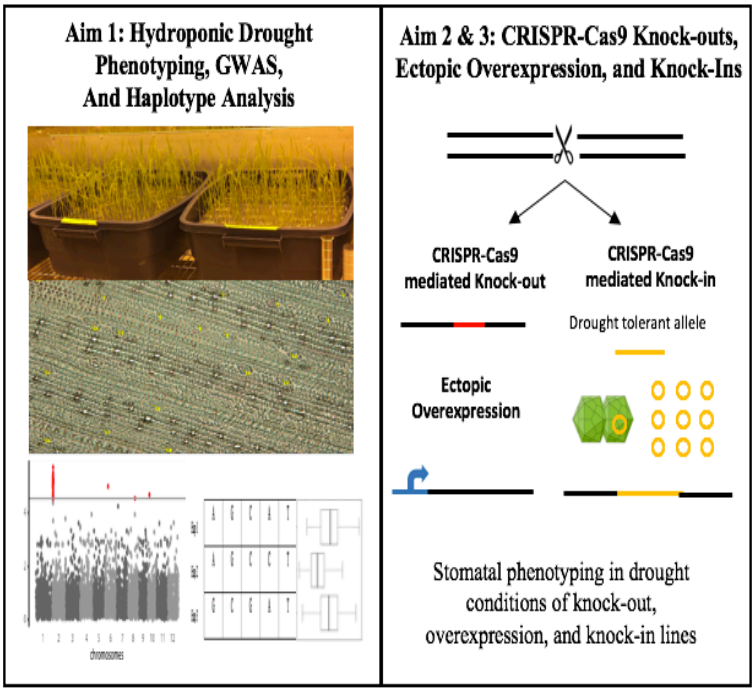
Characterizing the genetic underpinnings of stomatal development and physiology in grasses

Stomata are small pores located on the epidermis of aerial plant tissues necessary for $CO_2$ assimilation and $O_2$ release. Stomata are also the primary sites of transpiration, accounting for nearly 90% of all water loss in rice. Plant species can modulate their stomatal density and conductance on newly emerging leaves in response to environmental stimuli such as $CO_2$, water status, and temperature[1–3]. Despite many recent studies in dicots, limited attention has been paid to characterizing the genetic underpinnings of stomatal development and physiology in monocots, namely grasses. Grass stomata exhibit specialized anatomical and physiological attributes, such as subsidiary cells that flank the guard cells allowing for faster stomatal aperture rates[4]. Concerted efforts could provide insights into environmental adaptation mechanisms exclusive to monocots. The few studies that have characterized aspects of monocot stomatal biology have relied on mutant screens to identify key genes or have attempted to characterize homologs from the model plant organism *Arabidopsis thaliana*[4–6]. The use of quantitative genetic approaches as an alternative might reveal quantitative trait loci (QTLs) associated with this important trait. I will complete a genome-wide association study on a rice (*Oryza sativa)* diversity panel to characterize stomata-mediated drought response in rice. Currently available high density single nucleotide polymorphism (SNP) data allows for the resolution of discrete QTLs that are relevant in extant rice variation[7]. Further investigation of the most significant SNPs will enable the characterization of genetic variation involved in rice stomatal physiology and development in response to water deficit.

**Aim 1: Genome wide association study of stomatal traits in drought simulation**

A hydroponic platform incorporating polyethylene glycol 6000 (PEG)-induced drought stress will be used to yield a high-throughput and uniform assay of plant stomatal density and physiology in response to drought. The stomatal density differences will be measured in normal watering conditions and drought stress lines for each accession. A Li-6400 XT will be used to measure stomatal conductance of individual replicates. The optimized phenotyping platform will be applied to a rice diversity panel of 300 accessions. I will use principal component analysis to maximize coverage of total genetic diversity in the selected lines. Stomatal density and conductance differences between the two treatments will be used as the phenotypic parameters in the GWAS alongside a high-density rice array containing nearly 700k SNPs. Association mapping will be conducted using a custom python script that can account for subpopulation structure as a potential covariate. All loci above the significant p-value threshold will be further analyzed to identify likely candidate genes associated



with stomatal density and conductance modulations responsive to drought. I will then use haplotype analysis to determine the haplotypes and frequencies at the most significant QTL.

**Aim 2: Characterize candidate genes using CRISPR/Cas9 mediated knock-outs and ectopic overexpression**

The candidate genes most closely associated with the highest significance SNPs will be further characterized using CRISPR/Cas9 to induce mutations [8].Targeted mutagenesis will be executed in the Kitaake genetic background, in which I have already successfully produced knockouts. The short generation time of this accession makes it ideal for high-throughput research. Additionally, I will ectopically overexpress candidate genes with a strong promoter in the Kitaake background. The stomatal density and conductance of mutant overexpression lines will be measured to determine if these candidate genes play a role in stomatal development and physiology.

**Aim 3: Multiplexing knock-ins of drought adaptation alleles**

CRISPR/Cas9 and geminiviruses will be used to produce knock-ins of advantageous alleles in the homologous native location in the Kitaake background. Alleles selected will belong to the haplotype associated with the highest significance SNPs from the most drought tolerant accessions. Useful variation may exist in promoters, genes, or non-coding sequences. This variation will be leveraged using the high replication rates of geminivirus replicons to increase rates of homology-directed repair, with precise positioning enabled by CRISPR/Cas9 mediated double-stranded breaks[9]. Quantitative traits are governed by numerous QTL that contribute collectively to a phenotype[10]. Simultaneous knock-ins of alleles from drought adapted accessions into the Kitaake background will be confirmed and assayed for performance in drought. This approach highlights an avenue to leverage natural variation using targeted genome editing for allele swapping.

**Intellectual Merits:** Grass stomatal adaptations are currently understudied. Rice can serve as a model for other monocot species in investigating novel environmental adaptations that have evolved relative to dicots. Access to high density marker sets coupled with transformation facilities can enable biological investigations that go beyond the scope of model plant organisms. Results may be translated to species such as hexaploid wheat, where GWAS and transformation is more challenging, to explore the conservation of adaptive mechanisms among grasses. Furthermore, multiplexing adaptive allele knock-ins could bypass the high time investment and linkage drag inherent to traditional breeding approaches, and be broadly applied to a range of traits for which there is existing GWAS data[11].

**Broader Impacts:** Improved understanding of drought tolerance mechanisms in monocots can enable eventual crop improvements. These advancements will be necessary to improve plant performance in the face of impending global climate changes. International collaborators will assist with field testing of the most promising edited lines, integrating a broad community of plant scientists. I will leverage my connections in NPR Scicommers and local science museums to share findings of this study with the public and discuss data at conferences, thereby engaging with all individuals about plant-environmental interactions in the context of climate change.

**References: [**1]Hamanishi, E. T., Thomas, B. R. & Campbell, M. M. *J. Exp. Bot.* (2012). [2] Gray, J. E. *et al. Nature* (2000). [3].Zhu, J. *et al. Forests* (2018). [4].Raissig, M. T. *et al. Science* (2017). [5] Raissig, M. T. *et al. Proc. Natl. Acad. Sci.* (2016). [6] Hughes, J. *et al. Plant Physiol.* (2017). [7] McCouch, S. R. *et al. Nat. Commun.*(2016). [8]. Doudna, J.A. & Charpentier, E. *Science* (2014). 9.Wang, M. *et al. Molecular Plant* (2017). [10] Crowell, S. *et al. Nat. Commun.* (2016). [11]. Jacobsen, E. & Schouten, H.J. *Trends Biotechnol.* (2007).