required to study gene expression in plants.

7. **Project:** Understanding differences in root demographics of bioenergy crops
Mentor: Janith Chandrasoma
Faculty Member: Evan DeLucia
Location: University of Illinois Urbana-Champaign
Prerequisites: None

Project Description: The project will examine the fate of 13C-labeled plant litter in soil to assess litter turnover and mean residence time and its contribution to soil organic carbon sequestration. This project aligns with broader efforts to quantify the belowground total carbon allocation of bioenergy cropping systems.

Skills Overview: The student will gain experience with analytical laboratory methods related to 13C, experimental design, research planning, and scientific writing.

8. **Project:** Expanding the applications of biocatalysts in chemical synthesis
Mentor: Wesley Harrison
Faculty Member: Huimin Zhao
Location: University of Illinois Urbana-Champaign
Prerequisites: None

Project Description: This project aims to screen and develop biocatalysts for chemical reactions. The project will focus on screening and developing enzymes for amine synthesis.

Skills Overview: The student will gain experience in both molecular biology and in organic synthesis. The student will also learn how to conduct scientific research by actively managing a project, formulating research hypotheses, and testing their ideas with experiments.

9. **Project:** Investigating the role of plant-microbe-mineral interactions on soil carbon dynamics in response to drought in oil-enhanced sorghum bioenergy systems
Mentors: Yuan Liu and Katerina Estera-Molina
Faculty Member: Jennifer Pett-Ridge
Location: Lawrence Livermore National Laboratory

Prerequisites: Courses in general biology or chemistry helpful but not required

Project Description: Extracellular polymeric substance (EPS) production is an important microbial trait that contributes to soil aggregation and mineral-associated organic matter (MAOM) formation, which are fundamental to soil carbon stabilization. How oil-enhanced sorghum and drought impact microbial EPS production and its effect on MAOM formation remains unknown. This project aims to use a 13C labeling greenhouse experiment, combined with EPS extraction, density fractionation, and metagenomic sequencing, to understand the effect of oil-enhanced sorghum and drought on soil carbon stabilization.

Skills Overview: The student will gain experience processing soil samples from a stable isotope labeling greenhouse experiment. Skills developed will include but not be limited to molecular techniques such as DNA/RNA extraction and quantification; and biogeochemical analyses such EPS extractions, density fractionation, IRMS preparation, soil pH etc.

10. **Project:** Harmonizing techno-economic analysis assumptions for early stage biorefineries
Mentor: Lavanya Kudli
Faculty Member: Jeremy Guest
Location: University of Illinois Urbana-Champaign
Prerequisites: Experience with process design and basic knowledge of techno-economic analysis (TEA) helpful but not required

Project Description: BioSTEAM is a python-based open-source platform for heuristically modeling biorefineries and conducting techno-economic analysis (TEA) and life cycle assessment (LCA). Additionally, it can be used to conduct uncertainty and sensitivity analysis which is especially useful for early-stage processes. For this project, the student will use BioSTEAM to compare past TEA methodologies and provide a framework for selecting TEA assumptions for early stage biorefineries.

Skills Overview: The student will gain experience in conducting TEA using BioSTEAM (a python-based tool).

11. **Project:** Evaluation of nitrogen use efficiency using engineered sorghum genes under nitrogen deficiency
Mentor: Lalit Dev Tiwari
Faculty Member: Li-Qing Chen
Location: University of Illinois Urbana-Champaign
Prerequisites: None

Project Description: This project will focus on analyzing sorghum nitrogen metabolism-associated or transport genes to understand how root and shoot communicate about nutrient availability at the whole-plant level and adjust nutrient acquisition to plant growth requirements and to understand the influence of these genes on overall plant growth.

Skills Overview: The student will learn some molecular and phenotyping skills, such as polymerase chain reaction (PCR), gel electrophoresis, DNA isolation, stress treatment, and cloning (if required).

12. **Project:** Understanding soil moisture dynamics with depth and its impact on N_2O fluxes in bioenergy sorghum
Mentor: Toby Adjuik
Faculty Member: Marshall McDaniel and Andrew Leakey
Location: Iowa State University
Prerequisites: None

Project Description: This project will investigate the relationship between soil moisture with depth and nitrous oxide (N_2O) emissions in bioenergy sorghum. Using an advanced soil moisture probe, the student will measure soil moisture at various depths with accompanying measurement of N_2O fluxes using the LICOR N_2O trace gas analyzer. This data will enhance our understanding of how water availability at depth influences N_2O emissions and support our process-based N_2O modeling efforts.

Skills Overview: The student will gain experience deploying and operating advanced soil moisture sensing technology, collecting and analyzing N_2O fluxes and soil data, and understanding bioenergy crop greenhouse gas emissions dynamics. They will also gain skills for effective oral and written science communication.

13. **Project:** Dissecting how pH affects NAD+ metabolism in model and non-model yeast
Mentor: Alexandria Murphy
Faculty Member: Melannie McReynolds
Location: Penn State University
Prerequisites: Basic microbiology and pipetting skills helpful but not required

Project Description: This project will aim to elucidate the relationship between metabolism and environmental stressors (pH). Using isotope tracing and mass spectrometry, this relationship will be dissected in both *S. cerevisiae* (model yeast) and *Y. lipolytica* (non-model yeast).

Skills Overview: The student will gain experience with the RT-qPCR molecular biology method and will also learn how to perform metabolite extractions and analyze metabolomic data.

14. **Project:** Quantitating NAD+ flux through the life cycle of the yeast *R. toruloides*
Mentor: Alexandria Murphy

Faculty Member: Melannie McReynolds
Location: Penn State University
Prerequisites: Basic microbiology and pipetting skills helpful but not required

Project Description: This project will focus on how the NAD+ metabolome changes through the life cycle of the yeast *R. toruloides*. The student will use mass spectrometry to quantify NAD+ flux and visualize metabolome changes through the cell cycle.

Skills Overview: The student will gain experience with the RT-qPCR molecular biology method and learn how to perform stable isotope tracing and metabolite extractions and analyze metabolomic data.

15. **Project:** Development of a green chemistry approach to lignocellulosic biomass pretreatment for efficient lipid and sugar recovery
Mentor: Tirath Raj
Faculty Member: Vijay Singh
Location: University of Illinois Urbana-Champaign
Prerequisites: None

Project Description: The student will contribute to the development of an environmentally friendly, efficient process for the fractionation of lipids and fermentable sugars from biomass (e.g. oilcane, sugar cane, sweet sorghum) for biodiesel and ethanol production. The project will focus on preparation and evaluation of deep eutectic solvents derived from natural precursors (e.g., sugars, lactic acid, glycerol, choline chloride) for biomass pretreatment with the aim of providing a more environmentally friendly alternative to the organic solvents typically used for this step in biomass processing.

Skills Overview: The student will gain experience in laboratory scale biomass processing, compositional analysis, pretreatment, enzymatic hydrolysis, fermentation and yeast culture, reactor operation, media preparation, enzymatic assays, and product quantification via HPLC. Additionally, the student will have the opportunity to develop strong project management, time management, writing, and communication skills.

16. **Project:** Extraction of lipids from oleaginous yeasts
Mentor: Shivali Banerjee
Faculty Member: Vijay Singh
Location: University of Illinois Urbana-Champaign
Prerequisites: None

Project Description: Oleaginous yeasts are an emerging lipid source for producing a range of commercially valuable oleochemicals ranging from pharmaceuticals to lipid-derived biofuels such as biodiesel and sustainable aviation fuel. Lipid extraction from yeast biomass is a crucial step in the production pipelines of these products. Hence, the economically viable and environmentally friendly recovery of these lipids is essential. The student will support these efforts with a project exploring the use of enzymatic cell lysis for lipid recovery from oleaginous yeasts.

Skills Overview: The student will develop skills with wet chemistry techniques such as extractions, sample preparation for chemical analysis, data analysis, and presentation of experimental data.

17. **Project:** Optimizing fertilizer use in sorghum to reduce N2O emissions
Mentor: Andie Suratt
Faculty Member: Wendy Yang
Location: University of Illinois Urbana-Champaign
Prerequisites: Introductory chemistry and introductory biology/environmental science helpful but not required

Project Description: This project aims to test the effects of fertilizer quantity and application timing on sorghum yield and greenhouse gas emissions. This project will also explore mechanisms of microbial nitrogen cycling, particularly nitrogen mineralization rates early in the growing season.

Skills Overview: The student will gain experience collecting gas and soil samples in the field, analyzing those samples in the lab, and interpreting the data. Students will learn about soil biology, environmental chemistry, and data analysis as well as the general process of conducting a major research experiment from design to publication.

18. **Project:** Identification and utilization of stress-tolerance genes to improve machine learning models for predicting stress tolerance in *Issatchenkia orientalis*
Mentor: Ping-Hung Hsieh
Faculty Member: Yasuo Yoshikuni
Location: Lawrence Berkeley Lab
Prerequisites: Coursework in microbiology, molecular biology, and relevant laboratory experience are recommended. Coding experience in any programming language is highly beneficial.

Project Description: This project aims to validate genes previously predicted to be linked to stress tolerance in *Issatchenkia orientalis* through gene overexpression experiments. The resulting data from the phenotyping of engineered strains will enhance the training dataset for a machine learning model, improving predictions of stress tolerance in this species.

Skills Overview: The student will acquire hands-on experience in fundamental microbiology and molecular biology techniques. Additionally, they will gain an understanding of the principles of constructing a basic machine learning model, particularly in the context of biological data.

19. **Project:** Analyzing ploidy of multiple generations of an engineered *I. orientalis* strain to establish its stability
Mentor: Thasneem Banu Frousnoon
Faculty Member: Yasuo Yoshikuni
Location: Lawrence Berkeley Lab
Prerequisites: Basic molecular biology helpful but not required

Project Description: *Issatchenkia orientalis* is a non-model yeast that can be useful for production of high-value products such as organic acids due to its tolerance of environmental stressors such as low pH. Current challenges in engineering *I. orientalis* could be lessened in a haploid version of the yeast, which does not currently exist. Therefore, as part of a larger aim to create a stable haploid strain of *I. orientalis* for future engineering efforts, this project aims to determine whether the ploidy of an engineered *I. orientalis* yeast strain is stable after growing for multiple generations.

Skills Overview: The student will learn to grow multiple generations of the yeast, extract DNA from yeast cultures, determine ploidy using flow cytometry, and analyze genomic sequences.