Overview:

Understanding how plants adapt to survive and thrive under adverse environmental conditions is central to engineering sustainable solutions for agricultural production. While advanced phenotyping technologies have enhanced our understanding of agronomically important, naturally adapted responses to stress (e.g. flooding, drought, salinity, cold, and heat; Rahaman et al., 2015); surprisingly little is known about the sub-organismal tissue and organ system functions that contribute to these emergent traits. As a response, my research program is centered around the understudied molecular mechanisms underlying the emergence of phenotypes produced through plant grafting and the generation of periclinal chimeras (Fig. 1).

Both approaches, grafting and the generation of chimeras put forward my vison to disentangle complex organismal responses to environmental stress into functions on the sub-organismal level. Grafting can be used to unite genetically distinct root and shoot organ systems within a single individual (Fig. 1A), and periclinal chimeras (individuals that maintain genotypically distinct shoot meristem cell layers), can be used to generate genetically distinct tissue layers (Fig. 1B; Frank and Chitwood, 2016). Research on both techniques will allow me to: (1) deconstruct complex organismal responses to environmental stress into organ system and tissue-level functions, and (2) create a genomic, mobile molecular map of non-cell-autonomous "information" that coordinates growth and development between tissues and organ systems.

In summary, the overarching biological question of my research asks: "To what extend does a plant equal the sum of its parts?"

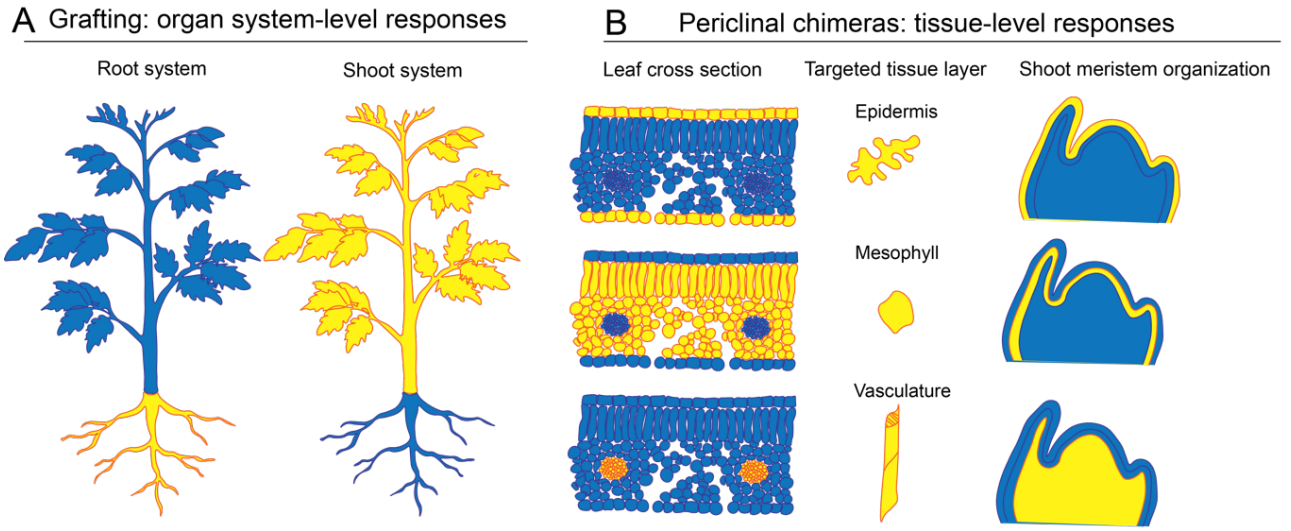

Fig. 1: Organismal mapping of organ system and tissue level adaptations to environmental extremes. Interspecific graft combinations (A) and periclinal chimeras (B) between domesticated tomato - *Solanum lycopersicum* (shown in blue) and its desert-adapted wild relative - *Solanum pennellii* (shown in yellow), will be uesd to identify tissue- and organ system-level contributions to drought responses.

Research Accomplishments:

Pre-doctoral Research

I have worked within the intersection of comparative development, evolution, and genomics for nearly a decade. My PhD, which I completed in Mike Scanlon's lab (at Cornell) in 2014, focused on macroevolutionary questions. I used comparative cell-specific transcriptomics to investigate the molecular relationships between structurally diverse shoot meristems from evolutionarily distinct land plant lineages. From this data I was able to address the molecular basis for three major developmental transitions that have shaped the evolutionary history of the land plants: (1) the transition from uniplanar cell divisions that give rise to filamentous growth, to triplanar divisions that give rise to three-dimensional growth (Frank and Scanlon, 2015a); (2) the rise of indeterminate apical growth in the sporophyte generation – an invention that enabled plants to grow upwards and dominate the aerial landscape (Frank and Scanlon, 2015b); and (3) the innovation of a distinctive stem cell anatomy called the "Apical Cell-type Meristem" that arose independently in two seedless vascular plant lineages (Frank et al., 2015). During my PhD, I developed as both a "wet lab" and "dry lab" scientist, capable of generating and analyzing large-scale genomic

datasets. As a Post-Doc, I am applying my experimental and computational skillsets to agronomically-relevant systems that have yet to be understood at the molecular scale.

Postdoctoral Research

I am independently funded through an NSF NPGI Post-Doctoral Fellowship, which I have used to develop my own research question in Dan Chitwood's lab at the Donald Danforth Plant Science Center. I use grafting as a tool to look at the intra-organismal coordination of growth between root and shoot systems with a focus on agronomically relevant grafting combinations. While certain graft combinations have been shown to increase tolerance to a variety of abiotic and biotic stresses, and significantly increase yield, the genetic and genomic mechanisms underlying the success of these graft combinations is poorly understood. My research addresses two processes that are crucial to the widespread success of this technique. First, I am using positron emission tomography (PET) and confocal imaging to investigate the dynamic reconnection of xylem and phloem cells within the graft junction. Second, I am taking a genetics and genomics approach to identify how particular genotypic graft combinations result in enhanced crop performance, with a special focus on a phenomenon called *grafting-induced vigor* (*GIV*), in which the vegetative and reproductive phenotypes of hybrid vigor are transmitted across the graft junction.

<u>Project 1: A dynamic investigation of graft junction formation using PET and confocal imaging</u>

I am the lead PI for a collaborative project (funded through NSF EPSCoR) with Dr. Yuan-Chuan Tai's lab at Washington University School of Medicine. This project focuses on the formation of the graft junction – a unique anatomical region that unites newly joined root and shoot systems into a single vascular conduit, and thus plays a pivotal role in determining the success of root-shoot combinations during grafting. Working together with physicists and biologists, I am developing an integrated imaging approach using positron emission tomography (PET) and laser scanning confocal microscopy to track the functional and anatomical stages of graft junction formation. Ongoing activities focus on uniting these anatomical and functional datasets into a unified model for graft junction formation. As a preliminary result, we have established that $^{11}CO_2$ and ^{13}NO- are dynamic and reliable markers for phloem and xylem conductance, respectively. Using these radionuclides, we have generated a quantitative timeline, demonstrating that phloem function is restored within five days post-grafting, while xylem restoration occurs within six days post-grafting. Our confocal image data shows that the appearance of elongated and stable vascular strand formation coincides the restoration of phloem transport, indicating that this anatomical stage is likely necessary for vascular function (Fig. 2). By extending this functional framework to problematic grafting combinations (e.g. – pepper x tomato grafts, which consistently fail), we can discover the anatomical and functional rules that govern successful versus failed root-shoot reconnections.

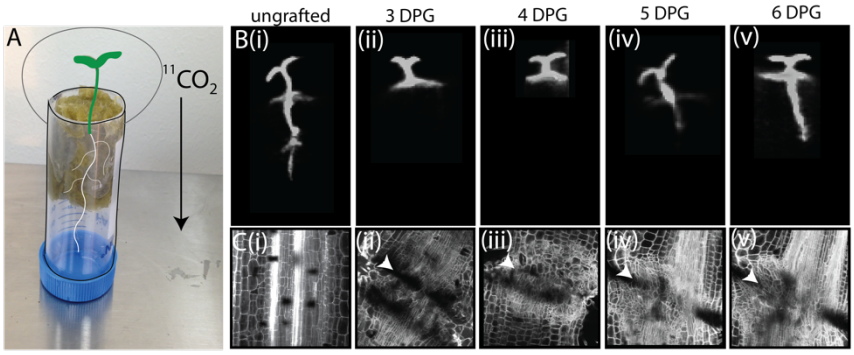

Fig 2: Concurrent Laser Scanning Confocal Microscopy (LSCM) and PET imaging experiments enable the anatomical and physiological dissection of graft junction formation. Schematic for radiolabeled $^{11}CO_2$ delivery to grafted plants (A). Timecourse imaging with PET (B(i-v)) and LSCM (C(i-v)) demonstrates that restoration of phloem transport occurs between 5-6 days post grafting (DPG) (B(iv-v)), which coincides with the first appearance of elongated seive elements (arrows in C(iv-v)). DPG = days post grafting.

<u>Project 2: Identifying the molecular and physiological mechanisms underlying *grafting-induced vigor*</u>

Although recent experimental evidence suggests that non-cell autonomous long-distance signals may play an important role in the mechanism through which grafting impacts plant growth and physiology (Albacete et al., 2014), the precise identity of these signals and the mechanisms by which they act to affect yield remain largely unexplored. In tomato, the grafting of elite fruit producing shoots (scions) onto vigorous, interspecific hybrid root systems leads to *grafting-induced vigor* (*GIV*), in which the domesticated scion exhibits a significant increase in both vegetative and reproductive biomass. I have developed a system that mirrors this agronomically-relevant graft combination and used this system to develop a fundamental understanding of the physiological and molecular responses that underlie *GIV*, with a special emphasis on non-cell autonomous RNAs that may be involved in coordinating this response. My work demonstrates that *GIV* can be reciprocally transferred between the root and shoot systems of interspecific hybrid (*Solanum lycopersicum* x *S. habrochaites*) and domesticated tomato (*S. lycopersicum*) genotypes. Associated with this reciprocal transfer of *GIV*, I have shown that there is a significant boost in photosynthetic efficiency, increased macro and micronutrient accumulation, and massive transcriptomic remodeling that occurs in hetero-grafted versus self-grafted plants. Using genotype-specific polymorphisms to

profile for the exchange of non-cell autonomous transcripts, I have discovered that there are thousands of mobile mRNAs exchanged between hetero-grafted root and shoot systems. The potential functionality and larger genomic impact of these mobile transcripts is unclear, and thus provides a launch point for my future investigations.

Future Investigations on grafting-induced vigor (Short-term): My future research seeks to answer three questions regarding the molecular function of non-cell autonomous RNAs and the longevity of the *GIV* phenotype:

First, we will use organ-specific RNA-sequencing to test whether graft-transmissible RNAs have a "zip-code" that directs their destination to specific organs within the shoot (e.g. flowers, young leaves, old leaves, etc). Transcripts that consistently exhibit organ-specific targeting will be analyzed for structural motifs that are likely to function in guiding RNA entry and exit through the phloem system. These motifs will be subsequently verified using an intracellular fluorescent *in situ* protocol developed in Blake Meyers' lab (unpublished), and functionally tested with an organ-specific fluorescence silencing approach. Briefly, we will graft shoots expressing organ-specific GFP reporters onto transgenic rootstocks containing candidate RNA motifs fused with miRNAs that target and silence the fluorophore (as described in Zhang et al., 2016).

My mobile transcriptomic dataset shows strong enrichment for gene families that are known targets of the 22-nucleotide phased, secondary, small interfering RNA "Phasi-RNA" pathway (Fei et al., 2013). My second aim will use small RNA-seq to detect whether these graft-transmissible mRNAs elicit a secondary, small RNA interference (phasiRNA and siRNA) response in their new location. If so, we will use DNA methyl-seq and Assay for Transposase-Accessible Chromatin "ATAC"-seq to test whether these small RNA pathways are triggering, genome-wide changes in the methylation and open chromatin status of hetero-grafted plants.

One of the long-standing questions in the field of grafting revolves around the stability, and potential trans-generational inheritance of grafting-induced phenotypes. My third aim, will test whether *GIV* phenotypes, associated non-cell autonomous RNAs, and epigenetic changes (in the form of DNA methylation status, histone modifications, and genome-wide open chromatin status) are inherited into the second generation of seedlings from hetero- versus self-grafted parents.

Concurrent with these molecular and physiological investigations into the *GIV* problem, I have designed a genetic mapping approach using *S. habrochaites* introgression lines that have been heterozygosed with *S. lycopersicum* to screen for quantitative trait loci underlying graft-transmissible hybrid vigor in greenhouse and field settings.

The overarching goals of this work are (1) to address the fundamental question of whether non-cell autonomous RNAs serve a functional role in coordinating growth and development between the two halves of the plant in a more general setting (i.e. – beyond grafting), and (2) to build an applied molecular and physiological framework that can be used to identify agronomically beneficial grafting combinations during future rootstock breeding efforts.

Future Vision - Mapping of organ system and tissue-level adaptations to abiotic stress (Long-term):
My long-term research vision revolves around the use of grafting and periclinal chimera lines that will allow us to: (1) deconstruct complex organismal responses to environmental extremes into organ system and tissue-level functions (Fig. 1), and (2) to create a genomic mobile molecular map of non-cell-autonomous "information" involved in coordinating growth and development between tissue layers and organ systems. As an initial test case for this approach, I will focus on sub-organismal responses to drought using the drought-sensitive, domesticated tomato – *Solanum lycopersicum* and its drought-tolerant, desert-adapted, wild relative – *S. pennellii*. In addition to being supported by outstanding molecular and genetic resources, these two species exhibit genotypic compatibility in both grafting and chimera experiments (Filippis et al., 2013). The two species will be transformed with contrasting fluorophores (e.g. – Cerulean and Venus), which can be used to screen for successful periclinal chimera formation and later, to separate the genotypes with fluorescence-activated cell sorting (FACS). Interspecific graft combinations and chimeras will be generated using standard grafting and callous co-culturing techniques. These genotypic combinations will then be functionally tested under well-watered and water limiting conditions to assess the relative "success" (e.g. - survivorship, growth rate, yield, etc) of each genomic combination and identify the effective contribution that independent organ systems and tissue layers make to the net organismal response.

The novelty of this approach, is that these same chimeras and grafting combinations can be used to profile for non-cell autonomous molecular "information" (small RNAs, mRNAs, and proteins) that may be involved coordinating intercellular developmental and physiological responses. The construction of this "mobile molecular map" is based on physically isolating the domesticated and desert-adapted cell types (through root and shoot harvesting for the

grafted plants, and FACS for the chimeras), and using genotype-specific polymporphisms to profile for non-cell autonomous RNAs and proteins. While numerous studies have indicated that mobile proteins and RNAs play an essential role in coordinating intercellular functions (Gallagher et al., 2014; Wu et al., 2016), this would serve as one of the first-ever genome-scale approaches to identifying such signals.

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