The Pennsylvania State University

The Graduate School

GENERATION OF GENETIC MATERIAL TO EVALUATE ARBUSCULAR MYCORRHIZAL SYMBIOSIS IN MAIZE

A Thesis In

Horticulture

by

Liza Nguyen

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The thesis of Liza Nguyen was reviewed and approved by the following:

Ruairidh Sawers
Assistant Professor of Plant Response to Abiotic Stress
Thesis Advisor

Liana Burghardt
Assistant Professor

Jesse Lasky
Assistant Professor of Biology

Erin Connolly
Professor of Plant Science
Head of the Department of Plant Science

ABSTRACT

The domestication of food crops has revolutionized society by fostering the development of large-scale agriculture. Maize (*Zea mays*) is a highly important domesticated crop grown globally which feeds millions of people. Demand for maize production as human populations grow in conjunction with climate change has put immense pressure on increasing maize yield. At the same time, increasing desire for sustainable agriculture is driving agricultural practices to require less inputs such as fertilizer and pesticides. Understanding maize's interactions with beneficial arbuscular mycorrhizal fungi and other microbiota can improve the crop's hardiness to environmental stressors while reducing the need for such inputs. In my thesis, I design and initiate the development of mapping populations to reveal the influence of mycorrhizae in maize cultivation. This work will provide important information to understand how current agricultural systems interact with mycorrhizae while allowing for better integration of fungal communities into agricultural practice. These mapping populations will enable mapping of QTL linked to increased maize response to mycorrhizae, which may be used in breeding programs. These mapping populations employ single gene mutations to generate mycorrhizae-free plants in the field, providing a 'built-in' non-mycorrhizal control for the evaluation of mycorrhizae response. To facilitate population development, I have optimized KASP SNP markers to follow mycorrhizal mutants. In the second part of my work, I performed a reverse-genetic screen to identify novel mutants in genes associated with maize strigolactone signaling. Uniquely in maize, the gene encoding the strigolactone receptor, *Dwarf14*, is duplicated, raising intriguing questions about the role of this gene compared to other cereal species. Addressing biological questions about mycorrhizal dynamics in maize will provide insight that can be leveraged to improve maize resilience to environmental stress.

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ACKNOWLEDGMENTS

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Chapter 1: Introduction to Root Systems and their Interactions with Fungal Communities

The generation of material in my thesis provides valuable resources which can be used to evaluate maize's relationship with a beneficial partner, arbuscular mycorrhizal fungi (AMF). Understanding fundamental concepts of forward and reverse genetics set the framework for my thesis, as I establish material from a dual approach following these two broad themes, which can answer similar but distinct questions about AMF in maize (Figure 1-1).

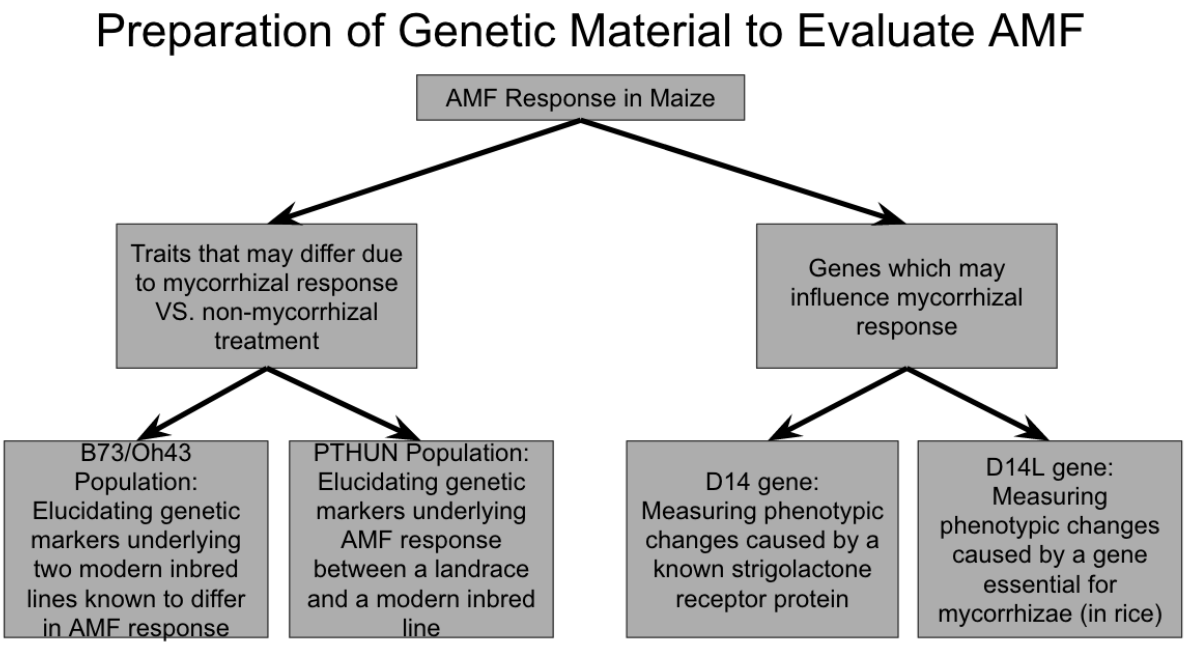


Figure 1-1. Overview of the approaches taken in my thesis to generate material which can be used to measure AMF response in maize. On the left are two mapping populations which take a forward genetics approach; on the right are two key reverse-genetics mutant genes believed to be tied to AMF response.

Researching root systems and belowground dynamics of crop systems can provide new methods to ensure food security. Climate change and increasing demand for food from a growing population has led to concerns over whether or not farmers will be able to sustain the high yields produced during the Green Revolution (Pingali 2012). Such concerns have directed research to look for ways to ensure consistently high crop yields in the face of increasing stress. One emerging avenue is to look more closely at

belowground dynamics of plant systems, such as root development, soil microbiome communities, and beneficial symbioses belowground (Hammer et al. 2009; Rasmann and Agrawal 2008; Shao et al. 2018; Smith and De Smet 2012). Advances in the technology to assess root systems has promoted ample new interest in this direction of study (McCormack et al. 2017; Erktan, McCormack, and Roumet 2018). Specifically, evaluating arbuscular mycorrhizal fungi (AMF) and the symbiosis they engage in with plant hosts is gaining interest (Bolduc and Hijri 2011; Preece and Peñuelas 2020; Thrupp 2000) as a means to shift towards more sustainable agriculture. Mycorrhizal symbiosis occurs in the roots of the plant and offers new avenues to study crop systems (Johnson and Gibson 2021; Parniske 2008). In addition to AMF, there is an abundance of morphological variation in root systems, shown to be tied to genetic differences (Wang et al. 2021). Cataloging and connecting genetic markers which influence root phenotypes and AMF symbiosis is the first step in utilizing belowground dynamics to optimize crops in the face of increasing environmental stress.

In order to study morphological variation in root systems, we can use both forward and reverse-genetics strategies. In forward genetics, genes are identified by genetic mapping following screening for mutant phenotypes in traits of interest. Genetic mapping uses linkage to DNA markers to narrow down the region where the gene responsible for the phenotype lies. Alternatively, these mutants' genomes (if available) may also be screened to discover genes connected to the altered phenotype observed (Peters, Cnudde, and Gerats 2003). In reverse genetics, genetic sequences believed to encode for genes of interest are mutated to render them non-functional for phenotypic evaluation.

One approach to generate mutants for reverse genetics studies that has been broadly used in plants is transposon-mediated mutation. This method uses a transposable element which can be activated to "jump" into genes, (Figure 1-2) causing mutations (Lisch 2002). Transposons are regions of DNA that can undergo transposition (i.e. move their physical location in the genome), originally discovered in maize (McClintock 1953). Transposons may be autonomous or non-autonomous. Autonomous transposons are capable of transposition by themselves, whereas non-autonomous transposons require the presence of an autonomous transposon in order to move.

Transposons can insert themselves into genetic sequences that encode genes, which can render the gene nonfunctional. The discovery of transposons by Barbara McClintock enabled experiments where scientists could purposefully insert transposons into genes, generating mutant individuals which can then be assessed for mutant phenotypes (Kolkman et al. 2005). Using a single autonomous transposon, scientists are able to generate a population of individuals in which transposons are actively moving across the genome and inserting themselves into gene sequences. Once ample coverage of the genome is achieved, segregation of the autonomous transposon can be done in order to leave the non-autonomous transposons fixed in their position (McCarty et al. 2005). These fixed transposons can then be mapped in the genome or populations screened to determine whether they lie close to or within a gene sequence of interest. Such individuals carrying this transposon insertion can then be selected for reverse-genetics studies (Ramachandran and Sundaresan 2001). Technical differences between systems, such as differences in transposon elements used (e.g. *mutator, mu* versus *Activator/Dissociation, Ac/Ds*) or methods in which the mutations were generated (e.g. in uniform inbred backgrounds or in heterogeneous populations) lead to variation in genome coverage and the use of the mutants identified (Marcon et al. 2020). In my thesis, I identify and confirm transposon-mediated mutants in genes believed to be impactful in mycorrhizal symbiosis.

My work uses both reverse and forward genetic systems to generate plant material which can be used in experiments to evaluate maize response to mycorrhizae. This material can serve as the basis for many experiments to answer multiple questions about mycorrhizae-maize dynamics, which may offer exciting avenues to promote sustainable agriculture.

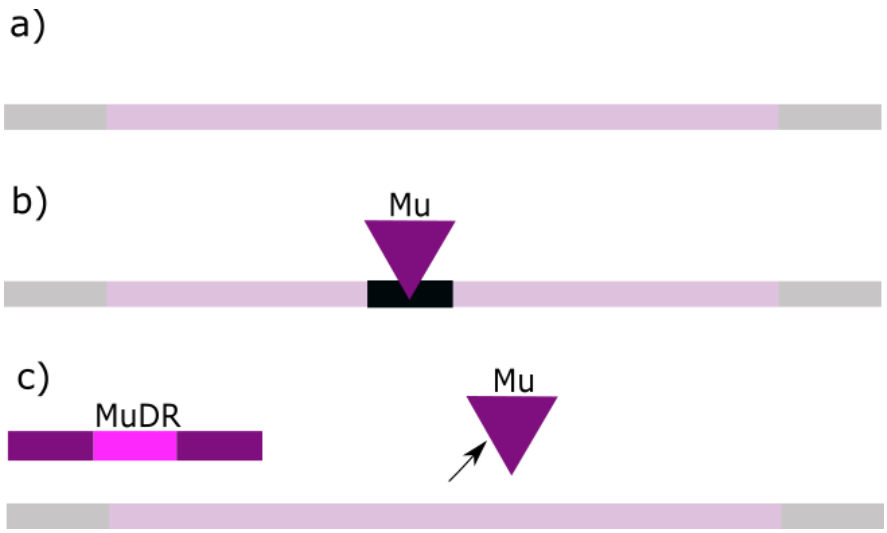


Figure 1-2. Schematic of *mutator (mu)* transposon. When autonomous MuDR is present, both MuDR and other Mu elements can "jump" out of their position and insert into a new position (c) possibly rendering a gene nonfunctional. When an autonomous Mu is not present, non-autonomous transposons are unable to "jump" and remain stable in their current position (b). Gene sequences with no transposon insertion are fully functional (a).

Over 70% of terrestrial land plants are able to enter symbiotic relationships with beneficial fungi to form 'mycorrhizae' (Parniske 2008). Arbuscular mycorrhizal fungi (AMF) are the most widespread fungal type that can partner with plants, with many agriculturally important crops being capable of this symbiosis (Öpik et al. 2006). While great advances have been made in elucidating the genes, proteins, and hormones required for mycorrhizal establishment, many important questions remain about how relationship dynamics change over time, whether fungal-plant preferences exist, and how mycorrhizae fit into overall plant systems with other hormones and plant response pathways. Answering these questions increases the possibility of leveraging mycorrhizae as a way to optimize plant nutrient capture while reducing fertilizer inputs, leading to cheaper and hardier agricultural systems.

Strigolactones (SLs) are a class of plant hormones that play diverse roles in plant development, including branching, root development and mycorrhizal symbiosis (García-Garrido et al. 2009; Kapulnik and Koltai 2014; Koltai 2011; Ruyter-Spira et al. 2011; Xie, Yoneyama, and Yoneyama 2010). As well as functioning as endogenous hormones, plant-produced SLs are exuded from root tips into the soil. The amount of SL

exudation varies due to fluxes in plant nutrient status to regulate mycorrhizal colonization to optimize nutrient capture (Yoneyama et al. 2012). Plants release SLs to recruit mycorrhizal fungi, while mycorrhizal fungal spores release signaling molecules of their own, known as myc factors, which signal to root cells to prepare for fungal hyphae penetration (Parniske 2008). This initial communication leads to a cascade of secondary signals and mechanical reactions from the cell and fungal spore. The fungal spore increases its mitochondrial activity in order to quickly form the physical formation of a hyphopodium before forming a pre-penetration apparatus (PPA) against a plant cell wall (Besserer et al. 2006). From the plant cell side, calcium spiking occurs within plant cells via calcium and calmodulin-dependent protein kinases (CCaMKs) in order to prepare for fungal penetration. Nuclear membrane proteins balance the quickly changing cytoplasmic charges due to Ca^{2+} by utilizing ion transport proteins CASTOR and POLLUX (Kassouar and Hamed 2014). Calcium spiking and the balancing of ions via CASTOR and POLLUX are required for mycorrhizal establishment, which cannot occur if either of the two proteins are rendered nonfunctional (Ramirez-Flores et al. 2020, Chen et al. 2009). By taking advantage of known cellular biological reactions required for mycorrhizal colonization, I was able to obtain mycorrhizae resistant maize plants. These single gene mutants (in the genes *castor* and *pollux*, respectively) can be used in experiments to evaluate plant performance without mycorrhizae in field conditions. While much is known about how mycorrhizal colonization occurs mechanistically (Figure 1-3), connecting how these proteins and cell responses are mediated by SLs remains unclear.

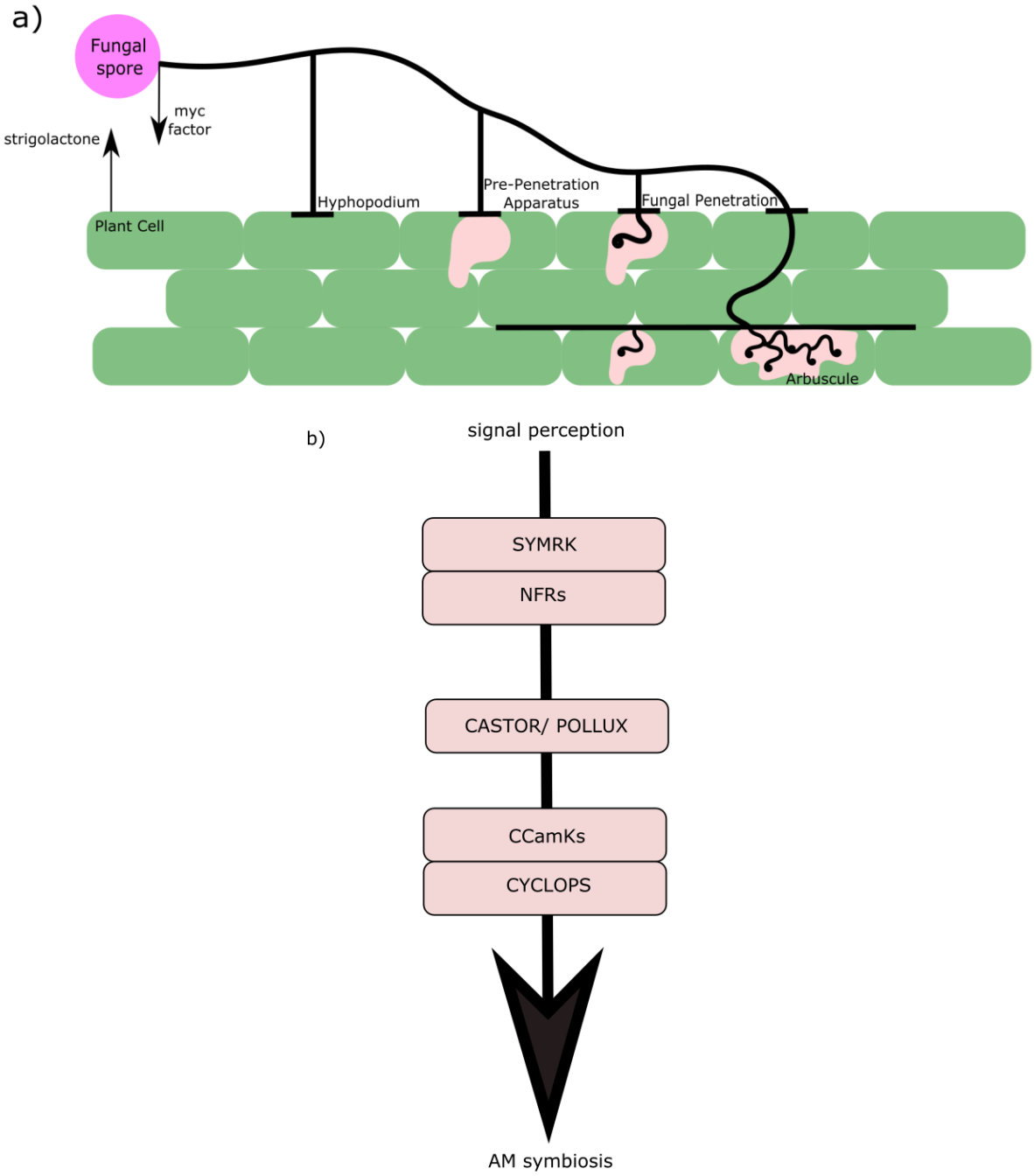


Figure 1-3. A generalized diagram of physical mechanisms of mycorrhizal colonization (a) and the gene pathway highlighting essential genes for mycorrhizal symbiosis to occur (b).

Understanding SL synthesis, as well as how plants recognize and perceive SLs as communication molecules, can help further elucidate their role in mycorrhizal symbiosis. SL synthesis begins with the production of carotenoids, a class of compounds plants produce for capturing light energy in order to perform photosynthesis

and deactivate harmful free radicals. Carotenoid beta-carotene is produced by the methylerythritol 4-phosphate (MEP) pathway in plastids and is the precursor of SL biosynthesis (Nisar et al. 2015). The OsDWARF27 (D27) isomerase converts beta-carotene from *trans*-beta-carotene to *cis*-beta-carotene (Alder et al. 2012). Further oxidative/ring-closure reactions are performed on *cis*-beta-carotene by carotenoid cleavage dioxygenases (CCD7 and CCD8) to yield carlactone (CL), the last common SL precursor (Saeed et al. 2017). Carlactone can be converted into either 5-deoxystrigol (5DS) or ent-2′-epi-5-deoxystrigol (ent-2′-epi-5DS), which are precursors of the two main strigolactone types, strigol and orobanchol (Xie et al. 2010). In *Arabidopsis*, the protein MAX1 converts CL into carlactonoic acid, which is able to interact with SL receptors (Abe et al. 2014). In rice, a MAX1 homolog (Os01g0700900) oxidizes CL into ent-2′-epi-5DS, and another homolog of MAX1 (Os01g0701400) converts this product into orobanchol (Zhang et al. 2014). Once synthesis is complete, SLs are shuttled to other regions of the plant, such as the rhizosphere or meristem (Lopez-Obando et al. 2015). The ATP-binding cassette (ABC) protein PDR1 is proposed to play a role in SL transport (Kretzschmar et al. 2012).

Plants utilize SLs throughout their system as a signaling molecule to balance root growth with shoot growth (Smith 2014). When nutrients are scarce, SL production increases to stimulate root growth and/or increase mycorrhizal colonization. Once nutrients are in adequate supply, plants decrease their amount of SL production, reducing root growth in favor of shoot development (Smith 2014). By using SLs to coordinate this balance of growth below-ground and above-ground, plants are able to optimize their rate of growth to maximize the nutrients and resources currently available to them. This feedback loop provides the rationale for why SLs are the signaling molecule that is responsible for mycorrhizal establishment.

This connection opens a plethora of more nuanced topics relating to mycorrhizae and SLs. Hundreds of species of arbuscular mycorrhizal fungi can be present at any given point in soil, and while prior studies have shown that these AMF are not host-specific (Klironomos and John 1999), advances in sequencing-based characterization have indicated that the microbiome of the root system in maize have heritable features in terms of community identity (Walters et al. 2018). Understanding how AMF fits in

conjunction with other soil biota is important to the understanding of how maize root systems function. Evaluating tradeoffs and relationship dynamics between these key characteristics will enable farmers to leverage maize cropping systems in ways that can ensure consistently sufficient yield.

One way to understand the mechanisms of mycorrhizal colonization is to determine how SL perception and signaling lead to this phenotypic response. The protein D14 (DWARF14) is responsible for the initial perception of SLs; understanding what downstream effects occur due to D14 perception of SLs can be informative of these mechanisms. When D14 perceives SLs, the protein performs a hydrolysis reaction on the SL D ring, resulting in a structural change in the D14 protein (de Saint Germain et al. 2013; Nakamura et al. 2013). This conformational change promotes binding of MAX2/D3 (MORE AUXILIARY GROWTH 2 / DWARF 3), an F-box protein. D14 and MAX2 assemble a SCF complex that targets the proteins SMAX (SUPPRESSOR OF MAX), also known as D53, and/or SMXL (SUPPRESSOR OF MAX-LIKE) for ubiquitination and degradation via the 26S proteasome (Wang et al. 2015). In *Arabidopsis*, SMXL6, SMXL7, and SMXL8 are all involved in regulation of shoot branching (Soundappan et al. 2015; Villaécija-Aguilar et al. 2019). Similar to D14, SMXL7 has been shown to be localized in the nucleus, strengthening the model that these SMXL genes are the targets of the SL-degradation pathway (Liang et al. 2016). SMXL proteins act as transcriptional repressors of downstream targets. Once SMXLs are degraded following SL perception, their target genes are free to express, leading to a cascade of downstream reactions that ultimately results in a plant response. Targets of SMXL6/7/8 include a TPR (TOPLESS-RELATED REPRESSOR) protein which can alter transcriptional activity of IPA1 (a SQUAMOSA PROMOTER-BINDING FAMILY LIKE transcription factor) known to regulate shoot branching (Villaécija-Aguilar et al. 2019). SLs have been proposed to influence auxin transport as a downstream effect, which could lead to modification of shoot architecture (Crawford et al. 2010; Shinohara et al. 2013).

The SL signaling pathway is similar to a second pathway responsible for perception of karrikins, which are compounds found in burnt plant material known to stimulate germination in some species (Figure 1-4). The similarity in receptor proteins

between SLs and karrikins has made uncoupling SL impacts from karrikin impacts difficult. Additionally, evidence shows there is overlap in downstream effects of the karrikin pathway with the SL pathway, specifically with mycorrhizae in rice (Gutjahr et al. 2015). Prior research in SLs has utilized mutants which overlap in the SL and karrikin pathway, creating a need for research on SL-specific genes such as D14 (Villaécija-Aguilar et al. 2019).

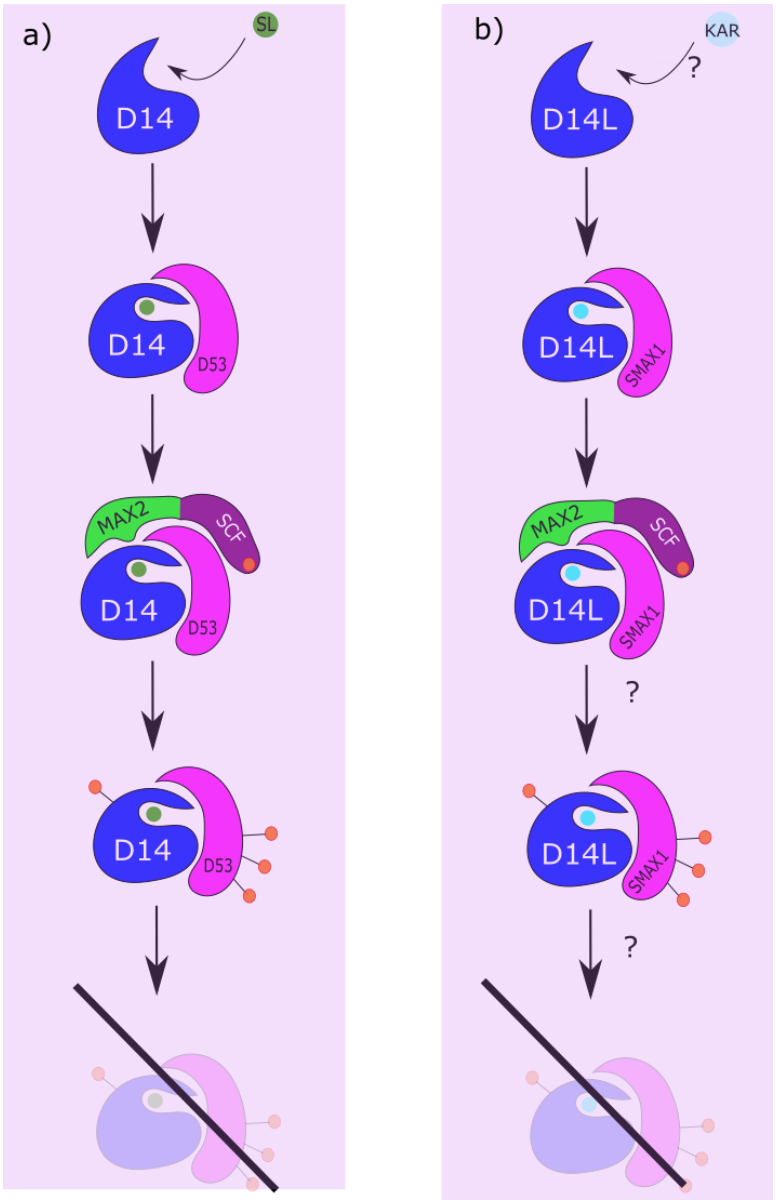


Figure 1-4. SL perception (a) leading to downstream applications, with a comparison to the karrikin pathway (b).

Collecting multiple mutants in the agriculturally important crop maize for various genes tied to SLs and mycorrhizae will enable finely tuned experiments that can answer the complex biological questions about below-ground plant dynamics. More broadly, understanding how AMF ties into whole plant systems and how this relationship can be exploited to optimize yield and ensure hardiness of crop systems can provide a means for agriculture today to face the impending environmental stresses to come. My work encapsulates these two broad tenets of forward genetics and reverse genetics, which frames the scope of my work in generating plant material to be used in addressing questions about plant response to mycorrhizae.

Chapter 2: A Forward Genetics Approach in Determining How AMF Affect Belowground Dynamics

Understanding mycorrhizae dynamics in agricultural crops can provide scientists with a new lens in which to optimize and improve crop yield. A long term aim of my team is to establish mapping populations where plant genetic variation can be linked to responsiveness to AMF. In my thesis, I initiate the first two crosses of a new mapping population (goal 1) while finishing another mapping population (goal 2). Specifically, my goals were to:

1. Initiate a mapping population where genetic variation in modern varieties can be tied to AMF response in realistic farming conditions using *castor/pollux* mutants.
2. Generate families to form a mapping population that will be informative of how AMF responsiveness may have changed over time by using an ancient landrace and a modern inbred line.

Using *castor/pollux* mutants in mapping populations enables non-mycorrhizal treatments in field trials

Conducting experiments to study the impact of mycorrhizae can be difficult, particularly in field trials, due to the presence of native fungal spores in the soil. Native fungal spores can influence plant performance, making it difficult to discern AMF effects caused by controlled treatments. Current methods to address this involve sterilization of soil and reintroduction of specific fungal species, but this method is not feasible for field trials where open air can blow spores and reinoculate soil throughout the timeline of an experiment. Another option is to sample soil and determine fungal communities before beginning experiments in order to determine a baseline. This however is very limiting, as it does not allow for the control of fungal species used, nor does it control for variation in fungal species' different levels of benefit or colonization to the host plant. To address this, my team has generated and validated transposon-mediated mutants in maize (*castor* and *pollux*, Figure 2-1) which are unable to establish mycorrhizal symbiosis. These mutants can be used to determine the effects of mycorrhizae by comparing between wild-type and mutant performance (Ramírez-Flores et al. 2020). Ramirez-Flores did not note any pleiotropic effects of the *castor* mutant which affected

plant development or yield, but this concern is addressed in my mapping population by inclusion of both *castor* and *pollux* mutants, which can be compared to each other to detect pleiotropic effects. To track these mutants, genotyping by PCR using gene-specific primers alongside a transposon-specific primer can be done throughout multiple generations. Genotyping by PCR is also informative in selection of individuals to use for crosses, depending on desired genotype (Figure 2-2). Integration of these mutants into my mapping population directly advances my first goal by providing a feasible way to conduct field trials with and without mycorrhizae in real farming conditions to determine the role of mycorrhizae in agricultural systems.

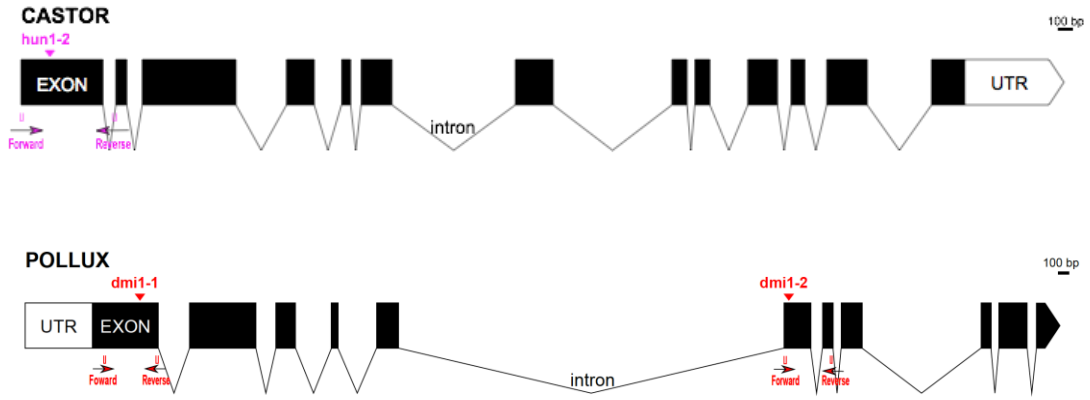


Figure 2-1. Gene models for CASTOR (hun) and POLLUX (dmi), showing insertions of transposons which render the genes nonfunctional. Primers used to identify the presence of the transposon (in *castor* is mutator, in *pollux* is dissociation) are also shown.

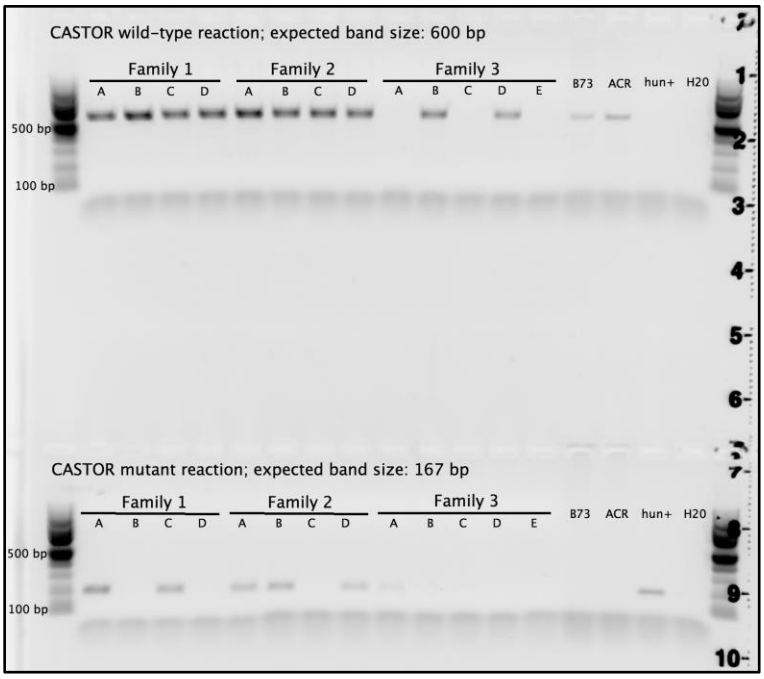


Figure 2-2. Genotyping for CASTOR. Three backcrossed families were genotyped by PCR, where only wild-type or heterozygous individuals are expected. A band in both reactions (top and bottom) indicates a heterozygous individual, whereas a band in only one indicates the individual is homozygous for that reaction. A positive control (hun+) is a known homozygous mutant for *castor*, which only yields a band in the mutant reaction.

Evaluating mycorrhizae requires the distinction between dependence and responsiveness, an issue taken into account by using parents with different levels of responsiveness

Plant responsiveness to mycorrhizal fungi can be distinguished from dependence (Sawers, Gutjahr, and Paszkowski 2008). Dependence is the inability for a plant to successfully grow and develop without mycorrhizae, whereas responsiveness is measured by the extent to which a plant's performance increases when mycorrhizae is introduced (Figure 2-3). These two traits are genetically distinct, but can be confounded when attempting to study multiple varieties or backgrounds. If the plants being evaluated are of different genetic backgrounds, they may vary in their dependence on mycorrhizae, which can skew any differences measured in their responsiveness. This makes evaluating mycorrhizae between different backgrounds difficult.

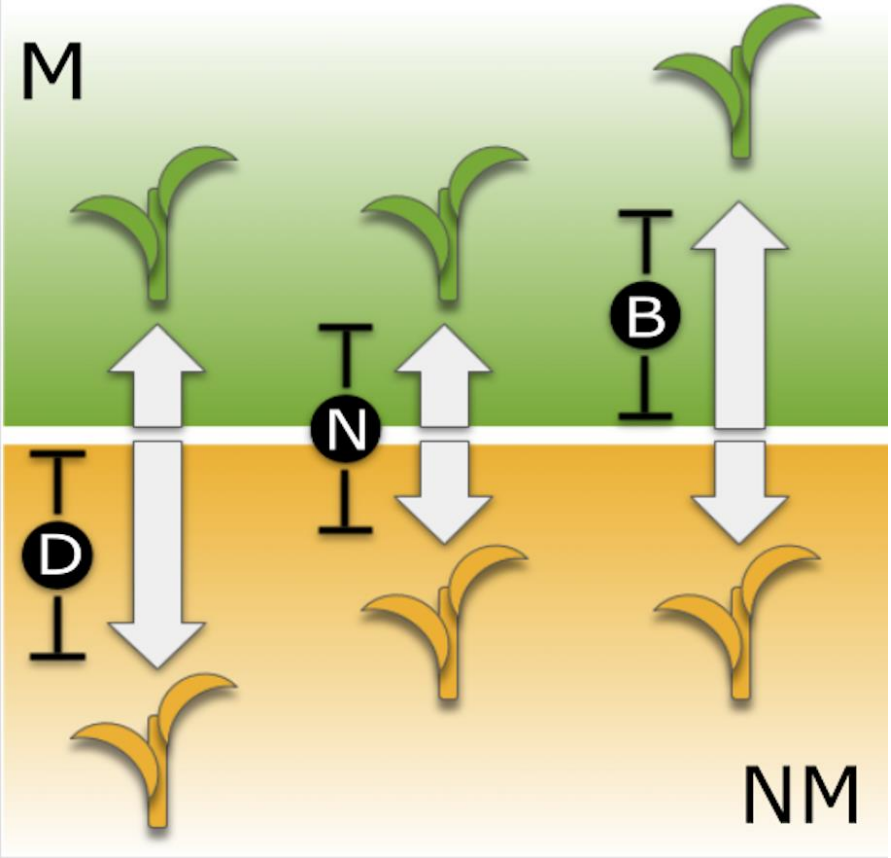


Figure 2-3. Distinguishing dependence (D) from benefit (B), or responsiveness. Plants which are more dependent on mycorrhizae (M) will perform worse in non-mycorrhizal (NM) conditions, whereas plants which are more responsive gain greater benefit with mycorrhizae. Plants may also be neutral (N) in their respective performance with and without mycorrhizae. Figure adapted from Ramirez-Flores 2020.

Therefore, to address this challenge in my first goal, two lines differing in their responsiveness to mycorrhizae are crossed together to generate a mapping population which takes into account responsiveness and dependence. Two different backgrounds, B73 and Oh43, have been shown to differ in their response to mycorrhizae (Sawers et al. 2008) and are the parental lines of my population. Integration of *castor/pollux* mutations will enable non-mycorrhizal treatments to be 'built-in' to this population. This allows for the assessment of mycorrhizal responsiveness between B73 and Oh43 by identifying genetic markers linked to AMF utility via QTL mapping. These QTLs can be compared between mycorrhizal or non-mycorrhizal treatments within one variety (B73 or Oh43) to discern whether a specific marker is correlated with dependence or with responsiveness. Many traits that are influenced by AMF are complex traits. Traits which

may be influenced by AMF may be tied to nutrient foraging, especially in phosphorus acquisition, or tied to flowering time, yield, and pollen development (Daft and Okusanya 1973; Eissenstat et al. 2015; Ramírez-Flores et al. 2020; Sawers et al. 2017; Zhang et al. 2019). QTL mapping is a useful tool to identify potential genetic markers for such traits, and will be conducted on the F2:3 population. By doing this, we can identify genetic markers that correlate to mycorrhizal capacity and observe how they influence phenotypic traits of agronomic interest (yield, flowering time, etc.) under stressful conditions (low phosphorus, drought, etc.) while controlling for confounding variables such as dependence. In Figure 2-4, a schematic of how the mapping population will be generated is shown.

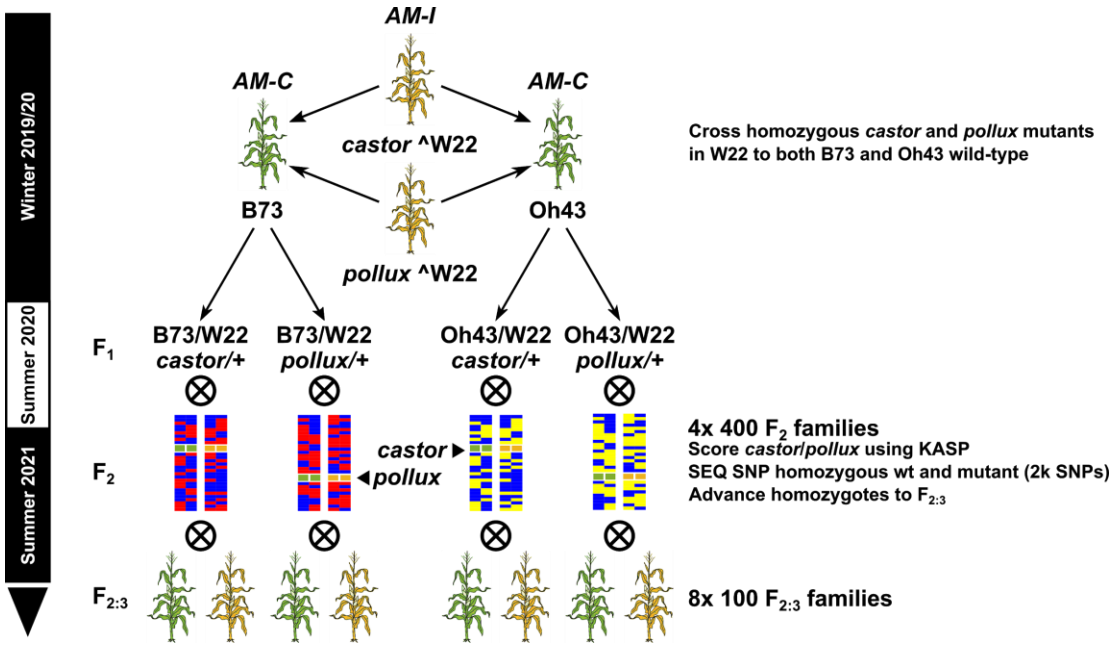


Figure 2-4. B73/Oh43 mapping populations were generated by generating a F1 cross of homozygous W22 mutants of *castor* and *pollux* with either B73 or Oh43. Subsequent families generated were selected for the mutation in order to leverage large populations which can be planted in field trials.

KASP genotyping provides an easy, large-scale method of determining background identity in mapping populations while accelerating QTL mapping

An accurate method of genotyping utilizing a method called KASP can help accelerate the generation of this mapping population. To initiate this mapping population, in the winter 2019-20, I crossed B73 and Oh43 inbred plants to homozygous mutants carrying

either *castor* or *pollux* mutations (both in an inbred W22 background). I confirmed the F1 cross was carrying these mutations via traditional genotyping by PCR. An alternative method of determining genotype which can be combined with genome-wide markers for QTL mapping involves utilizing KASP genotyping (‘k’ompetitive allele-specific PCR, developed by the company LGC). KASP genotyping can be used to determine genotype by distinguishing between the W22, B73, and Oh43 background using a polymorphic SNP marker within the gene or as close as possible to the gene. An individual in which the presence of W22 is detected can be assumed to carry the mutant *castor* or *pollux* as there are no other wild-type W22 copies of these genes present in the population. As long as the SNP marker is polymorphic between W22 vs B73 or between W22 vs Oh43 (or W22 vs both), determining the genotype of individuals is possible simply by distinguishing whether individuals have copies derived from these three backgrounds. KASP genotyping depends only on a single reaction, thereby reducing error calls compared to traditional genotyping by PCR which relies on two separate reactions that must be interpreted together. The higher accuracy for KASP genotyping provides high confidence to screening hundreds of plants, which is critical in the F2 generation. I selected 10 candidate SNP markers to validate their viability on 72 individuals each for *castor* and *pollux* and compared KASP results to traditional PCR genotyping results. Traditional PCR genotyping varied greatly in its accuracy, a problem not seen from the SNP markers from KASP genotyping.

Testing 10 SNP markers for KASP shows segregation patterns of F2 families following Mendelian expectancies

To determine suitable SNP markers for KASP genotyping, I selected 10 markers based off of SNPVersity searches (available on MaizeGDB) for polymorphic SNPs between W22 vs B73 and Oh43. Candidate SNPs were run on 4 different F2 families for both CASTOR and POLLUX. For each family, 18 individuals were sampled, for a total of 72 individuals for each gene. Controls W22, B73, and Oh43 were also sampled and genotyped in order to make calls on the genotype of individuals from each family. In addition to testing these 10 markers to determine whether they could distinguish between the three backgrounds, segregation patterns for each gene were also checked

(see Table 2-1) to ensure Mendelian segregation before advancement to the F3 stage in summer of 2021.

df = n - 1 = 3-1 = 2 using P=0.05 → critical value is 5.991

CASTOR	Observed (O)	Expected (E)	$(O - E)^2$	$(O - E)^2/2$
Wild-type	20	17	9	0.5294
Heterozygotes	34	34	0	0
Homozygous Mutants	14	17	9	.5295
Total Individuals	68	68		$\chi 2 = 1.0588$

df = n - 1 = 3-1 = 2 using P=0.05 → critical value is 5.991

POLLUX	Observed (O)	Expected (E)	$(O - E)^2$	$(O - E)^2/2$
Wild-type	20	16.5	12.25	0.742
Heterozygotes	33	33	0	0
Homozygous Mutants	13	16.5	12.25	.742
Total Individuals	66	66		$\chi 2 = 1.4848$

Table 2-1. Chi-square analysis of segregation patterns for CASTOR and POLLUX genes in AMF mapping population based on KASP genotyping calls. Analysis was performed on a total number of individuals called from KASP results. Degrees freedom (df) using a p-value of 0.05 indicates a chi-square value of 5.991 for both genes. A chi-square value ($\chi 2$) less than the critical value indicates that the genes are segregating at an expected 1:2:1 Mendelian ratio.

Landraces represent "old" maize varieties, and may differ in their responsiveness to AMF than modern varieties

Landraces (traditional, locally adapted varieties of maize) may be more or less responsive to mycorrhizae than modern agricultural varieties. During the domestication of maize, and over time as maize has been cultivated, the genetic diversity of the species decreased (Liu et al. 2019). Selection for traits of interest (yield, uniformity, etc) may have been prioritized over more nuanced traits such as mycorrhizal capacity. Current agricultural systems for maize are high-input, in which extensive fertilizer is

applied to promote plant growth and yield. Studies have shown that saturation of nutrients minimizes the responsiveness of plants to mycorrhizae (Sawers et al. 2017, 2008), and that colonization in these situations can lead to a net performance cost. Given these conditions, it is uncertain whether modern-day agriculture has selected against maize utilization of mycorrhizae, and that more “wild” varieties of maize have the capacity to benefit more from AMF than modern-day lines. Comparing a landrace (representing a more “wild”, or genetically diverse, variety of maize) to a modern variety can help resolve this question. Palomero Toloqueno (PT) is an ancient popcorn landrace that represents the indigenous genetic diversity of maize (Prasanna 2010), and is grown under harsh conditions compared to modern varieties (acidic soils, high elevation, etc). I hypothesize that PT is able to sustain adequate yield in its harsh environment because it is able to utilize AMF to overcome environmental stressors, leading to better responsiveness than a modern variety. PT plants may utilize AMF for phosphorus (P) acquisition, as acidic soils tend to have low P availability (Magalhaes et al. 2018). To test this hypothesis, I complete generating a mapping population in which a landrace of maize (Palomero Toluqueno, PT) is introgressed into a modern day inbred (B73).

Generation of a mapping population (PTHUN) using an ancient landrace and a modern variety can help determine whether plant responsiveness to AMF has changed over time

My team crossed a single PT parent to B73, and subsequently crossed this F1 to a BC5 B73 plant carrying the *castor* mutation before I initiated selfing down these individuals. The incorporation of *castor* in this population enables progeny to be planted in field conditions without native mycorrhizal fungi influencing plant performance. With two rounds of backcrosses to B73, each family generated carries a unique 12.5% portion of the PT genome. Families generated were selected for segregation of the *castor* mutation (i.e. heterozygotes) and continuously selfed to reduce heterozygosity at other loci while still maintaining wild-type and homozygous *castor* progeny. Since each family in the PTHUN population is segregating the *castor* mutation, near-isogenic lines (NILs) pairs from each family are also present and can be identified. Each NIL pair will

be wild-type and homozygous for *castor* while being homozygous at nearly all B73 loci, and contain the same 12.5% portion of the PT genome. This provides a comparison of how a distinctive region of the PT genome may impact mycorrhizal-related performance with and without AMF in a uniform background of B73. By determining what effects (if any) the PT portion of the genome in each family has in mycorrhizal and non-mycorrhizal treatments, a correlation between genetic background (PT or B73) and plant performance in relation to mycorrhizal benefit can be seen. An overview of the generation of the PTHUN population is shown below in Figure 2-5.

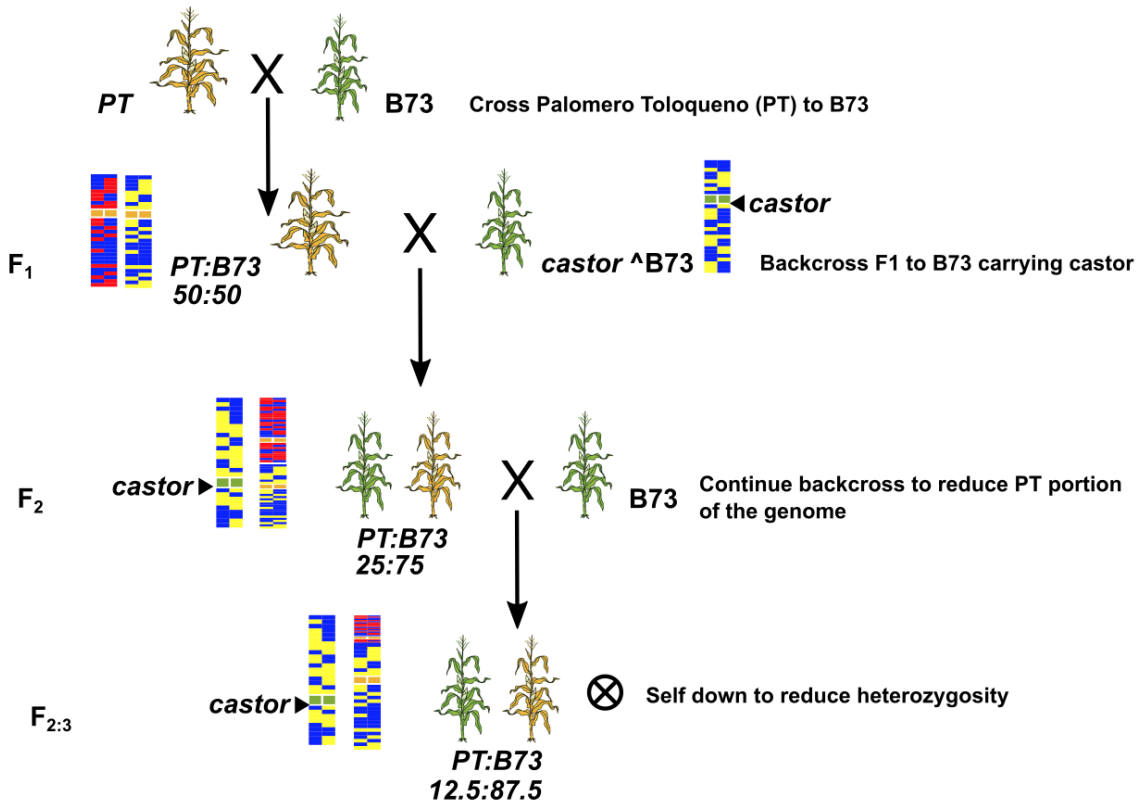


Figure 2-5. PTHUN mapping populations were generated by generating a F1 cross of PT with B73 carrying a mutation of *castor*. Subsequent families generated were selected for the mutation in order to leverage large populations which can be planted in field trials.

Chapter 3: A Reverse Genetics Approach in Evaluating AMF Regulation via Strigolactones

How strigolactones direct mycorrhizae establishment while influencing root system development is still unclear. To help elucidate the role of SLs in maize, I identified, obtained, and validated mutations in key genes linked to the SL signaling pathway in terms of mycorrhizae, such as D14 and D14L. I also began moving validated mutants to various inbred backgrounds to generate material for experiments which can evaluate AMF capacity and root traits. Specifically, my goals were:

1. Elucidate the phylogenetic relationships of maize D14 and D14L orthologs while leveraging transposon-mediated mutation systems to identify at least two independent mutation alleles for each gene.
2. Initiate introgression of D14 and D14L mutants into various backgrounds (B73, Oh43, W22, etc) to enable downstream experiments that can assess impacts of these genes.

Orthologs of D14 and D14L in maize are duplicated: D14a, D14b, D14La, and D14Lb

D14 and D14L are excellent candidate genes to elucidate SL signaling effects in maize for several reasons. The D14 protein has been well characterized in rice and *Arabidopsis thaliana* as a SL perception protein, and annotated so that searching for orthologs in other species is relatively easy. Since SLs are messenger molecules in a feedback loop to regulate mycorrhizae, D14 is implicated in this feedback loop as plants require the D14 protein in order to perceive SLs. Interestingly, while SLs have been shown to be a major signaling molecule in mycorrhizae across multiple species, including sorghum (Lanfranco et al. 2018; Lendzemo et al. 2007), this is not seen in other cereals. Rice is dependent on the D14L protein for mycorrhizal colonization, not the D14 SL-perception protein, and *d14* mutants in rice exhibit higher colonization levels than normal (Gutjahr et al. 2015; Yoshida et al. 2012). D14L genes, also known as KAI genes in *Arabidopsis*, detect karrikins, and have been reported to play a parallel and additive influence alongside strigolactones on mesocotyl elongation in rice (Zheng et al. 2020). Therefore, *d14* and *d14l* mutants may have impaired mesocotyl development.

Mutants in *d14* and *d14l* may also play additive roles in cell membrane maintenance and leaf cuticle development (Li et al. 2020). Additionally, evidence of an uncoupling of strigolactones to branching and other plant architecture features (Guan et al. 2012) raises questions about whether D14 in maize will have the same function as in rice.

Furthermore, I confirmed two copies of D14 existed in the maize genome (on Chr1 and Chr9) when I BLASTed the rice sequence of D14 to search for orthologs. A similar scenario emerged for D14L, and a phylogenetic tree I constructed (Figure 3-1) shows that while similar, D14 and D14L are distinct from one another and separate into two separate clades. Validation of D14 and D14L as candidate genes is important because determining whether D14 (as a SL receptor) or D14L (as a karrikin receptor) is responsible for mycorrhizae in maize is still unknown. This question may be answered by evaluating *d14* and *d14l* mutants. While D14 has been shown to be a major gene involved in SL signaling (Yao et al. 2018), the dual roles of the two copies in maize is unresolved, creating a need for *d14* maize mutants for both copies. Similar questions about the dual copies of D14L in maize also arise. Answering these questions can be informative of symbiosis-hormone dynamics. My identification of mutants in D14 and D14L in maize is the first step in elucidating the roles these genes play to help answer such questions.

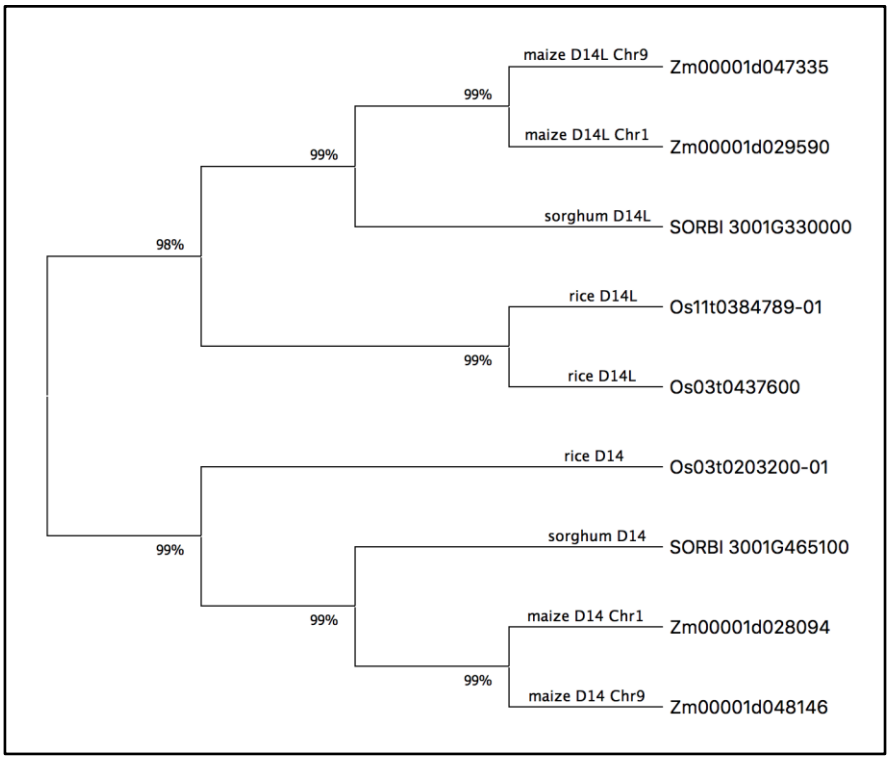
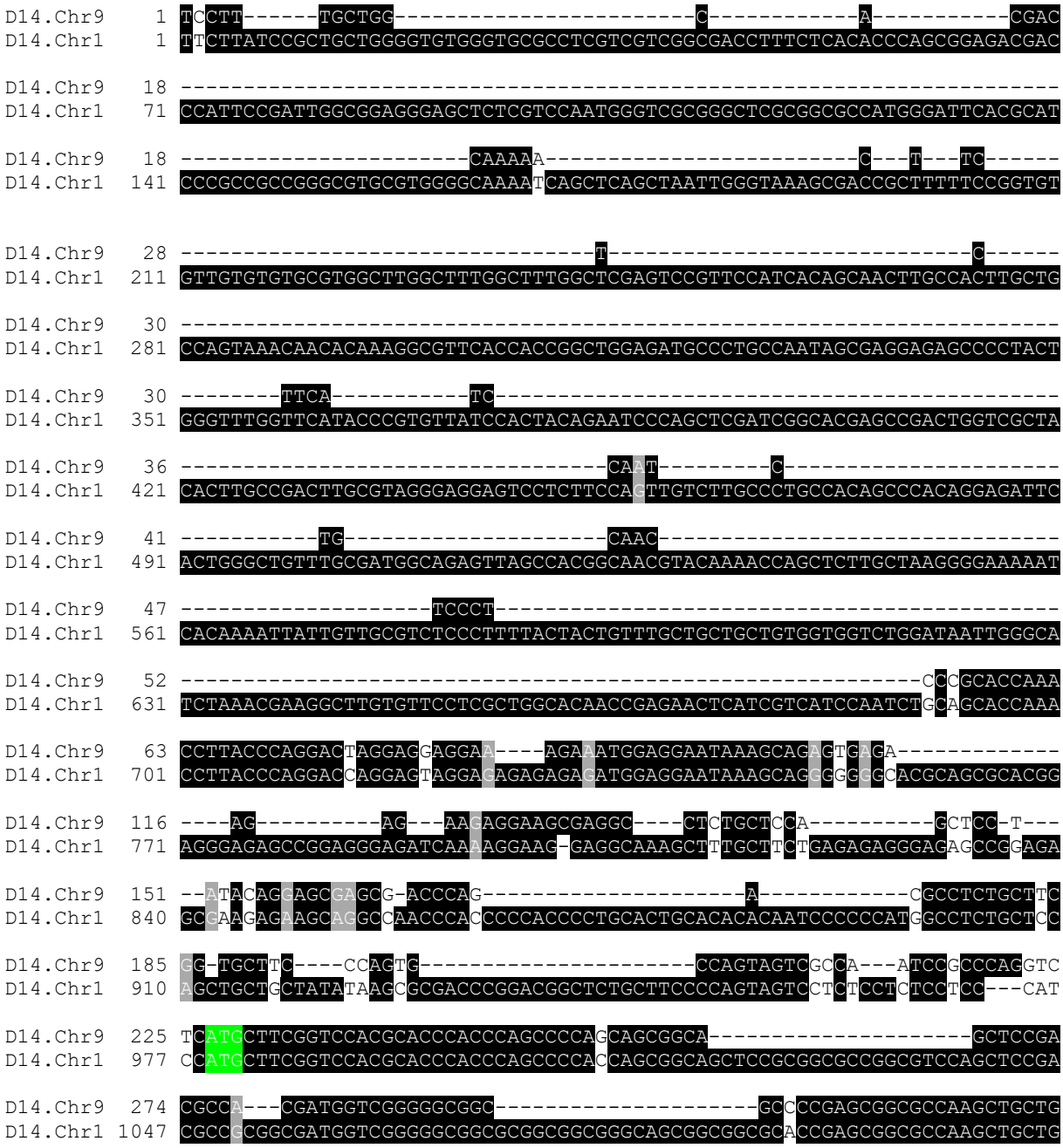


Figure 3-1. Gene tree alignment of D14 and D14-like (D14L) genes between cereals rice, sorghum, and maize. Rice DNA sequences were pulled from the Gramene database and BLASTed on MaizeGDB to identify maize orthologs. D14 and D14L are distinct from one another and separate out into two distinct clades. Tree was constructed with MEGAX software using protein sequences of each gene.

Utilizing multiple transposon mutation systems ensures ample coverage of the SL signaling pathway at critical points

I scoured pre-existing transposon-mediated mutant systems to obtain and validate desired mutants in D14 and D14L for reverse-genetics studies: TUSC (Trait Utility System in Corn), UniformMu, BonnMu and Ac/Ds. The first three use Robertson's mutator, an autonomous transposon in order to promote activity of non-autonomous mutator elements to generate a population of individuals with mutator insertions in genes. After segregating away Robertson's mutator, these insertions are then considered stable, fixed in their position in or nearby genes. This establishes a resource for maize geneticists to obtain mutant stocks for genes of interest. By screening through these mutation systems, I acquired potential candidates with transposon insertions near or within D14 and D14L sequences. I confirmed the insertion of transposons with traditional genotyping by PCR. D14 ortholog (on Chr1 and Chr9) alignments and

positions of mutant alleles are shown in Figure 3-2. In mutants in which *mutator* was the transposon used, I used primers designed for a universal feature of mutator elements, called terminal inverted repeats (TIRs), to evaluate whether the transposon was present or not. Gene-specific primers were used to determine if an individual carried a 'wild-type' copy, or functional copy of the gene. One gene-specific primer was then paired with the TIR primer to evaluate whether a mutant copy of the gene was present.




D14.Chr9 320 CAGATCCTCAACGTGCGGGTGGTGGGCAGCGGCGACCGCGTGGTGGTGCTGTCCCACGGGTTCGGCACGG
D14.Chr1 1117 CAGATCCTCAACGTGCGGGTCGTGGGCAGCGGCGACCGCGTGGTGGTGCTGTCCCACGGGTTCGGCACGG
D14.Chr9 390 ACCAGTCGGCGTGGAGCCGCGTGCTCCCCTACCTCACCCGCGACCACCGCGTGGTGCTCTACGACCTCGT
D14.Chr1 1187 ACCAGTCGGCGTGGAGCCGCGTGCTCCCCTACCTCACCCGCGACCACCGCGTGGTGCTCTACGACCTCGT
D14.Chr9 460 CTGCGCCGGCAGCGTCAACCCGGAGCACTTCGACTTCCGCCGCTACGACACGCTGGACTCGTACGTCGAC
D14.Chr1 1257 CTGCGCCGGCAGCGTCAACCCGGAGCACTTCGACTTCCGCCGCTACGACACGCTCGACTCGTACGTCGAC
D14.Chr9 530 GACCTGCTCGCCATCCTCGACGCGCTCCGCGTCTCGCGCTGCGCCTTCGTCGGGCACTCCGTGTCCGCCA
D14.Chr1 1327 GACCTCCTCGCCATCCTCGACGCGCTCCGCATCGCGCGCTGCGCCTTCGTCGGCCACTCCGTCTCCGCCA
D14.Chr9 600 TGATCGGCATCCTCGCCTCCATCCGCCGCCCCGAGCTCTTCGCCAAGCTCGTCCTCATCGGCGCGTCGCC
D14.Chr1 1397 TGATCGGCATCCTCGCCTCCATCCGCCGCCCCGAGCTCTTCGCCAAGCTCGTACTCATCGGCGCGTCGCC
D14.Chr9 670 CAGGTAACATGGCGTGATCGGAT----------TCGTTCGGTTCGGTCTCACTCTC----ACCAACCGGC
D14.Chr1 1467 CAGGTATGCCGCCGTATGCGTATGAATATGATGCCGACCGAATCCATCCATCCATCCCGATTCGATCAAC
D14.Chr9 726 CCAGCCTCTGGAG--AGTTT-AATTTGATGAATTCCGGCCCGGGCACTGCACTGCAGGTTCCTGAACGAC
D14.Chr1 1537 CCAACCTCTGGAAGCAATTTGAGTTTGTTTTAA--AATTCCGGGC--------ACAGGTTCCTGAACGAC
D14.Chr9 793 CACGACTACCACGGCGGGTTCGAGCTGCCGGAGATCCAGCAGGTGTTCGACGCGATGGCGGCCAACTACT
D14.Chr1 1597 CACGACTACCACGGCGGGTTCGAGCTGCCGGAGATCCAGCAGGTGTTCGACGCGATGGCGGCCAACTACT
D14.Chr9 863 CGGCGTGGGCGACCGGGTACGCCCCGCTGGCGGTTGGCGCGGACGTGCCCGCGGCGGTGCAGGAGTTCAG
D14.Chr1 1667 CGGCGTGGGCGACCGGGTACGCCCCGCTGGCGGTGGGCGCGGACGTGCCGGCGGCGGTGCAGGAGTTCAG
D14.Chr9 933 CCGCACGCTCTTCAACATGCGGCCGGACATCTCGCTCCACGTCTGCCGCACCGTGTTCAACACGGACCTC
D14.Chr1 1737 CCGCACCCTGTTCAACATGCGGCCGGACATCTCCCTCCACGTGTGCCGCACCGTGTTCAACACCGACCTC
D14.Chr9 1003 CGCGGCGTGCTGGGCATGGTGCGCGCCCCCTGCGTGGTGGTGCAGACCACCCGCGACGTCTCCGTCCCGG
D14.Chr1 1807 CGCGGCGTGCTGGGCATGGTGCGGTCCCCCTGCGTGGTGGTGCAGACCACCCGCGACGTCTCCGTCCCGG
D14.Chr9 1073 CCTCCGTCGCCGCCTACCTCAAGGCCCACCTCGGCGGCCGCACCGCCGTCGAGTTCCTCCAGACCGAGGG
D14.Chr1 1877 CCTCCGTCGCCGCCTACCTCAAGGCCCACCTCGGCGGCCGCACCGCCGTCGAGTTCCTCCAGACCGAGGG
D14.Chr9 1143 CCACCTCCCGCACCTCAGCGCCCCCGGCCTCCTCGCCCAGGTGCTCCGCCGCGCGCTCGCCCGGTACTAG
D14.Chr1 1947 CCACCTCCCGCACCTCAGCGCCCCCGGCCTCCTCGCGCAGGTGCTCCGCCGCGCGCTCGCCCGGTACTAG
D14.Chr9 1213 CAGCC---GGGCCCGCCA--GGCC-------------TG-TTTTTACCGCCTCGATTTACCCC-TGCCGA
D14.Chr1 2017 CGGAGCGCCTGCCACCTACGGTCCTGGTGGTCATCGGTATTTTTTACCGCCTCGATTTACCCCGTGCCGA
D14.Chr9 1263 GACCGA------GAATCAAGAAATGGCGCGACTCGCGCCGTTTGCTGTGCCT---ATGCGCGCCATGACT
D14.Chr1 2087 GACCGGGGACGGGGATCAGATAATGTGGCGACCCGCGCCGTTTGCTGTGCGCGCCATGAC--TGTAGACT
D14.Chr9 1324 GTAG--------TACAACTTGTTCTGGTAGGTAGTGGTACCCGATTTCTTTTC-GCTTTTT-ATGAGCAG
D14.Chr1 2155 GTAGTACGAGTGTACGACTTGTTCTGGA--CTTCTGGTACCCGATTTTTTTACTGCTTTTTAATGGAGAG
D14.Chr9 1384 TTGCAGTGTTGCACTGCTACTCCGCATCTTGCCTTCGTCTCCTGCACCTCCACCCAACCCAAGGACAGAT
D14.Chr1 2223 CCGAGCAGTAGCACTGGTACTCCGCATCTTGCCCTCTTCTCCTGCACCTCCACCCAGG------TCC---
D14.Chr9 1454 AAGTGGGCCTGCTCGGGACACGTGGCGAGTAGTCGAGGCGGCGCACGAGCTCGTTCCGCCTTCGCCTTCG
D14.Chr1 2284 TGGTGGGCCTGCTCGCGACACGTGGCGAGTACTCGAGGCGGTGCACGAGCTCGGTCCAGATTCGCCTTCG
D14.Chr9 1524 CCCTGTCCCATCCGCAAC---TGCAA--CTAGTGCTGGGAATTCAG--CC-----------GGACCGCCC
D14.Chr1 2354 CCTTGTCCTACCTGCTACCTTTGTTATTTTACTGCTGGTAGTTTACTGCTATGAGAATATGAGATAGGGA
D14.Chr9 1576 TCATC-G-TCATTCGT--GTTAAACAGATTAGGGTTAGGAGAGTCTAA-----ATTTTTTTATATACCGTG
D14.Chr1 2424 TCAGCCGCCGCCCCGTCGGATATTCTGACTTTGATTAAGCGGGTAGTAATGGATTGGTTTGGTTGGCAT
D14.Chr9 1638 CTAGTCTGAAGTATAAA-----AGATCCAGCTCGGTCCTAAATT-ATATCATGC--ATTTGTTGTACCGGT
D14.Chr1 2494 ATTACGTGACGTCCATAGTTGGGTTCTGGATAAGTCCTGCCTGCCTCCTGGGGAAGGGCAAAACCGAG

Figure 3-2. DNA sequence alignment for D14 gene orthologs in maize. The two copies of D14 are found on separate chromosomes (1 and 9); different transposons and their point of insertion (highlighted in pink) in the D14 genes are shown, alongside primers (forward primers in orange, reverse primers in blue) designed to flank the transposon. Start and stop codons for each gene are shown in green and red, respectively.

By screening through various transposon systems, I was able to procure mutants for both D14 and D14L. Both genes carry multiple transposons inserted in different positions, thereby providing multiple independent events in which these genes may have been rendered nonfunctional. Insertion points for transposons in D14/D14L genes in maize are shown in Figure 3-3, highlighting the use of different transposon systems in order to obtain multiple independent mutant events.

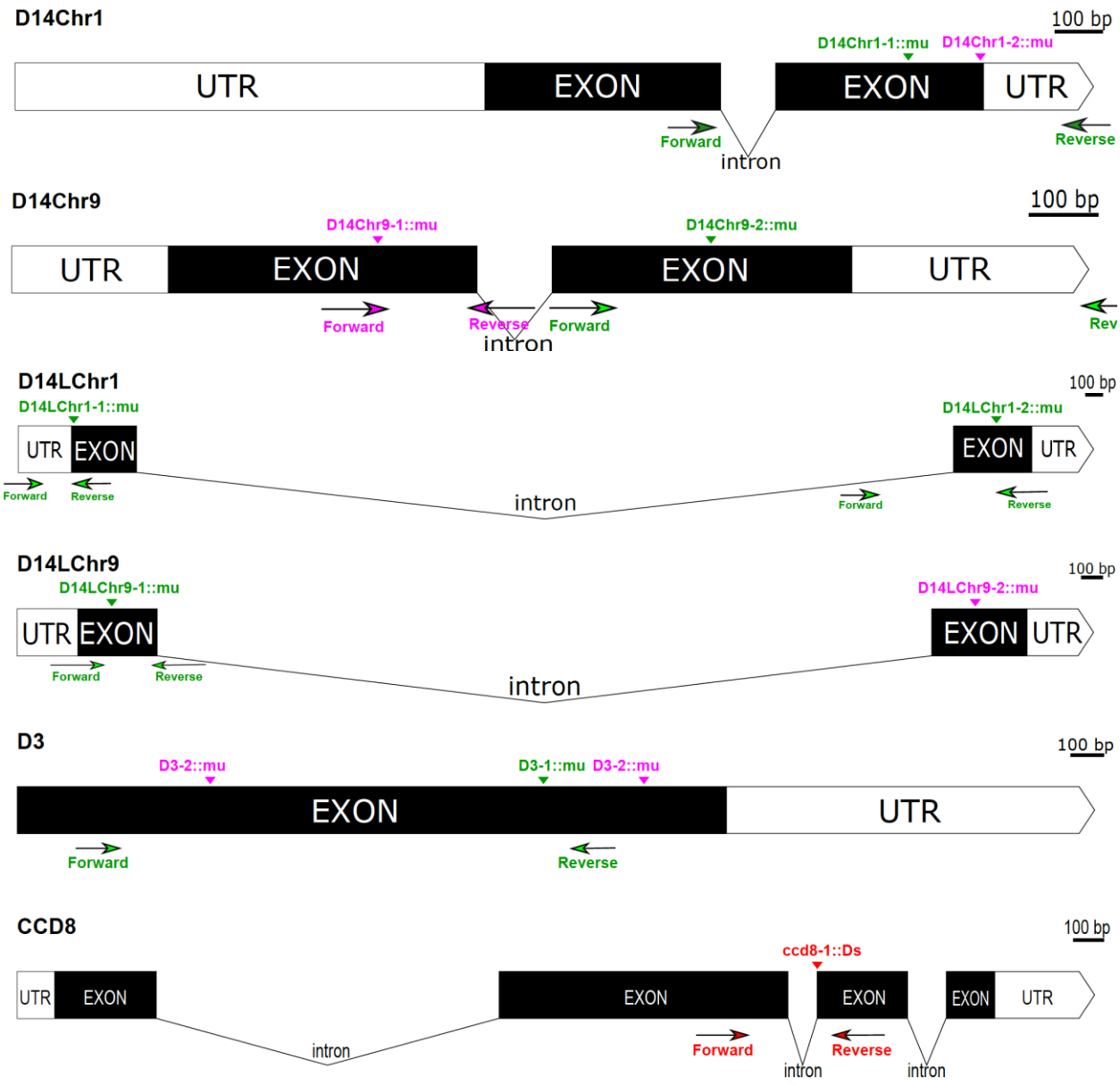


Figure 3-3. Gene models for various genes linked to SL pathways. Events in pink indicate mutation was sourced from the UniformMu/BonnMu population, green indicates TUSC, and red indicates Ac/Ds, alongside forward and reverse primers to test for presence of the transposon.

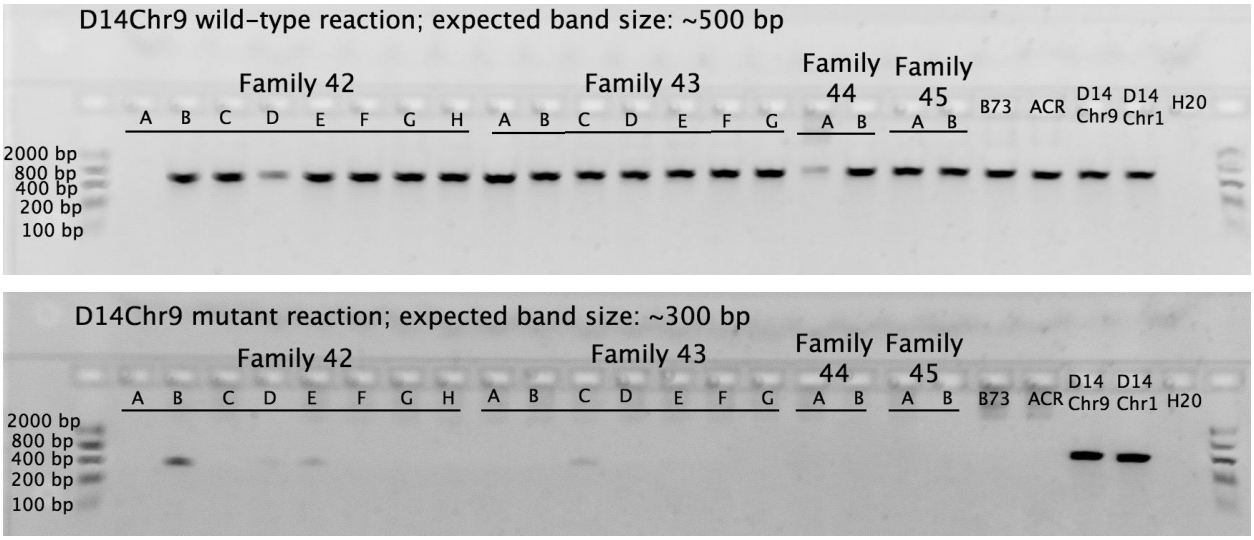


Figure 3-4. Genotyping by PCR for gene D14 on Chromosome 9. The wild-type reaction (top) shows gene-specific amplification, while the mutant reaction (bottom) detects the presence of the transposon (here, mutator) or not. Here, only families 42 and 43 carry the mutation of interest. Gene specificity (between chromosome 1 and 9) can pose problems for identifying unique mutations; sequencing the point of insertion will resolve such problems.

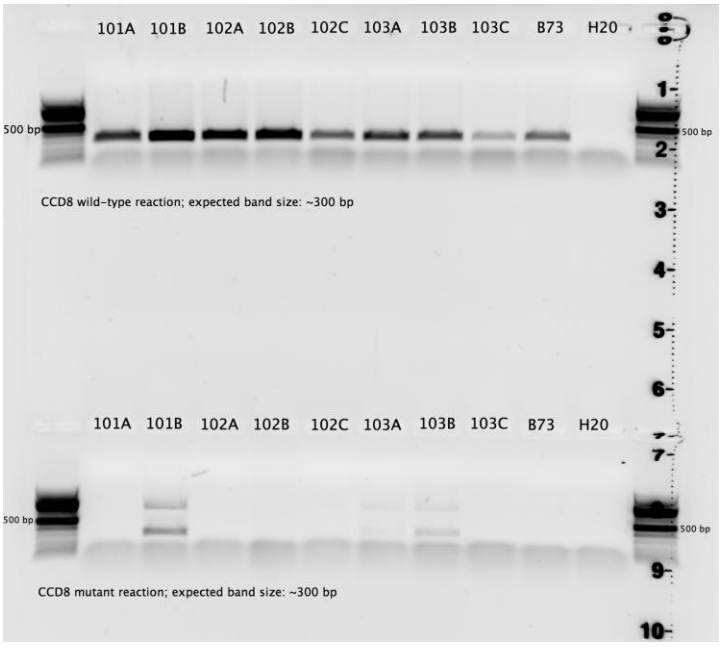


Figure 3-5. Genotyping by PCR for gene CCD8. The wild-type reaction (top) shows gene-specific amplification, while the mutant reaction (bottom) detects the presence of the transposon (here, dissociation).

Expression of D14 and D14L in maize peaks during the transitional period from vegetative to reproductive stage

To formulate hypotheses about what *d14* and *d14l* mutants might look like, I examined expression data for D14 and D14L wild-type copies in maize. Higher expression levels were observed for D14 and D14L on Chromosome 1, approximately 5 and 3 times higher, respectively (Figure 3-6 and Figure 3-7). In all four genes, expression levels peak in the thirteenth leaf during the reproductive stage of the plant. Expression levels are also noticeably higher in later stages of vegetative growth just before the reproductive stage and in silks of ears. Transcript analysis of mutants in D14 and D14L will be measured against reported wild-type levels of expression to determine the extent of the transposon rendering the gene nonfunctional.

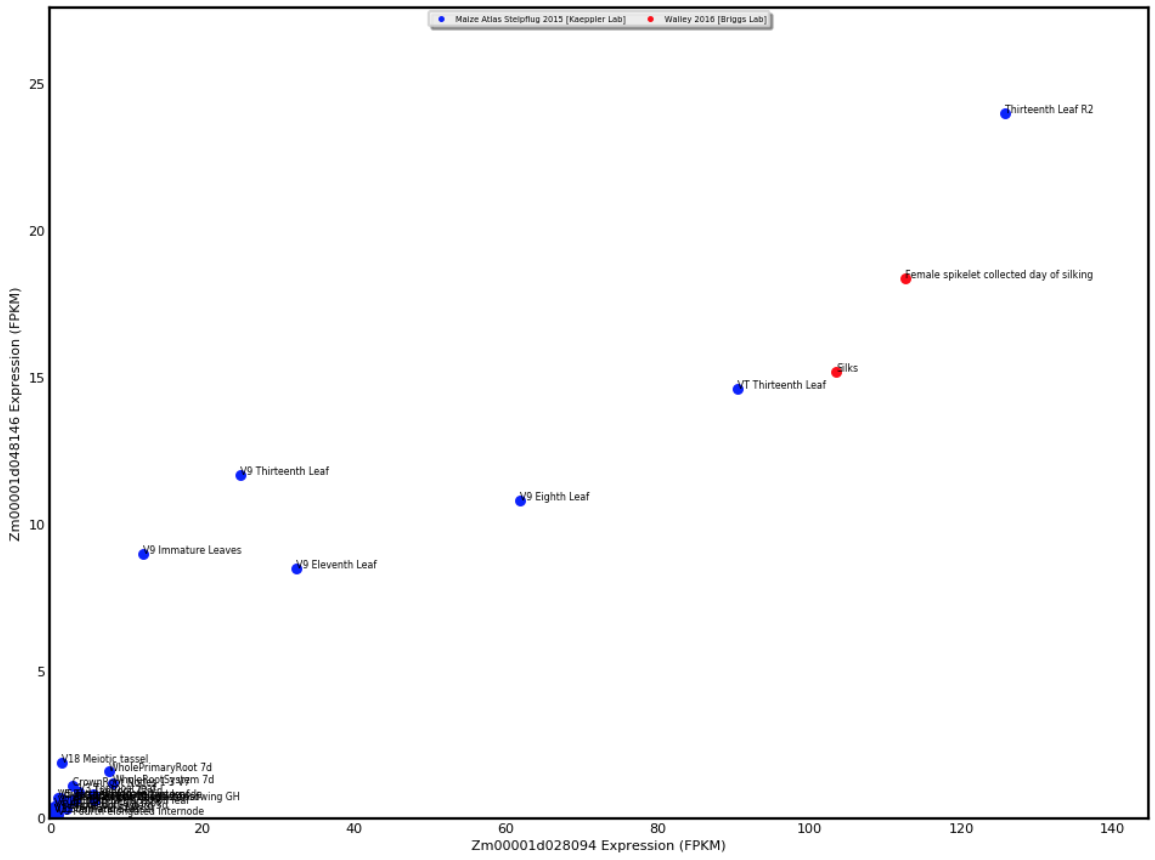


Figure 3-6. Expression level for D14Chr1 (x-axis) in various tissue organs compared to D14Chr9 (y-axis) expression levels. Note that the expression quantity for D14Chr1 is over 5 times higher than that of D14Chr9.

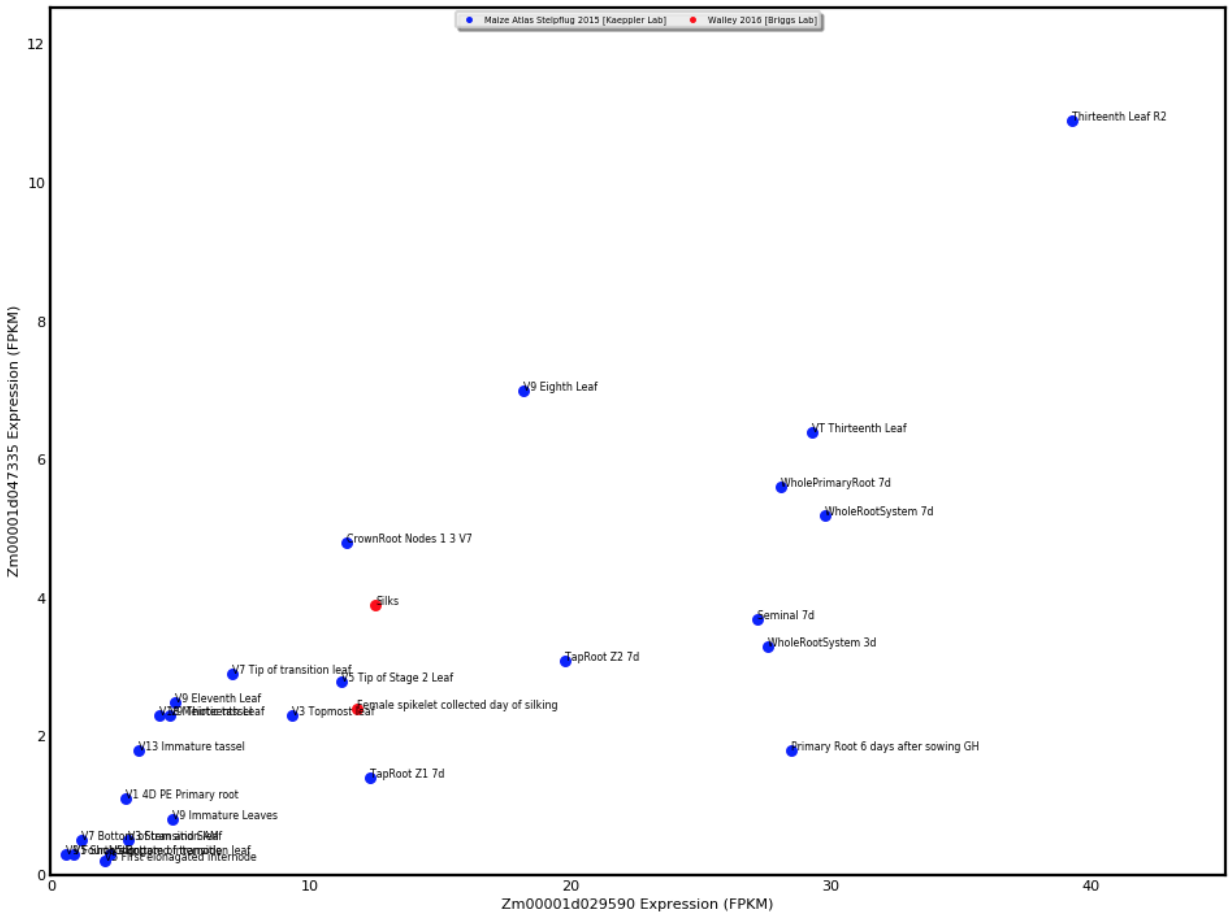


Figure 3-7. Expression level for D14LChr1 (x-axis) in various tissue organs compared to D14LChr9 (y-axis) expression levels. Note that the expression quantity for D14LChr1 is over 3 times higher than that of D14LChr9.

Comparing SL mutants can reveal key roles the SL signaling pathway plays in root architecture, mycorrhizal symbiosis, and other belowground dynamics

These mutants provide a valuable resource in which to study the impacts of SLs on downstream applications. Current status of where I have moved each mutation of interest into common backgrounds is summarized in Table 3-1 below. Evaluating whether these mutants have the same phenotype in regard to mycorrhizal capacity will elucidate the roles these genes play in AMF symbiosis, and help understand this complex relationship. By utilizing multiple transposon systems, a complete coverage of

the four D14/D14L genes is made possible. While evidence indicates that D14 and D14L are implicated in SL and mycorrhizal traits, other genes tied to the SL-signaling pathway and mycorrhizae (e.g. CCD8 and D3) can also help fine-tune where these genes play a specific role.

Gene Name	**Mutation System**	**Validated (by genotyping via PCR)**	**Initial Background**	**Current Background**
CCD8::Ds	Ac/Ds	Yes	W22	BC2 in B73
D3::Mu	TUSC	Yes	Mo17	Mo17
D14Chr1-1::Mu	TUSC	Yes	B73	BC7 in B73 BC6S1
D14Chr1-2::Mu	BonnMu	Received Seed	W22	W22
D14Chr9-1::Mu	TUSC	Yes	B73	BC7 in B73 BC6S1
D14Chr9-2::Mu	UniformMu	Yes	W22	W22
D14LChr1	TUSC	No	F1 (x B73)	F1 with B73
D14LChr9-1::Mu	TUSC	Yes	F1 (x B73)	BC1 in B73 F1 with W22
D14LChr9-2::Mu	BonnMu	Received Seed	W22	W22

Table 3-1. Current generation times of validated mutants, alongside source population and initial background.

Chapter 4: General Discussion and Conclusions

The B73/Oh43 mapping population can help elucidate genetic markers responsible for differences in responsiveness to AMF between modern varieties

The generation of the AMF mapping population in my thesis can help answer fundamental questions about maize's current relationship dynamics with AMF. Understanding differences between modern varieties' responsiveness to AMF can be informative to future breeding efforts to select for higher responsiveness, which can ameliorate nutrient or environmental stress. The two lines used to generate this population have been shown to differ in their performance under mycorrhizal treatments, indicating there are underlying genetic mechanisms responsible for this difference. Identifying these genetic markers is the first step in implementing them into breeding pipelines. Integrating these markers might boost plant responsiveness to AMF and could reduce the amount of fertilizer applied in agricultural systems, as mycorrhizae may supplant some of the required nutrient capture, thereby making sustainable agriculture more feasible. The integration of *castor/pollux* mutants in this mapping population establishes a mycorrhizal and non-mycorrhizal treatment in which to measure plant performance, while enabling field trials in real agronomic conditions (e.g. denser planting). Prior studies which assessed mycorrhizal benefit to plant performance indicated about one third of grain production is tied to mycorrhizal benefit (Ramírez-Flores et al. 2020), revealing important connections of AMF to agronomically important traits. A similar approach to Ramirez-Flores' work is taken here with the AMF population, but population size is increased substantially in order to increase power to detect important QTLs tied to mycorrhizal performance. Inclusion of *pollux* in addition to *castor* in the AMF population generated in my thesis takes into account possible pleiotropic effects that *castor* might have, which was not taken into account in Ramirez-Flores' work. Additionally, optimization of KASP genotyping will facilitate population generation. The potential gains for improved maize breeding can help generate resilient maize lines that can endure changing climate, soil, and nutrient conditions in agricultural systems.

The PTHUN mapping population can help determine whether plant responsiveness has changed over time or in different environments by using an ancient landrace cultivated in harsh conditions introgressed in a modern variety background

The PTHUN population can help determine whether maize interactions with AMF have shifted as agricultural practices and cultivars of maize have changed. Studies have shown that the diversity of fungal communities in agricultural soils is low compared to more natural systems (Verbruggen and Kiers 2010), due to changes such as monoculture, tillage, and high input fertilizers implemented within the last few decades (Johnson and Pfleger 1992; Oehl et al. 2003; Tawaraya 2003). Prior studies conducted in wheat revealed a decrease in AMF colonization in modern varieties compared to landraces or wild varieties (Hetrick, Wilson, and Cox 2011), but a similar result was not observed in a study of maize germplasms (An et al. 2010). These studies did not report information on yield, a critical connection to AMF for future utilization in agricultural systems. An also noted a huge amount of variation in mycorrhizal colonization of varieties, making any discernible conclusions about plant responsiveness to mycorrhizae difficult. Additionally, levels of mycorrhizal colonization may not necessarily be indicative of plant responsiveness (Ramírez-Flores et al. 2020).

Therefore, introgressing portions of a landrace into a uniform background can more precisely determine the influence of older varieties to AMF response. In the PTHUN population, genetic regions of the PT genome which correlate to their adaptation to their local environment are intermixed within the B73 genome. These regions, if influential to changes in plant response to AMF, can then be identified with QTL mapping. A unique feature of this population is the reduction of heterozygosity during each generation where families are selfed. Once heterozygosity is low enough, wild-type and homozygous *castor* individuals in each segregating family can be identified to create near-isogenic lines (NILs) for each family. Each NIL pair per family will have the same 12.5% PT genome and be homozygous at nearly all B73 loci, with one pair being capable of mycorrhizal symbiosis (wild-type) or non-mycorrhizal (homozygous mutant for *castor*). If a particular segment of the PT genome has implications for mycorrhizae, this should be apparent when evaluating the NIL pair that

contains this region. Pleiotropic effects between multiple regions of the PT genome will be minimized, as there is only a reduced portion present (12.5%) and all other loci will be homozygous and uniform between the mycorrhizal and non-mycorrhizal NIL pair. Being able to compare PT to B73 alleles in the same growing environment against a uniform genetic background with and without mycorrhizae allows for differences in performance between the two to be accurately identified as due to differences in mycorrhizal benefit.

Mutants in genes important for strigolactone signaling will be informative as to the roles SLs play in mycorrhizae and other plant traits

While evidence indicates that SLs regulate mycorrhizae, the mechanisms by which they do so remain unclear. By obtaining mutants at different points in the SL synthesis/perception pathway (e.g. first at CCD8, then at D14, then at D3), pinpointing where this regulation occurs can be elucidated. Furthermore, the degree to which SLs influence plant traits via mycorrhizae can be observed by comparing between mutants (i.e. a D14 mutant performs “better” than that of D3 or whether they are equivalent). SLs influence mycorrhizal dynamics by inducing fungal spore germination and hyphal elongation (Lanfranco et al. 2018), thereby implicating themselves in the modulation of mycorrhizae. However, in rice, it is not the SL receptor protein D14 that is tied to mycorrhizal colonization but rather D14L (the karrikin receptor) that is essential for mycorrhizal colonization to occur (Gutjahr et al. 2015). Whether maize, as another cereal crop like rice, will have the same phenotype in *d14* and *d14l* mutants remains to be seen. The duplication of D14 and D14L genes in maize in particular raises many other questions about what kind of phenotypes may be seen in mutants of these genes.

Research has indicated that mycorrhizae impacts traits such as plant yield, pollen production, and disease resilience (Daft and Okusanya 1973; Olawuyi et al. 2014; Ramírez-Flores et al. 2020), but discerning how these connections are determined mechanistically through SL-mediated means remains unclear. The mutant stocks generated in my thesis will enable various types of experiments that can help answer these questions about mycorrhizae while focusing on the influence SLs play on these characteristics. Prior research has implicated SLs in the regulation of flowering

time by means of crosstalk with melatonin (Zhang et al. 2019); *d14* mutants in maize may therefore also exhibit earlier flowering, but whether both copies will exhibit the same phenotype remains to be seen. Additional research has implicated SLs with other plant compounds such as nitric oxide (Kolbert 2019), but the role of D14 in this interaction remains unclear. Research has defined a common symbiosis signaling pathway (CSSP), in which a conserved set of proteins, signals, and compounds are responsible for symbiotic relationships across various genera of plant species with beneficial microbes, fungi, and others (Genre and Russo 2016). Understanding the role of SLs in this CSSP can also be informative of the pathways and processes in which other compounds influence plant-soil communities. Coumarins, compounds that facilitate iron uptake in soil, have recently been dubbed ‘new kids on the block’ for their identification in impacting soil microbiome dynamics (Stassen et al. 2021); analogies and comparisons between coumarins and SLs can help determine the specific niches that these two similar yet distinct categories of compounds play in plant-soil dynamics. By using the mutant stocks generated in my thesis, we can facilitate understanding how SLs tie together mycorrhizae with phenotypic performance.

Exploring belowground dynamics opens new avenues to improve, increase, and interpret modern day agricultural practices

As environmental stresses increase, the pressure to sustain yield has pushed plant science research to explore unprecedented avenues in order to meet demands. Studying belowground dynamics in crop systems provides a unique and expansive field in which farmers, growers, and scientists can learn more about how agricultural systems currently behave and leverage this information to improve and increase crop yield. The mapping populations outlined here provide a valuable resource to evaluate plant performance in response to mycorrhizae and can be informative in advancing the goal of increasing and optimizing crop yield. Discerning the various components and compounds responsible for the intricate communications and dynamics of plant-soil communities will require a herculean effort and begins with understanding the basic building blocks for such complex interactions. Much remains to be learnt concerning SL signaling, from discerning synthesis/perception pathways to understanding its

downstream effects. The reverse-genetics mutants I have generated will enable studies which can help elucidate these effects. Teasing apart the intricate dynamics and network of SLs in relation to other hormone crosstalk will confer a more holistic understanding of plant systems as well as plant-microbe interactions within a larger biological community. The material generated in my thesis can help scientists understand the mechanisms by which AMF symbiosis influences plant performance and how this process is regulated in plant systems.

Appendix A: PCR Genotyping Additional Information

All PCR genotyping uses the following PCR cocktail mix and is run at 35 cycles:

	x1
DNA	1 uL
5x GC buffer	5 uL
DMSO	2.5 uL
Betaine (5M)	2.5 uL
dNTPs (10 mM)	2 uL
Primer1 (50 uM)	0.25 uL
Primer2 (50 uM)	0.25 uL
H20	11.38 uL
Phusion	0.12 uL
Total Volume:	25 uL

1. CASTOR
    a. 98° 30 sec
    b. 98° 15 sec
    c. 65° 20 sec
    d. 72° 30 sec
    e. 72° 5 min
    f. Hold at 10°
2. POLLUX
    a. 94° 3 min
    b. 94° 30 sec
    c. 50° 30 sec
    d. 72° 30 sec
    e. 72° 10 min
    f. Hold at 10°
3. D14 (Chr1)
    a. 98° 30 sec
    b. 98° 15 sec
    c. 64° 20 sec
    d. 72° 45 sec

   e. 72° 5 min
   f. Hold at 10°

4. D14 (Chr9)
   a. 98° 30 sec
   b. 98° 15 sec
   c. 64.5° 20 sec
   d. 72° 30 sec
   e. 72° 5 min
   f. Hold at 10°
5. D14-Like (Chr1) *not yet confirmed
   a. 98° 30 sec
   b. 98° 15 sec
   c. 64° 20 sec
   d. 72° 45 sec
   e. 72° 5 min
   f. Hold at 10°
6. D14-Like (Chr9)
   a. 98° 30 sec
   b. 98° 15 sec
   c. 66° 20 sec
   d. 72° 30 sec
   e. 72° 5 min
   f. Hold at 10°
7. D3
   a. 98° 30 sec
   b. 98° 15 sec
   c. 58° 20 sec
   d. 72° 45 sec
   e. 72° 5 min
   f. Hold at 10°
8. CCD8

a. 94° 3 min
b. 94° 30 sec
c. 61° 20 sec
d. 72° 30 sec
e. 72° 5 min
f. Hold at 10°

Primers Used for Genotyping by PCR:

1. CASTOR (Zm00001d012863)
   F: 5’ CGCGAAGAAACGCAGACATTCC 3’
   R: 5’ TAACCTGGAGCGAACAGAATCCAC 3’
   Mu: 5’ CGCCTCCATTTCGTCGAATCCSCTT 3’
2. POLLUX (Zm00001d042694)
   F: 5’ AAGTCGCGCACCATCTCT 3’
   R: 5’ GTGAGAGATACGGAGAGAGATCA 3’
   Ds: 5’ TCATATTTAACTTGCGGGACG 3’
3. D14 (Chr1) (Zm00001d028094)
   F: 5’ AGCTCTTCGCCAAGCTCGTACTCATC 3’
   R: 5’ GATGCAAAGGTTAGGTGGCACGGTCAA 3’
   Mu: 5’ CGCCTCCATTTCGTCGAATCCSCTT 3’
4. D14 (Chr9) (Zm00001d048146)
   TUSC:
   F: 5’ AGGTTCCTGAACGACCACGACTACCA 3’
   R: 5’ GGGTAAATCGAGGCGGTAAAAACAG 3’
   Mu: 5’ CGCCTCCATTTCGTCGAATCCSCTT 3’
   UniformMu:
   F: 5’ CAACCCGGAGCACTTCGACT 3’
   R: 5’ GTAACATGGCGTGATCGGATT 3’
   Mu: 5’ CGCCTCCATTTCGTCGAATCCSCTT 3’
5. D14L (Chr1) (Zm00001d029590)
   F: 5’ ACCAATTCAGCTCTGTCTTTGACC 3’

R: 5’ GTCTTGGAGGAACGATTGACCAAC 3’

Mu: 5’ CGCCTCCATTTCGTCGAATCCSCTT 3’

6. D14L (Chr9) (Zm00001d047335)

   F: 5’ GCCCGCGTACCATTTGCAATTCGCAA 3’

   R: 5’ TCCGTACCGGTACCTTCTAGTGCCTA 3 ‘

   Mu: 5’ CGCCTCCATTTCGTCGAATCCSCTT 3’

7. D3 (Zm00001d045563)

   F: 5’ CGTTTCTCTTCCTCAGGCCGACTTTC 3’

   R: 5’ TTCATCTTGGCCAGCACTGGGAAACCT 3’

   Mu: 5’ CGCCTCCATTTCGTCGAATCCSCTT 3’

8. CCD8 (Zm00001d043442)

   F: 5’ CGATCATCGCCGACTGCTGCGAG 3’

   R: 5’ GGGTTGATGCTGCACATGTCCAT 3’

   Ds: 5’ CCGGTTCCCGTCCGATTTCG 3’

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