5-13-2019

The Effects of Meloidogyne incognita on JAZ1, JAZ12, and JAZ13 Gene Expression in Arabidopsis

Emily Green
Bridgewater State University

The Effects of *Meloidogyne incognita* on JAZ1, JAZ12, and JAZ13 Gene Expression in *Arabidopsis*

Emily Jordan Green

Submitted in Partial Completion of the

Requirements for Commonwealth Honors in Biology

Bridgewater State University

May 13, 2019

Dr. Heather Marella, Thesis Advisor

Dr. M. Caitlin Fisher-Reid, Committee Member

Dr. Kenneth Adams, Committee Member

Abstract

Meloidogyne incognita, a root-knot nematode (RKN), poses a threat to the well-being of the global agricultural industry. These endoparasites rely on plant roots and drain nutrients from crops intended for human or livestock consumption. Root knot nematodes cost the agricultural industry billions of dollars in lost crops each year and threaten efforts to meet the growing demand for food. Though plants are susceptible to dangers, such as these parasites, many have evolved defense and/or repair mechanisms to compensate for this vulnerability. The goal of this research is to study one gene family involved in such defenses. Specifically, I studied the effects of root-knot nematodes on JAZ gene expression in *Arabidopsis thaliana.* JAZ genes are the focus of this study due to their role as transcriptional regulators; however, it is uncertain if each gene has a distinct role or if they are interchangeable. To date, 13 JAZ genes have been identified in *Arabidopsis* and I focused on the roles of JAZ 1, 12, and 13 during root-knot nematode infection. In order to look at expression and localization of JAZ1 and JAZ12, I used promoter GUS lines to observe staining in the roots during nematode infection between six hours and twenty-seven days post infection. Staining was variable between transgenic lines and time points, though JAZ1::GUS A2 showed to be the most sensitive to RKN infection. To identify *jaz1* mutant plants, I conducted PCR and visualized products via gel electrophoresis to ensure T-DNA insertion in the gene was successful. I then isolated a *jaz1* mutant and tested it for nematode infection. Mutant seedlings were inoculated with RKN eggs and I compared the number of egg masses between the mutant and Columbia wild-type after 8 weeks of incubation. There was no significant difference between wild-type and *jaz1* seedlings. This was followed by qPCR on JAZ 1, JAZ12, and JAZ13 genes to test their expression during nematode infection in both uninfected portions roots and root knots over various stages of infection. These results indicate that expression of JAZ1 and JAZ12 is highest around 13-14 days of infection, suggesting these two genes may work in combination to attempt to rid the host of the pathogen. These results differ from JAZ13 expression, which peaked at three weeks of infection, indicating its role takes place after JAZ1 and JAZ12 expression has peaked. The goal of this research is to contribute to existing JAZ/RKN data and eventually exploit host defense pathways, so that the agricultural industry may reduce nematicide use and better fulfill food demands of our global population.

Introduction

Due to their sedentary nature, plant defense mechanisms differ from those of mobile organisms. While some plants have physical defense characteristics, such as thorns, most are limited to chemical mechanisms regulated through hormones. These hormones include jasmonic acid (JA), ethylene, and salicylic acid, all of which defend plants from pathogens. Specifically, JA and ethylene defend plants from insects, fungi and parasites. JA is a lipid derived hormone, essential for growth, development, and defense and is found in many species of plants (Chini et al., 2007; Nahar, Kyndt, De Vleesschauwer, Höfte, & Gheysen, 2011). It is the main defense hormone against parasites. JA also regulates many additional processes, such as seed germination, stamen development, and senescence (Wasternack & Song, 2016).

JA and its jasmonate derivatives are formed in the membranes of chloroplasts and are members of the oxylipin family (Wasternack & Song, 2016). They are synthesized via the lipoxygenase pathway from α-linolenic acid (α-LeA). Galactolipids release α-LeA by phospholipase A1 SN1 hydrolysis (Ishiguro, Kawai-Oda, Ueda, Nishida, & Okada, 2001). The stereochemistry of the intermediates and products is a critical component in JA synthesis. In the allene oxidase cyclase portion of the reaction, the enantiomeric form on 7C is established to form the *cis*-(+)-7-*iso*-JA. This is a precursor for the (+)-7-*iso*-JA-Ile isoleucine conjugate required for JA signaling (Fonseca et al. 2009). (+)-7-*iso*-JA-Ile promotes CORONATINE INSENSITIVE1 (COI1) interaction with JAZ1, a member of the JASMONATE ZIM-domain (JAZ) family. In *Arabidopsis*, JAZ is a 13-member family classified under the TIFY family involved in plant hormonal signal transduction pathways (Howe, Major, & Kloo, 2018; Ikeda et al. 2001; Sasaki et al. 2003; Ulmasov, Hagan, & Guilfoyle, 1999;). As TIFY proteins, JAZ act as transcriptional repressors of JA-induced genes. NOVEL INTERACTOR OF JAZ (NINJA) and TOPLESS

(TPL) are bound to JAZ and this complex is degraded by the ubiquitin proteasome pathway via Skp1-Cullin-F-box (SCF)-type E3 ubiquitin ligases (Chini et al., 2007; Dharmasiri, Dharmasiri, Jones, & Estelle, 2003; Kepinski & Leyser 2005; Pauwels & Goosens, 2011; Thines et al., 2007). Specifically, the F-box protein, COI1 ubiquitinates JAZ (Chini et al., 2007; Thines et al., 2007).

The JAZ family plays a role in a variety of plant functions. JAZ1 is responsible for embryogenesis, formation of somatic embryos, male fertility, and resistance to pathogenic fungi (Figueroa & Browse, 2015; Grunewald et al., 2009; Jiang & Yu., 2014; Mira et al., 2016). JAZ2 is responsible for regulation of defense metabolites (Yan et al., 2014). JAZ3 plays a role in insect resistance, root growth inhibition, growth of lateral organs, and activity in the stem apical meristem (Boter et al., 2015; Valenzuela et al., 2016; Moa et al., 2017). JAZ4 is responsible for senescence and cold stress responses, while JAZ5 and JAZ10 control pathogen growth (de Torres Zabala et al., 2016; Hu, Jiang, Wang, & Yu, 2013; Jiang & Yu, 2014). JAZ6 is responsible for rhythmic susceptibility in plant defense, while JAZ7 controls root length, senescence, flowering, and pathogen defense (Ingle et al., 2015; Thatcher et al., 2016; Yan et al., 2014). The functions of JAZ8, JAZ9, JAZ11, JAZ12, and JAZ13 are not yet known, though Smith (2017) suggests JAZ12 is isolated in root tips and knots.

According to Pauwels & Goosens (2011), JAZ proteins block MYC2 activity. MYC2 is a basic helix-loop-helix Leu zipper transcription factor that differentially regulates JA-responsive pathogen defense and wound response (Dombrecht et al., 2007). TPL and NINJA work in conjunction to block MYC2 and are released by JA-Ile-induced COI1 ubiquitinating JAZ after binging to the S-L-X(2)-F-X(2)-K-R-X(2)-R C-terminal region (Fig. 1; Pauwels & Goosens, 2011; Yan et al., 2007). After the release of the JAZ complex (Fig. 1), MYC2 recruits RNA polymerase to transcribe the JA target gene (Pauwels & Goosens, 2011). The JA-associated (Jas)

COI1 binding domain amino acid residues differ between JAZ members, thus, cooperativity differs (Chung, Phillips, & Vierstra, 2010). The Jas COI1 binding domain is present in all JAZ members; however, JAZ7 and JAZ8 lack conservation at residues 205 and 206 essential for COI1 binding (Chung et al., 2010). A TIFY domain, containing a ZML [ZIM (Zinc-finger like protein inflorescence meristem)] or an additional Jas domain, follows the initial Jas domain to recruit NINJA and TPL (Vanholme et al., 2007; Bai, Meng, Huanga, Qib, & Chen, 2011).

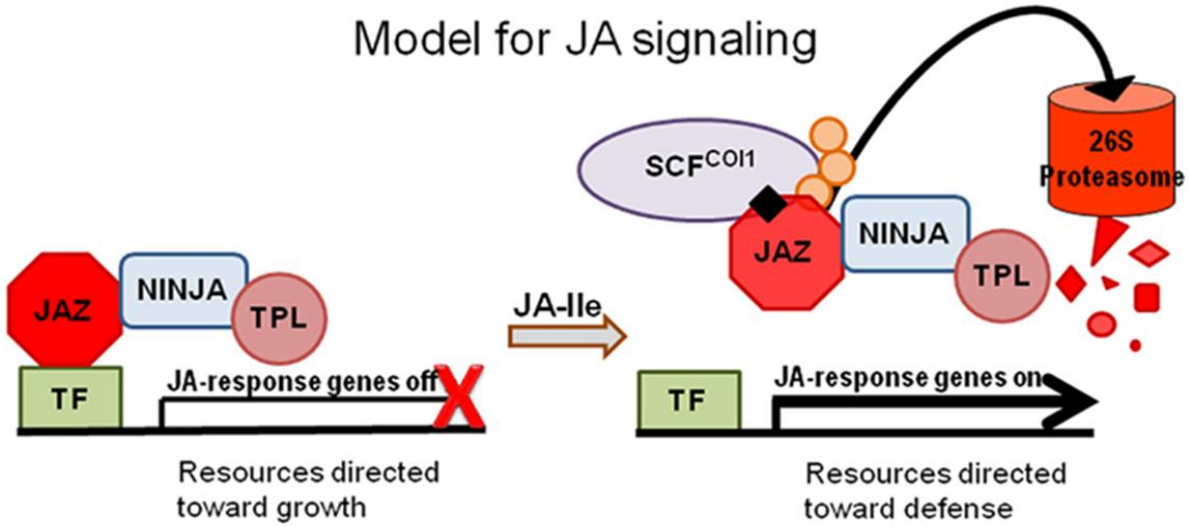


Figure 1: JAZ is a Transcriptional Repressor for Jasmonic Acid Response Genes
In this figure, JAZ proteins block the JA response gene transcription factor on the left. Due to the transcription being blocked, plant resources are directed to growth and reproduction. NINJA and TOPLESS are bound to JAZ. The JA-Ile signals for SCF^{COI1} to bind JAZ at the SCF^{COI1} S-L-X(2)-F-X(2)-K-R-X(2)-R C-terminal region binding domain, as shown on the right. This triggers ubiquitination, shown by the three orange circles on JAZ, and degradation by the 26S proteasome. The amino acid components that make up JAZ is represented by the variety of red shapes below the 26S proteasome. JAZ degradation allows the JA-response genes to be readily transcribed, and the product plays a role in plant defense. Under times of stress, such as parasitic attack, this mechanism occurs so more resources are allocated towards defense (Wager & Browse, 2012).

Nematodes are just one of many organisms that trigger plant defense hormones like JA. Over 4100 species of plant-parasitic nematodes have been identified and pose a significant annual threat to food security and the global agricultural industry, as they have spread worldwide and are typically host nonspecific (Jones et al., 2013). As of 2011, global annual crop loss due to nematodes is estimated at US $80 billion (Nicol et al., 2011). This is a conservative estimate, as

those growing crops in developing countries may be unaware of parasitic nematodes presence in the soil. Additionally, the use of nematicide adds to this cost (Bird & Kaloshian, 2003).

Meloidogyne, commonly referred to as root-knot nematodes (RKNs), is just one of many examples of parasitic nematodes (Curtis, Robinson, & Perry, 2009). This genus consists of 98 species and is named for the galls or "root knots" they cause their host to develop. Like most plant-parasitic nematodes, *Meloidogyne* are non-host specific endoparasites dependent on vascular plants, and *Meloidogyne incognita* alone can infect over 2000 plant species (Curtis et al., 2009). *M. incognita* infects their hosts at the root tip in the second juvenile stage (J2) in their lifecycle (Fig. 2; Curtis et al., 2009). The J2s use a combination of cellulolytic and pectolytic enzymes and stylet thrusting to break down the cell walls of host roots. Typically, this penetration is done directly behind the root cap; however, *M. incognita* is not entry site specific. Sixty-one host cell wall-degrading, carbohydrate-active enzymes are used by *M. incognita* (Abad et al., 2008). These enzymes consist of cellulases, xylanases, polygalacturonases, pectate lyases, arabinases, and invertases, which together are used to degrade cellulose, hemi-cellulose and pectin. The proteins that bind cell wall components are adapted to enable or accelerate cell wall digestion. *M. incognita* use these enzymes to enter plant roots because more pliable cell wells allow easy movement between plant cells.

Once the J2s enter the hosts' roots, they travel intercellularly to the root tips. At the root tip, they then make a 180° turn at the apical meristematic region and move up the stele (Fig. 2; Curtis et al., 2009). Upon reaching the zone of differentiation, the J2s establish permanent feeding sites, called giant cells. Root cells become giant cells after the J2s inject substances produced in their dorsal and subventral pharyngeal glands into several surrounding root cells (Curtis et al., 2009). Giant cells are much larger than normal plant cells and typically contain

over 100 nuclei. These giant cells are embedded in proliferated tissue, creating the root knot (Fig. 2; Gheysen & Mitchum, 2011). Organelles of giant cells are altered, specifically the mitochondria and ribosomes, increasing overall metabolic rate (Castagnone-Sereno, Danchin, Perfus-Barbeoch, & Abad, 2013). The root knots allow the now stationary J2s to continue their life cycle by providing a secure nutrient source and causes a nutritional sink for the host (Fig. 2; Gheysen & Mitchum, 2011).

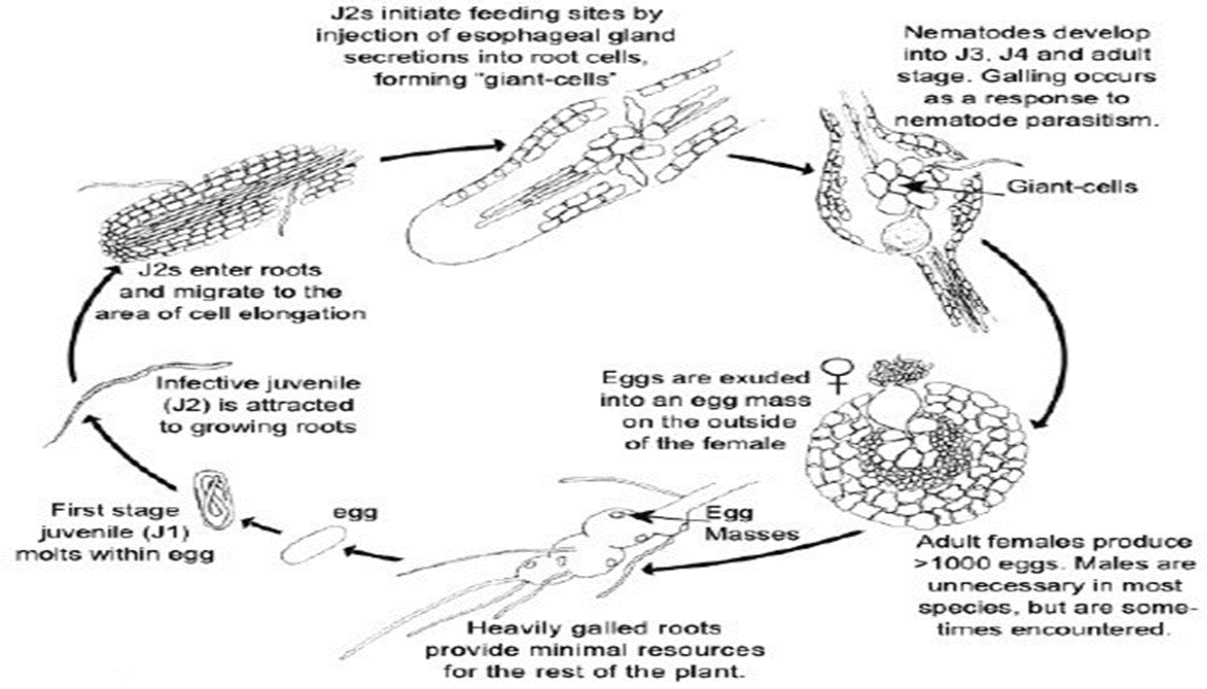


Figure 2: The *M. incognita* Life Cycle with a Generic Vascular Plant Host
This image shows how *M. incognita* infects and reproduces as a vascular plant root parasite, moving clockwise. The top, center portion shows *M. incognita* in the J2 stage, injecting enzymes from their esophageal glands into plant root cells. This initiates giant cells, which have altered metabolic functions that feed nematodes and is a nutritional sink for the host. The next stage shows the that the J2s matured into J3s and then into J4s. This is followed by the adult stage, which occurs after two moltings. The giant cells continue to swell, and this area creates a noticeable gall on the roots. The next portion shows that the majority of adults are female and reproduce asexually by excreting egg masses on the outside of the roots. This image also notes that males are sometimes encountered, but unnecessary. In *Meloidogyne incognita*, J2s will mature into males in times of stress. These males leave the host and will die, leaving more resources to reproductive females. The next portion in the bottom center shows the egg masses positioned on the galls and notes that very few resources are left for the host. The next portion focuses on the egg or J1 phase, which hatch, releasing J2s. The J2s entering the host root is shown in the following portion (top left). The J2's may infect the same or nearby hosts' root, where they travel to the area of cell elongation and will restart this life cycle by inducing giant cell formation (Hyer, Jorgenson, Garber, & Smith, 1979).

In addition to the increased allocation of photosynthetic products and nutrients being transported from shoots and consumed by giant cells, amino acid transporter gene expression is altered under parasitic nematode infection (Bird & Loveys, 1975; Hammes et al., 2005; McClure, 1977). This alteration increases nematode essential amino acid levels (Marella, Nielsen, Schachtman, & Taylor, 2013) as well as levels of aquaporins, sucrose transporters and ATPases by allocating them to the giant cells and reducing nutrient transport to developing seeds (Harrington et al., 1997; Tegeder, Wang, Frommer, & Patrick, 2000; Wang, Offler, & Patrick, 1995). The transfer of these nutrients and transporters decreases carbon, ion, sugar, and phosphate exchange between host roots and their symbionts (Flemetakis et al., 2003; Harrison, Dewbre, & Liu, 2002; Rausch et al., 2001; Tyerman, Whitehead, & Day, 1995). Hammes et al. (2005) found expression differences in 50 transport proteins in *Arabidopsis* plants under *M. incognita* infection.

After J2s establish this feeding site, they undergo two molting cycles before entering the adult, female reproductive stage (Fig. 2; Castagnone-Sereno et al., 2013). Due to their reproduction being asexual, male RKNs are rare and typically only occur in stressful environments. These males leave the root and die, and more nutrients are allocated to the remaining reproductive females (Davide & Triantaphyllou, 1967; Davide & Triantaphyllou, 1968; Snyder, Opperman, & Bird, 2006; Jones et al., 2013). Female adults lay egg masses through mitotic parthenogenesis outside the root knots (Fig. 2; Jones et al., 2013; Triantaphyllou, 1981, and Triantaphyllou, 1985). These egg masses contain hundreds of eggs, which will hatch to re-infect the same root or migrate to neighboring plant roots, so the cycle may continue.

In 2007, 133 million pounds of nematicide active ingredients were added to United States' crops (Grube, Donaldson, Kiely, & Wu, 2011). Nematicide as a long-term solution is not

feasible because these highly volatile, broad-spectrum chemicals pose negative impacts on mammalian, avian, and environmental health (Sasser & Freckman, 1987). What were once commonly used ingredients in nematicides have been banned in various countries because of these negative impacts (Bird & Kaloshian, 2003). Many effective fumigants, including 1, 2-dichloropropane, 1, 3-dichloropropene, ethylene dibromide, and 1, 2-dibromo-3-chloropropane are no longer used as nematicides because of ground water contamination and acute toxicity (Aspelin & Grube, 1999). Additionally, ingredients in nematicides disrupt host-symbiont relationships. Methyl bromide and aldicarb, which are carbamate insecticides, reduce mycorrhizae and rhizobacteria populations, which reduces overall plant growth. Due to the richness of the Nematoda phylum, these nematicides, while effective on some species, are not effective on others. Until safer and more effective nematode management chemicals are developed, exploiting host resistance and defense remains the best tactic to combat nematode parasitism (Bird & Kaloshian, 2003).

Though it is not fully understood, there is evidence that JA plays a significant role in defense against *M. incognita* and its expression may be altered due to the exoenzymes *M. incognita* inject into giant cells (Zhou et al., 2019). Through understanding the role of all 13 JAZ genes and other additional defense hormone pathways, we may eventually be able exploit internal defense mechanisms against plant parasites. By doing so, fewer crops will be lost because of parasites like RKNs, which could increase farmers' profits and the social welfare of growing human and livestock populations. According to Gheysen & Mitchum (2011), it is likely that JAZ proteins play a role in the manipulated jasmonic acid pathway. The purpose of this research is to contribute to the pool of JA data so that we may eventually exploit host resistance

to plant hormone-altering nematodes. Exploitation of these genes, such as JAZ1, JAZ12, and JAZ13 genes, may allow us to reduce pesticide usage in agricultural crops.

Materials and Methods

Arabidopsis Media

Twenty grams of D-sucrose, three grams of Gamborg Basal Salt Mixture, and one liter of ultra-pure water were combined in a two-liter glass flask. I adjusted the pH to 6.1 +/- 0.05 with KOH while continuously mixing to ensure even distribution of the reagents throughout the media. I added six grams of Phytagel and thoroughly mixed the solution before autoclaving for 20 minutes on liquid cycle. Twenty-five mL of liquid medium was pipetted into each petri dish in a laminar flow hood to prevent contamination. The agar solidified on a flat surface and then was chilled until it was used.

Arabidopsis Seed Sterilization and Plating onto Media

Seeds in 0.1 mL aliquots soaked in 1 mL of water for approximately 10 minutes before being brought into a laminar flow hood. In the laminar flow hood, the 1mL of water was discarded and 1 mL of 95% ethanol solution was added and mixed. The seeds soaked in the ethanol for five minutes before it was poured off. I added 1 mL of 10% bleach solution to the seeds, mixed, and poured off the solution after soaking for five minutes. One mL of sterile water was added, mixed, and poured off. This rinse step was repeated three times for a total of four. I resuspended the seeds in 1 mL of sterile water and pipetted approximately 0.5 mL seed/water onto each agar plate in a straight line. The plates remained level until the water soaked into the medium and the seeds adhered. The plates were then wrapped in parafilm and incubated under 8 hours day/16 hours night at 23°C and rested at an approximate 45° angle until root systems were established. Seedlings were transferred in fives to the center of fresh plates in a horizontal line

for GUS staining and counting egg masses. The plates were wrapped in parafilm and incubated under the same conditions as described until use.

GUS Staining

β-glucuronidase (GUS) staining is a reporter gene system that allows visualization of specific gene family members (Jefferson et al. 1987). This assay allows one to study the expression of individual genes separate from the other members of the gene family. The GUS enzyme cleaves 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc). The blue product is present in cells where this cleavage occurs, thus they express the gene of interest. JAZ1 promoter::GUS and JAZ12 promoter::GUS transgenic jaz1 A1, jaz1 A2, jaz1 C, jaz12 B1, and jaz12 B2 lines underwent GUS staining to visualize and localize JAZ1 and JAZ12 promoter activity. Each plate was inoculated with 500 *M. incognita* J2s, per Marella et al. (2013). After being infected with RKN for nine, twenty, and twenty-seven days, inoculated seedlings and uninoculated controls were removed from the medium and placed in empty petri dishes. I submerged the seedlings in GUS staining solution (Jefferson, 1987) overnight and decolored the seedlings in ethanol until the chlorophyll was removed. I repeated GUS staining assay for jaz1 A2, jaz1 C, and jaz12 B2 seedlings on the growth media to eliminate transfer-induced GUS expression. These seedlings were stained after six, twenty-four, and forty-eight hours, and seven days post inoculation. Due to limited access of transgenic seeds, JAZ1 promoter::GUS and JAZ12 promoter::GUS transgenic jaz1 A1 and jaz12 B1 lines were omitted from the second GUS staining assay. After the plants were sufficiently blue, I submerged them in ethanol in individual wells to remove the chlorophyll. Seedlings from both assays were analyzed and photographed using the Olympus

DP25 camera mounted on the Olympus SZ61 brightfield microscope to explore the localization of JAZ1 and JAZ12 activity.

DNA Extraction and PCR

DNA was extracted with the Qiagen DNeasy Plant Mini Kit from leaves of putative *jaz1* (SALK_011957) and *jaz12* (SALK_055032) mutant *Arabidopsis* grown in soil. I conducted two PCR experiments per BioRad recommendations in the GeneAmp PCR System 9700 machine to validate T-DNA insertion and positively identify mutants with 35 cycles of 94°C for 15 seconds, 51°C for 30 seconds and 68°C for 60 seconds. This was done using New England Biolab One-Taq super mix and T-DNA LP, LBb1.3 (LB) (Alonso et al. 2003), and RP SALK primers (Table 1). Successful amplification of these genes with the LB and RP primers indicate the T-DNA was successfully inserted. Amplification in reactions with LP and RP primers indicate the gene is intact and the T-DNA was not successfully inserted. I assessed the PCR products via 1.0% agarose gel electrophoresis in 1X sodium boric acid buffer to ensure DNA extraction and amplification was successful. Results were visualized via ethidium bromide on the Spectroline TI-213E UV lamp, against 1 kb ladder. The *jaz12* PCR had to be repeated with the annealing temperature adjusted to 53°C. The *jaz13* seeds failed to germinate and unfortunately had to be omitted from this portion of this project.

Table 1: Primers Used in PCR
These primers were used in PCR to evaluate if the T-DNA was successfully inserted.

Gene	Forward Primer	Left Border Primer	Reverse Primer
JAZ1	5’-AGGTAAAATGCGGAGAGAGAGG-3’	5'-ATTTTGCCGATTTCGGAAC-3"	5’-AAGCACCGCTAATAGCTTAGC-3'
JAZ12	5’-AGTTATGGCACACTCCCATTG-3’	5'-ATTTTGCCGATTTCGGAAC-3"	5’-AGCATCAGTCCTGTCTCATCG-3’

Egg Masses

Columbia wild-type and *jaz1* mutant seedlings on media were inoculated, per Marella et al. (2013), with 1000 *M. incognita* eggs per plate. The RKNs infected *Arabidopsis* roots for eight weeks in incubation conditions described above. Due to media contamination, only eight wild-type and nine *jaz1* plates were viable. Egg masses were marked with permanent marker in the bottom side of the media under the Olympus dissection microscope. I counted and averaged the markings to evaluate if RKNs reproduce better in Columbia wild-type or *jaz1* roots.

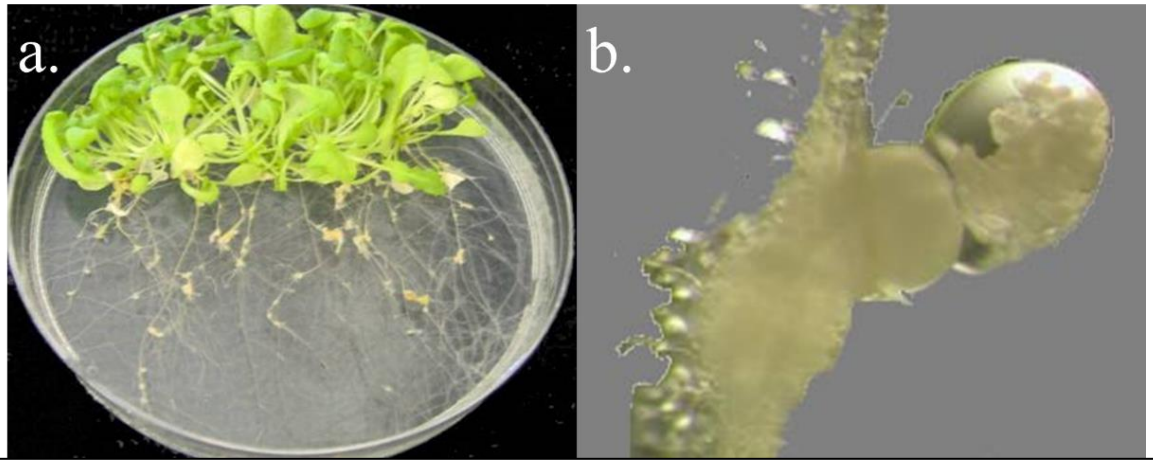


Figure 3: Egg masses on *Arabidopsis*
a. This image shows RKN infected Columbia wild-type *Arabidopsis* on complete media. The large, yellow growths on the roots are the RKN knots. These growths may be one or multiple egg masses. b. This image shows an individual RKN egg mass, containing hundreds of RKN eggs (Hammes 2005).

RNA Extraction and qPCR Analysis

Seedlings used to obtain the RNA were inoculated with 500 RKN J2s per plate, as done by Marella et al. (2013). Roots were harvested from RKN-infected and control seedlings at intervals of two, thirteen, and twenty days, inserted in Eppendorf tubes and placed in liquid nitrogen for one minute. After the roots were sufficiently frozen, they were crushed with a plastic pestle in the Eppendorf tube. A Qiagen RNeasy kit was used to extract the RNA. The RNA concentration of each sample was determined on a Nanodrop 1000 spectrophotometer.

Equivalent amounts of RNA were used for cDNA synthesis. RNA was converted to cDNA using the Invitrogen SuperScriptIII cDNA Synthesis Supermix through oligo dT as the primer using, manufacturer recommendations. Additionally, RNA was extracted from knots or uninfected portions of the same roots from RKN J2-infected *Arabidopsis* seedlings at one, two, and three weeks post-inoculation, following the same protocol described above.

Comparative qPCR was conducted in the Life Technologies' Applied Biosystem QuantiStudio 6 Flex. I diluted root and knot cDNA with sterile water in a 1:10 ratio. Primers were also diluted with sterile water to 4 µM (Table 2). Trials were run in 10µL reaction triplicates that contained 1 µL of diluted cDNA, and 9 µL of master mix. The master mix contained the diluted primers, sterile water, and BioRad Sso SYBRgreen, as per BioRad suggested protocol. I used PP2AA3 or QUBC1 as the housekeeping gene, based on sample amplification profile. The qPCR was performed for 40 cycles of 95˚C for fifteen seconds then at 60˚C for one minute on the Applied Biosystems QuantStudio 6 qPCR machine using the ΔΔCT Gene Expression method on the system software. The melt curve was obtained to verify a single product (Appendix 1). qPCR was performed with three technical replicates for each sample.

Table 2: Primers used in qPCR
These primers, with the exception of those for JAZ13, were obtained from Demianski et al. (2012). The PP2AA3 and QUBC1 genes were used as housekeeping genes. The JAZ13 primers were prepared using the Integrated DNA Technologies' PrimerQuest Design Tool.

Gene	Forward Primer	Reverse Primer
JAZ1	5'-GTCGTGGCTCGGTTTAGCAG-3'	5'-GGCTTGAGGGTGGTTAGCATT-3'
JAZ12	5'-TCTCGTTTTGCAGATCCACG-3'	5'-GCGAATGCCTCCTTGCAATA-3'
JAZ13	5'-CGTTAGGATGTTCGGTGAAGA-3'	5'-GAGAGGAGGATGATGAGGAGGTA-3'
PP2AA3	5'-AACGTGGCCAAAATGATGC-3'	5'-AACCGCTTGGTCGACTATCG-3'
QUBC1	5'-TACCTCCATCCAGTCCTTGC-3'	5'-GCTCAACAACATCACGCACT-3'

Results

GUS Staining to Visualize JAZ Gene Expression

In this project, GUS staining was used on two separate occasions to visualize and localize expression of JAZ1 and JAZ12 in transgenic lines of *Arabidopsis.* The results of the jaz1 A2, jaz1 C, and jaz12 B2 transgenic seedling GUS stain differed throughout the infection time line (Figs. 4-5). Various shades of blue were present in all except jaz1 A1 in the control (Fig. 4). At nine, twenty, and twenty-seven days post inoculation, root knots are extremely dark, despite the overall root appearing light blue at twenty-seven days. The jaz1 A2 and jaz1 C roots are also lighter than the knots, however, there is less of a contrast and more of a gradient. In the jaz1 seedlings, the blue color was observed throughout the plant. These counter the scarcity of blue observed in the jaz12 seedlings. In the jaz12 B1 seedlings, the blue is observed in small spots in knots, with a few spots throughout the roots. The jaz12 B2 images show dark knots, like the jaz1 seedlings; however, the blue is not observed throughout the entire plant and only on roots with knots.

Due to GUS expression potentially being induced by transferring plants to empty Petri dishes for staining, the GUS assay was repeated on growth media for jaz1 A2, jaz1 C, and jaz12 B2 seedlings, as jaz1 A1 and jaz12 B1 lines were unavailable. During the second GUS stain, blue product was only observed in jaz1 A2 seedlings (Fig. 5). Interestingly, it was only observed in leaves at six, twenty-four, and forty-eight hours post inoculation, and was nonuniform. Due to being inoculated only six hours prior, no knots were observed at the six-hour mark between any of the seedlings. As expected, jaz1 A2 knots were a darker blue than the surrounding portion of roots and the contrast was conserved between both assays (Figs. 4-5).

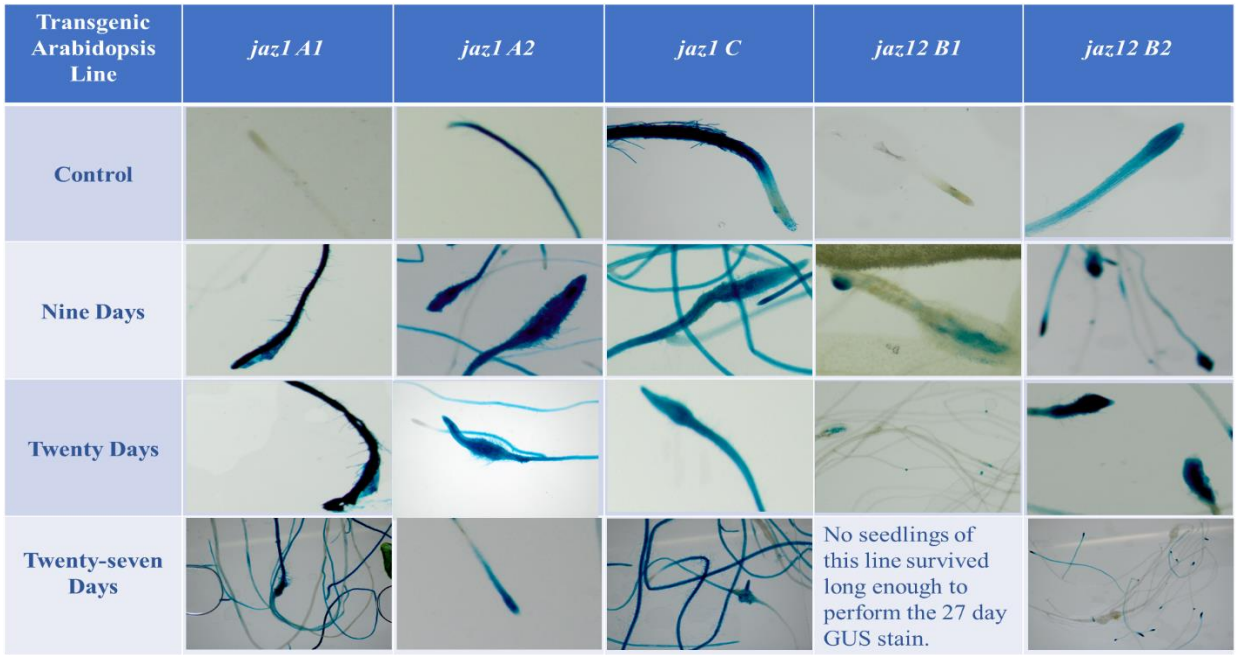


Figure 4: GUS Stain Assay at Done Seven, Nine, Twenty, and Twenty-seven Days Post Infection
The top row of this image shows uninoculated jaz1 A1, jaz1 A2, jazi1 C, jaz12 B1, and jaz12 B2 *Arabidopsis* seedlings after GUS staining as described in the methodology. Seedlings of the same mutant lines underwent the same GUS staining process as described after being infected with RKNs for seven, nine, twenty, and twenty-seven days. Observation of the individual root knots are the focus of this experiment and all lines showed various shades of blue in various plant cells. Due to the limited amount of seeds available, no jaz12 A was available to inoculate and stain for the twenty-seven-day interval.

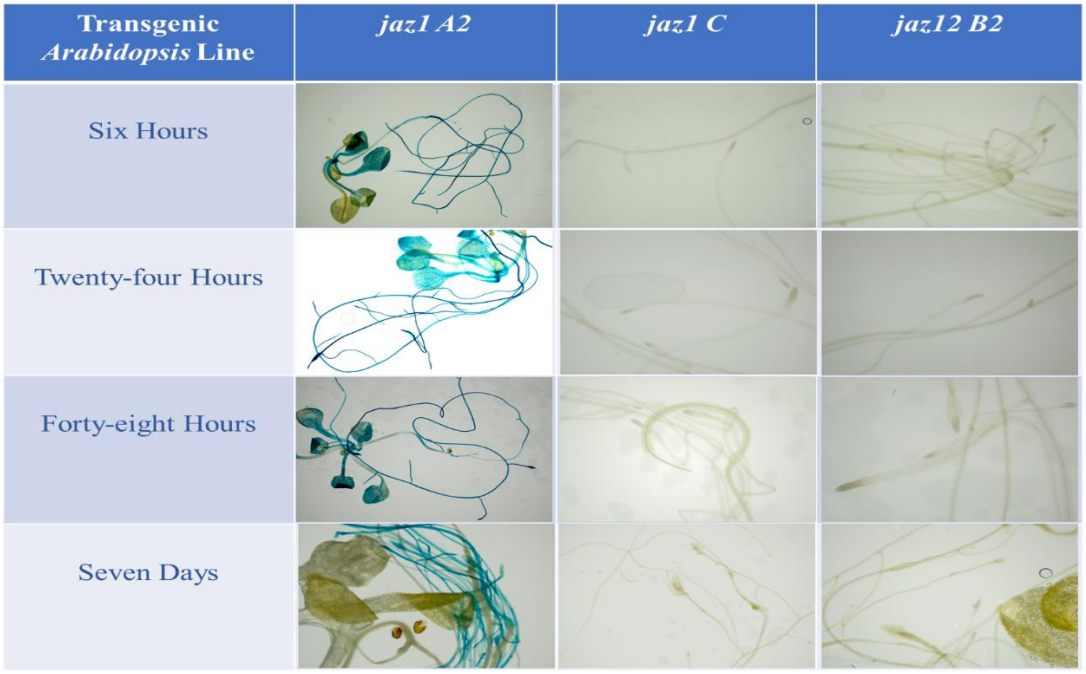


Figure 5: GUS Stain Assay 2 Performed Six, Twenty-four, and Forty-eight Hours and Seven Days Post-Infection
Only jaz1 A2, jaz1 C, and jaz12 B2 were used during the second GUS stain, as jaz1 A1 and jaz12 B1 were unavailable. These three lines were stained after being infected for six, twenty-four, and forty-eight hours and seven days. Though the focus was on the individual root knots, leaf localization was also noted can be seen in this figure.

PCR Verification of *jaz* Mutants

To generate transfer DNA (T-DNA) mutants, T-DNA must be inserted into the target gene, blocking or altering its transcription. To verify this insertion, PCR can be performed using a primer set designed to amplify the intact gene and a primer set to detect the T-DNA insertion in the gene (Table 1). T-DNA insertion was successful in *jaz1*, as shown by bands in lanes loaded with the LB primer in the first PCR analysis (Fig. 6). No bands were observed in either the wild-type or *jaz12* lanes, regardless of primer combinations, indicating that the PCR failed to amplify JAZ12. This is shown below in Figure 6 by bands in lanes that correlate with the forward and reverse primers (LP/RP) (Table 1). Samples with the left border primer of the potential T-DNA insertion and the reverse primer (LB/RP) did not amplify. Due to these results, *jaz12* PCR was

repeated to evaluate the mutant status of this seed line (Fig. 7). Bands are observed in wells 9-14, including the 1 kb DNA ladder (Fig. 7). These wells contained PCR products amplified with (LP/RP), indicating JAZ 12 was intact and not disrupted by the T-DNA.

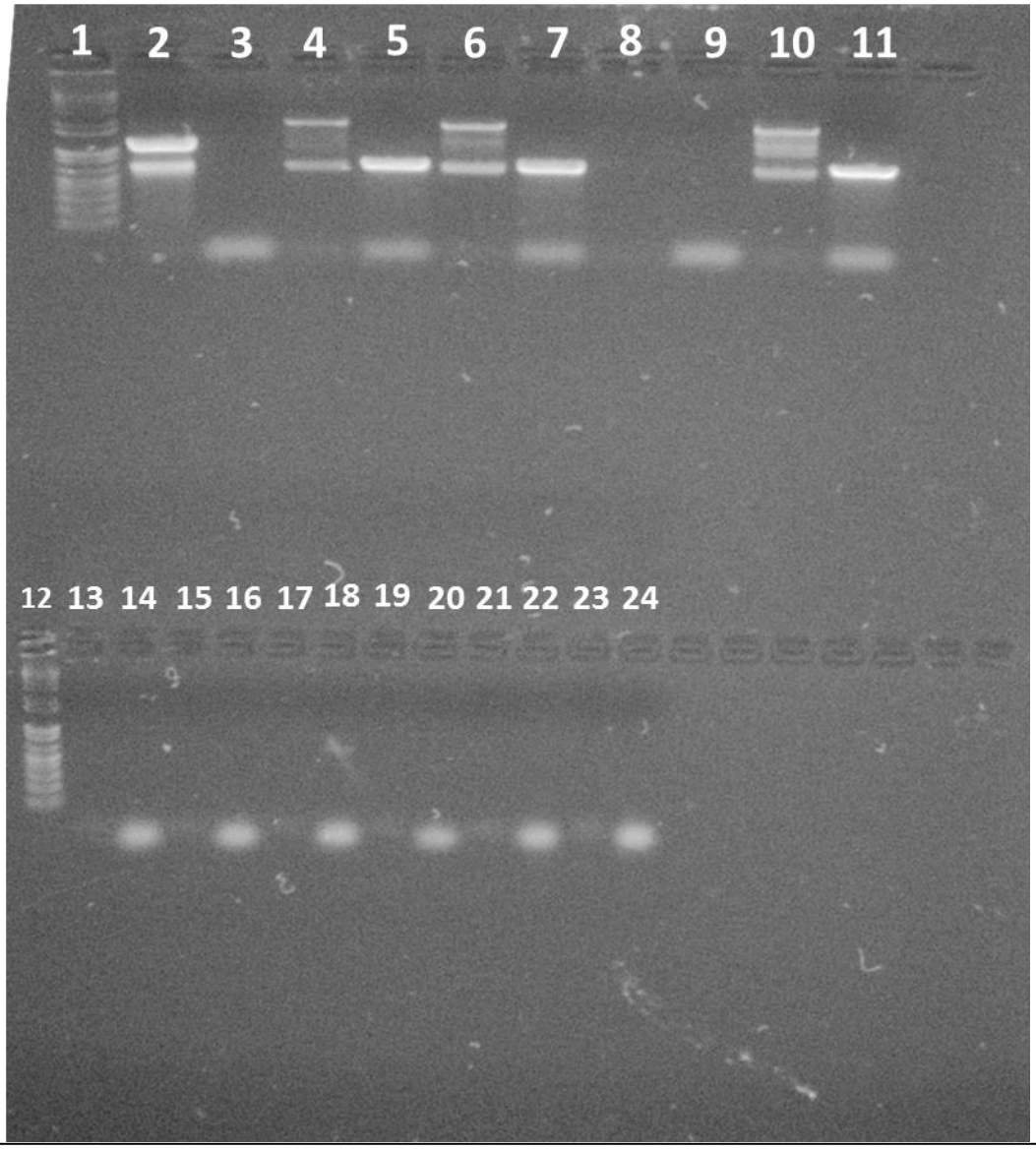


Figure 6: T-DNA Insertion PCR Visualization of *jaz1* and *jaz12*
Numbers in the figure above are correlated to wells/lanes with contents. Wells 2-11 contain the *jaz1* SALK primers and wells 13-24 contain *jaz12* SALK primers, shown in Table 1. Specific PCR products are listed below. Letters A-E represent DNA from individual and potentially mutant seedlings.

1- 1 kb Ladder **2**;- COL LP/RP; **3**- COL LB/RP; **4**- *jaz1* A LP/RP; **5**- *jaz1* A LB/RP; **6**- *jaz1* B LP/RP; **7**- *jaz1* B LB/RP; **8**- *jaz1* C LP/RP; **9**- *jaz1* C LB/RP; **10**- *jaz1* D LP/RP; **11**- *jaz1* D LB/RP; **12**-Ladder; **13**- COL LP/RP; **14**- COL LB/RP; **15**- *jaz12* A LP/RP; **16**- *jaz12* A LB/RP; **17**- *jaz12* B LP/RP; **18**- *jaz12* B LB/RP; **19**- *jaz12* C LP/RP; **20**- *jaz12* C LB/RP; **21**- *jaz12* D LP/RP; **22**- *jaz12* D LB/RP; **23**- *jaz12* E LP/RP; **24**- *jaz12* E LB/RP

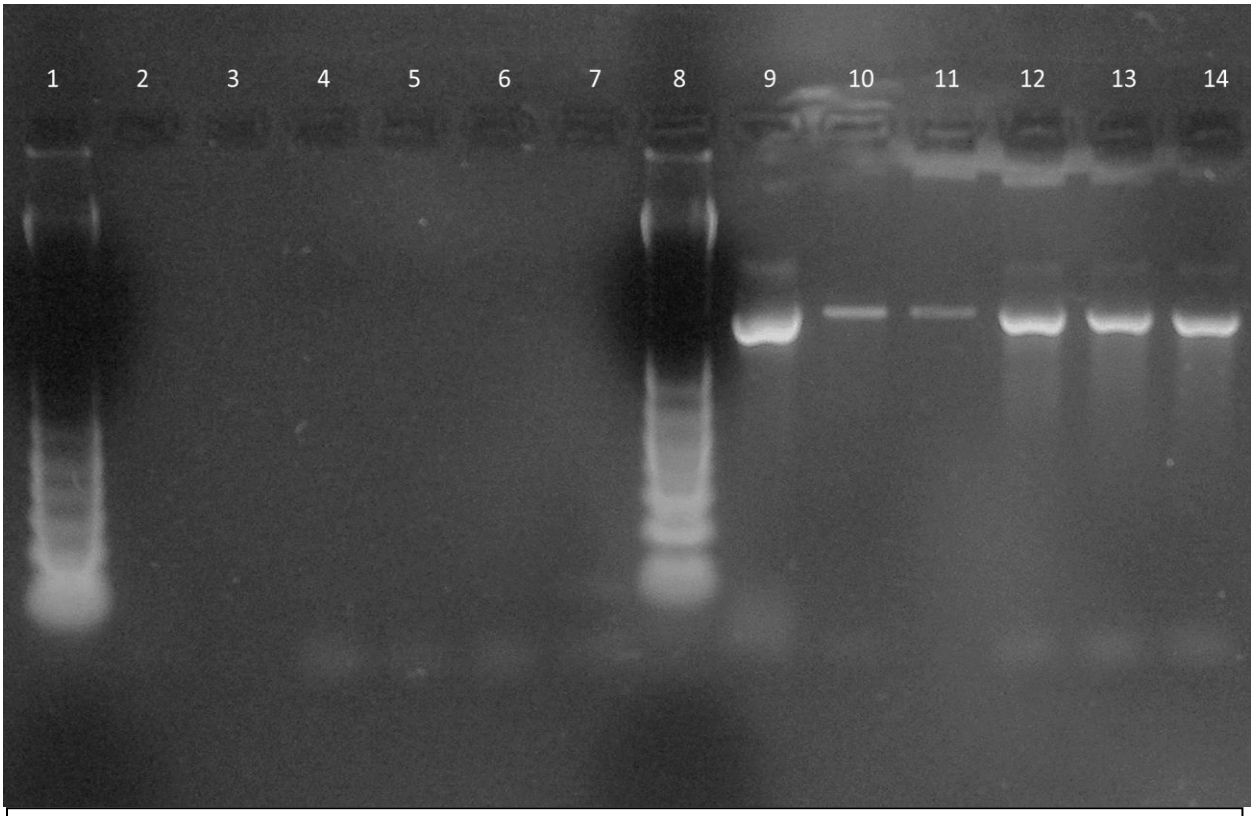


Figure 7: T-DNA Insertion PCR Visualization of *jaz12*
Numbers in the figure above are correlated to wells/lanes with jaz12 SALK primers, shown in Table 1. Specific PCR products are listed below. Letters A-E represent DNA from individual and potentially mutant seedlings.

1- 1 kb ladder; **2**- COL LB/RP; **3**- *jaz12* A LB/RP; **4**- *jaz12* B LB/RP; **5**- *jaz12* C LB/RP; **6**- *jaz12* D LB/RP; **7**- *jaz12* E LB/RP; **8**- 1 kb ladder; **9**- COL LP/RP; **10**- *jaz12* A LP/RP; **11**- *jaz12* B LP/RP; **12**- *jaz12* C LP/RP; **13**- *jaz12* D LP/RP; **14**- *jaz12* E LP/RP.

RKN Infestation is Not Altered in the *jaz1* Mutant

RKN reproduction was used to evaluate resistance of the isolated *jaz1* mutant. Comparing this resistance to that of the wild-type was done by evaluating the average number of egg masses on each plate. There was no significant difference in the number of egg masses in these two lines (Fig. 8).

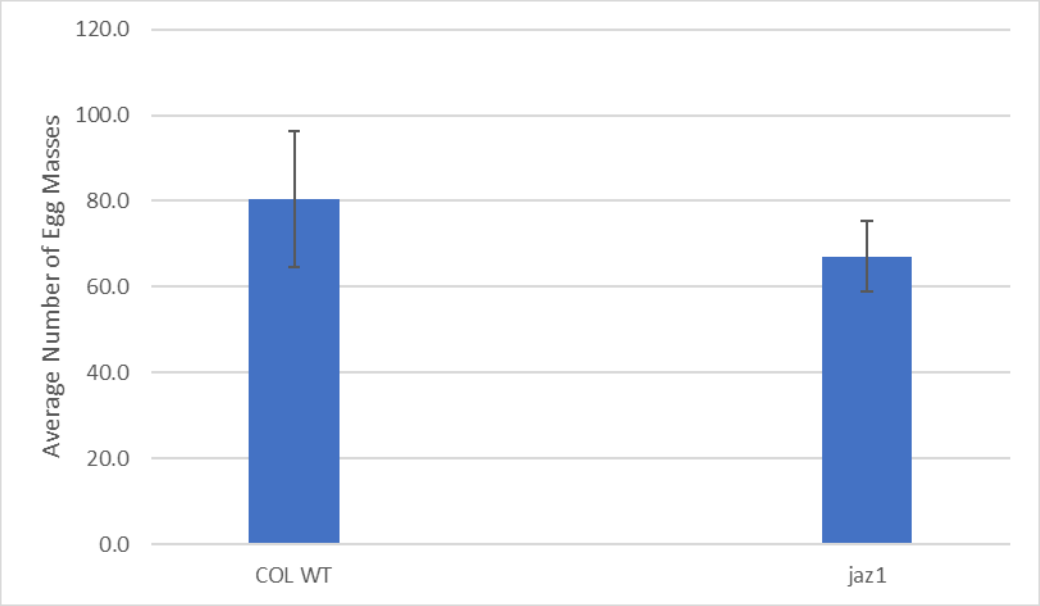


Figure 8: Comparing Average Egg Masses Between Wild-type and *jaz1 Arabidopsis*
This graph shows the mean RKN egg masses on Columbia wild-type *Arabidopsis* compared to that of *jaz1 Arabidopsis.* The error bars indicate standard deviations.

qPCR Analysis of JAZ1, 12, and 13 Gene Expression in Infected Roots

To follow up on the results of the GUS staining with JAZ1 and JAZ12, and to begin investigating the role of JAZ13, qPCR was used to monitor gene expression during RKN infection. Each qPCR analysis produced one amplicon, indicating the primers facilitated the replication of the target genes only (Appendix 1). Only cDNA constructed from knots amplified successfully for all three genes. PP2AA3 was used as housekeeping genes for JAZ1 and JAZ 12 and QUBC1 was used to normalize JAZ13.

In whole roots, JAZ12 expression levels were lower two days after inoculation than those of the uninoculated control (Fig. 9). Expression levels drop in both infected and uninfected seedlings after being infected for thirteen days compared to their respective samples at two days; however, the infected and uninfected expression levels do not differ from each other at this time. Likewise, there is a small increase of JAZ12 expression after 20 days of infection compared to

the 13-day expression levels, though there is no significance between expression in the 13- and 20-day control or the 20-day infected and control cDNA (Fig. 9).

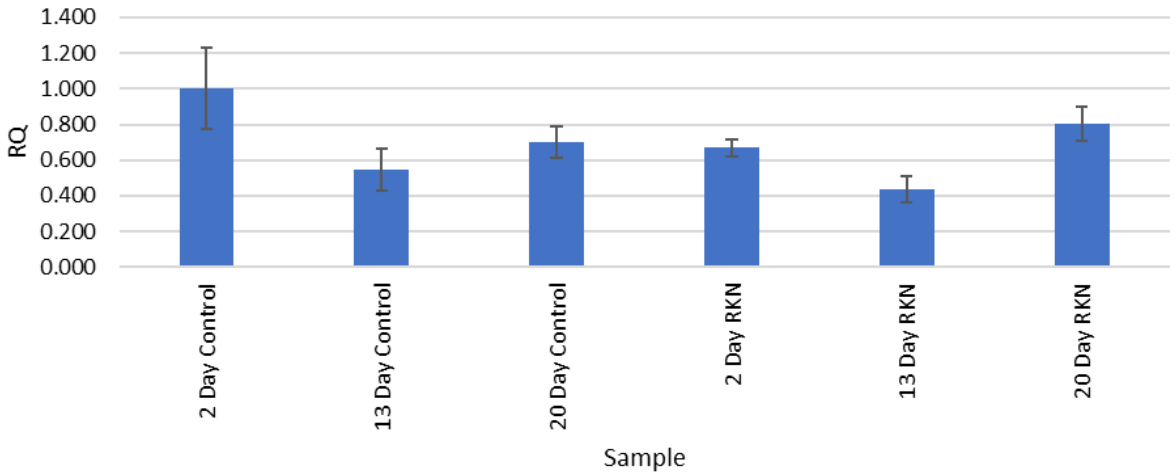


Figure 9: JAZ12 qPCR Results from Root cDNA
JAZ12 expression in roots infected for two, thirteen, and twenty days post inoculation are compared to expression in uninoculated seedlings of the same age. Error bars indicate standard deviation. RQ: relative quantity.

Due to the potential of whole roots diluting JAZ12 expression levels, qPCR was repeated using knot cDNA (Fig. 10b). JAZ1 and JAZ13 were also evaluated using knot cDNA (Figs. 10a and 10c). In both the uninfected and infected portion of the roots, JAZ1 gene expression increases in week two and decreases in week three, compared to the respective week prior (Fig. 10a). JAZ12 expression opposes this pattern in uninfected portions of the roots, as expression remains consistent over the three-week period. In knots, JAZ12 expression pattern is similar to that of JAZ1 (Figs. 10a-10b). JAZ13 expression differs from this pattern in knots, as expression decreases slightly in week two and greatly increases in week three (Fig. 10c). At two weeks, JAZ12 expression is higher in knots than in all JAZ1 and JAZ12 uninfected controls (Fig. 10a-10b). Additionally, JAZ12 expression is higher than JAZ1 and JAZ13 expression in the knots

after one week of infection (Fig. 10a-10b). Expression of both JAZ1 and JAZ12 peaks at week two (Fig. 10a-10b). These results differ from the results of JAZ13 expression (Fig. 10c). JAZ13 is only higher in knots after the host has been infected for three weeks, compared to expression in knots after one and two weeks of infection (Fig. 10c).

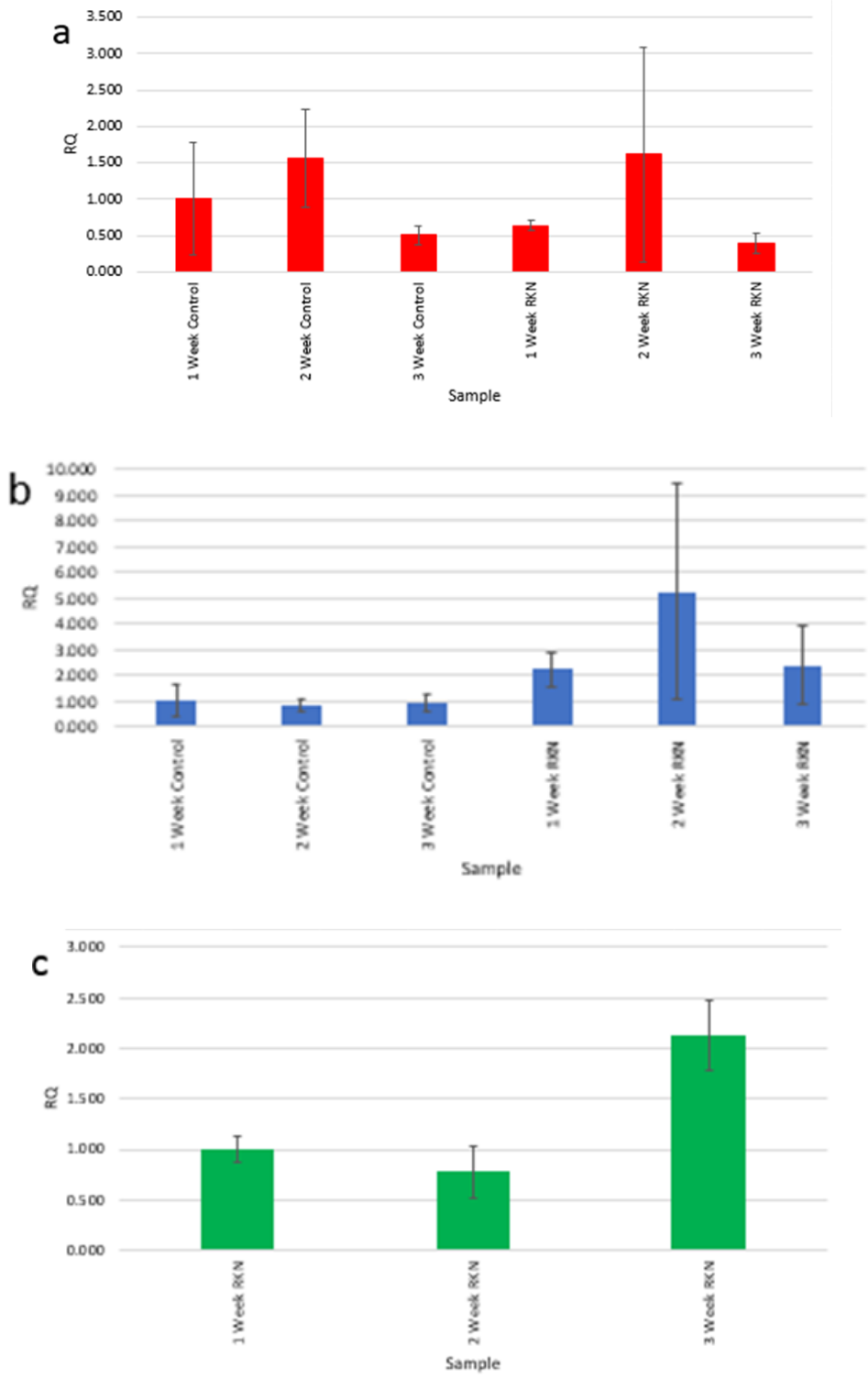


Figure 10: Relative expression levels of JAZ1, JAZ12, and JAZ13 in knots measured by qPCR
a JAZ1, b JAZ12, and c JAZ13 expression levels measured over three weeks and compared to uninfected samples. In week three, JAZ1 levels in both the control and sample were significantly lower than those of JAZ 12 and JAZ13. JAZ13 expression in week three was significantly higher than that of weeks one and two. RQ: relative quantity. Bars represent expression levels and error bars represent standard deviation.

Discussion

JAZ plays a vital role in regulating JA signaling in plants; thus, it is, at least in part, responsible for how plants combat biotic stress. Such stress can be induced by RKNs, as RKNs create nutritional sinks in plants, such as *Arabidopsis.* Though JAZ genes are of great interest to scientists, we have yet to completely understand the functions of all 13 genes and their relationship with each other in the model organism *Arabidopsis*. Additionally, RKNs alter the metabolic defense pathways in hosts during giant cell synthesis (McClure, 1977). Thines et al. (2007) studied JAZ1 and JAZ6 through GUS staining in *Arabidopsis.* Doing so, they were able to visualize and conclude that jasmonate-mediated degradation of JAZ1 and JAZ6 is dependent on COI1 function. I conducted two GUS fusion assays on a total of five JAZ1 and JAZ12 lines to visualize and localize expression after being infected for nine, twenty, and twenty-seven days (Fig. 4). All JAZ1 mutants showed a widespread, dark blue color, indicating that JAZ1 is widely expressed throughout the plant. These support Smith's (2017) findings, as a GUS staining assay was conducted on jaz1 A1 and jaz1 C transgenic *Arabidopsis* seedlings at five, seven, and eleven days post inoculation. Smith (2017) also performed this assay using a JAZ12::GUS line and found JAZ12 was mainly expressed in knots. This project supports these findings through GUS staining, as expression was localized in knots between nine- and twenty-seven-days post inoculation (Fig. 4). One confounding aspect of the first GUS stain in this project was the expression of the JAZ genes in all but the jaz1 A1 controls. It is possible that JAZ gene expression may have been induced by transferring seedlings from media to empty Petri plates for staining.

When the GUS staining assay was repeated on jaz1 A2, jaz1 C, and jaz12 B2, gene expression was only observed in jaz1 A2 (Fig. 5). This suggests that expression during the first

assay was likely greater influenced by seedling transfer, rather than RKN infection for the jaz1 A1, jaz1 C, jaz12 B1, and jaz12 B2 seedlings. This hypothesis is also supported by Smith's (2017) findings, as overlapping staining time lines yielded different results when seedlings were transferred rather than staining being performed on the media plate. Due to jaz1 A2 drastically expressing JAZ1 during every stain and every time point, I conclude that this line is sensitive to JAZ1 inducing stress and more transgenic lines should be tested to confirm the patterns observed with on-plate staining. Additionally, I suggest exploring the localization of JAZ13 through GUS.

Between PCR trials, I found amplification occurred in *jaz1* and *jaz12* samples amplified using LB/RP and LP/RP primers, respectively (Table 1). This was observed through gel electrophoresis, as bands were only present in lanes with wells containing samples amplified using LB/RP primers for *jaz1* and LP/RP primer for *jaz12* (Figs. 6-7). These results indicate the T-DNA was only successfully inserted into the *jaz1* line, making these seedlings true mutants. Additional SALK lines will be tested to isolate a *jaz12* mutant. There was no significant difference in the number of egg masses on the wild type, compared to *jaz1* (Fig. 8). Based on this data, *jaz1* is not better, nor worse, as a host compared to the Columbia wild-type; however, this may be the result of other JAZ genes compensating for the loss of JAZ1. Unfortunately, contamination of multiple plates restricted the sample size in this study, which limits the ability to detect significant patterns. Replication of this test with more samples, double mutants, or RNAi seedlings to explore how well other *jaz* mutants host RKNs is suggested.

Smith (2017) found no change in JAZ1 and JAZ12 expression during qPCR and additional qPCR was conducted to validate or challenge these results. JAZ1, JAZ12, and JAZ13 expression levels between infected and uninfected whole *Arabidopsis* roots, as well as just the knots, were compared using qPCR analysis. Whole root cDNA was only amplified in the

presence of JAZ12 primers. Between the infected and control samples at the two-day point, JAZ12 was expressed less in infected plants. Though no other expression between infected and uninfected samples differ, there was a decrease, followed by an increase in JAZ12 expression at 13- and 20-days post inoculation, respectively. This may be explained by giant cell formation. It is possible that the decrease of JAZ12 expression at between two days and two weeks is due to the altered metabolic pathways in giant cells, which were induced by RKN exoenzymes (Hammes et al., 2005). Additionally, it is likely that each JAZ gene is not being expressed independently at various times. Though it is not well understood, it is likely that JAZ proteins work with other JAZ proteins, and this relationship potentially plays a role in plant pathogen defense.

This idea that JAZ proteins work together and are upregulated at various times throughout RKN infection was explored in qPCR analysis with knot DNA (Fig. 10). During infection, JAZ1 and JAZ12 show a similar pattern, peaking in expression at approximately two weeks after inoculation (Figs. 10a-10b). This pattern of co-expression indicates that JAZ1 and JAZ12 genes are being expressed almost simultaneously (Fig. 10a-10b). Figure 10 suggests JAZ1 and JAZ12 potentially have a relationship and the two-week mark in infection is likely when the increase in giant cell altered metabolic activity peaks (Hammes et al., 2005). Interestingly, JAZ13 expression did not follow suit (Fig. 10c). Expression of JAZ13 peaked at three weeks, indicating the role of JAZ13 occurs later in infection, after JAZ1 and JAZ12 expression has peaked. Extending infection time to at least four weeks to further analyze JAZ13 expression patterns through qPCR is suggested. Additionally, more research in the relationship of JAZ protein interactions should be conducted to understand the JAZ gene expression timeline.

Further study of this field is crucial, as RKNs, as well as other plant parasitic nematode species, strain the agricultural industry (Nicol et al., 2011). Nematicides are not a feasible long-term solution, as they have shown to be causative agents of detrimental environmental effects, such as ground water contamination and reduced mammalian and avian fitness (Aspelin & Grube, 1999; Sasser & Freckman, 1987). By studying the natural defense mechanisms in plants, we may eventually be able to exploit these mechanisms to reduce both the plant parasitic nematode population and gallons of nematicides used. JAZ is a candidate of potential exploitation, and through GUS staining, egg mass comparisons, and qPCR analysis, more information on the role of JAZ has been added to the pool of plant pathogen defense. Though this is a small fraction of information that may eventually lead the agricultural industry to make use of altered natural defense mechanisms, we are closer to understanding the role of three of the thirteen JAZ genes.

Acknowledgements

I would like to thank the Office of Undergraduate Research at Bridgewater State University (BSU) for funding my research over three semesters. I would also like to thank the faculty of BSU Department of Biological Sciences and the Center for Transformative Learning, as well as my family and peers for their unconditional support. I am grateful for my laboratory colleagues: Lacey Battles and Leslie Aviles for creating and characterizing the JAZ1::GUS and JAZ12::GUS transgenic plants, Alison Palasek for synthesizing cDNA from knots, Davriana Fontes for extracting leaf DNA, and Ashley Smith for paving the way to this research project. I would also like to thank Dr. Gregg Howe for donating *jaz13* seeds to this project. Though they failed to germinate, his generosity is not taken lightly. A large thank you goes to Dr. Ken Adams and Dr. M. Caitlin Fisher-Reid for serving on my reading committee and bringing the final copy of this thesis to fruition. Last but far from least, I would like to thank my mentor, Dr. Heather Marella. Words cannot describe how grateful I am for her investment in me and my academic goals. Through her patience and expertise, I can now proudly call myself a biologist.

References

Abad, P., Gouzy J., Aury, J. M., Castagnone-Sereno, P., Danchin, E. G., Deleury, E., Perfus-Barbeoch, L., Anthouard, V., Artiguenave, F., & Blok, V. C. (2008). Genome sequence of the metazoan plant parasitic nematode *Meloidognye incognita. Nature Biotechnology*, 26, 909-915.

Alonso, J. M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H., Shinn, P., Stevenson, D. K., Zimmerman, J., Barajas, P., Cheuk, R., Gadrinab, C., Heller, C., Jeske, A., Koesema, E., Meyers, C. C., Parker, H., Prednis, L., Ansari, Y., Choy, N., Deen, H., Geralt, M., Hazari, N., Hom, E., Karnes, M., Mulholland, C., Ndubaku, R., Schmidt, I., Guzman, P., Aguilar-Henonin, L., Schmid, M., Weigel, D., Carter, D. E., Marchand, T., Risseeuw, E., Brogden, D., Zeko, A., Crosby, W. L., Berry, C. C., Ecker, J. R. (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science,* 5633,653-657.

Aspelin, A. L. & Grube, A. H. (1999). Pesticides Industry Sales and Usage, 1996 and 1997, U.S. Environmental Protection Agency Publication, 733–R-99-001.

Bai, Y., Meng, Y., Huanga, D., Qib, Y., & Chen, M. (2011). Origin and evolutionary analysis of the plant-specific TIFY transcription factor family. *Genomics*, 98(2), 128 – 136.

Bird, D. M., & Kaloshian, I. (2003). Are roots special? Nematodes have their say. *Physiological and Molecular Plant Pathology*, 62(2), 115-123.

Bird, A. F., & Loveys, B. R. (1975). The Incorporation of Photosynthates by *Meloidogyne javanica*. *Journal of Nematology*, 7(2), 111–113.

Boter, M., Golz, J. F., Gimenez-Ibanez, S., Fernandez-Barbero, G., Franco-Zoreilla, J. M., & Solano, R. (2015). FILAMENTOUS FLOWER Is a Direct Target of JAZ3 and Modulates Responses to Jasmonate. *The Plant Cell*, 27(11), 3160-3174.

Castagnone-Sereno, P., Danchin, E. G. J., Perfus-Barbeoch, L., & Abad, P. (2013). Diversity and Evolution of Root-Knot Nematodes, Genus Meloidognye: New Insights from the Genomic Era. *Annual Review of Phytopathology*, 51, 203-220.

Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J. M., Lorenzo, O., García-Casado, G., López-Vidriero, I., Lozano, F. M., Ponce, M. R., Micol, J. L., & Solano, R. (2007). The JAZ family of repressors is the missing link in jasmonate signaling. *Nature*., 448(7154), 666 – 671.

Chung, T., Phillips A.R., Vierstra R.D. (2010). ATG8 lipidation and ATG8-mediated autophagy in Arabidopsis require ATG12 expressed from the differentially controlled ATG12A AND ATG12B loci. *Plant Journal* 62, 483–493.

Curtis, R. H. C., Robinson, A. F., Perry, R. N. (2009). Hatch and host location. In: Perry R, Moens M, Starr J, (eds) *Root-knot nematodes,* 1, 139-162. Wallingford, UK: CABI Publishing.

Davide, R. G. & Triantaphyllou, A. C. (1967). Influence of the environment on development and sex differentiation of root-knot nematodes II. Effect of host nutrition. *Nematologica,* 13, 111-117.

Davide, R. G. & Triantaphyllou, A. C. (1968). Influence of the environment on development and sex differentiation of root-knot nematode III. Effect of foliar application of amleic hydrazide. *Nematologica,* 14, 37-46.

de Torres Zabala, M., Zhai, B., Jayaraman, S., Eleftheriadou, G., Winsbury, R., Yang, R., Truman, W., Tang, S., Smirnoff, N., & Grant, M. (2015). Novel JAZ co-operativity and unexpected JA dynamics underpin Arabidopsis defence responses to *Pseudomonas syringae* infection. *New Phytologist Trust,* 209, 1120-1134.

Dharmasiri, N., Dharmasiri, S., Jones, A. M., & Estelle, M. (2003). Auxin action in a cell-free system. *Current Biology,* 13, 1418–1422.

Dombrecht, B., Xue, G. P., Sprague, S. J., Kirkegaard, J. A., Ross, J. J., Reid, J. B., Fitt, G. P., Sewelam, N., Schenk, P.M., Manners, J. M., & Kazan, K. (2007). MYC2 differentially modulates diverse jasmonate-dependent functions in Arabidopsis. *Plant Cell,* 19, 2225–2245.

Figueroa, P. & Browse, J. (2015). Male sterility in Arabidopsis induced by overexpression of a MYC5-SRDX chimeric repressor. *Plant Journal,* 81(6): 849-860.

Flemetakis, E., Dimou, M., Cotzur, D., Efrose, R. C., Aivalakis, G., Colebatch, G., Udvardi, M., & Katinakis, P. (2003) A sucrose transporter, LjSUT4, is upregulated during Lotus japonicus nodule development. *Journal of Experimental Botany,* 54, 1789–1791.

Gheysen, G., & Mitchum, M. G. (2011). How nematodes manipulate plant development pathways for infection. *Current Opinion in Plant Biology*, 14(4), 415-421.

Grube, A., Donaldson, D., Kiely, T., & Wu, L. (2011). *Pesticides Industry Sales and Usage 2006 and 2007 Market Estimates.* Retrieved from https://www.epa.gov/sites /production/files/2015-10/documents/market_estimates2007.pdf.

Grunewald, W., Vanholme, B., Pauwels, L., Plovie, E., Inzé, D., Gheysen, G., & Goossens, A. (2009). Expression of the Arabidopsis jasmonate signaling repressor JAZ1/TIFY10A is stimulated by auxin. *EMBO Reports*, 10, 923-928.

Hammes, U. Z., Schachtman, D. P., Berg, R. H., Nielsen, E., Koch, W., McIntyre, L. M., & Taylor, C. G. (2005). Nematode-Induced Changes of Transporter Gene Expression in Arabidopsis Roots. *Molecular Plant-Microbe Interactions*, 18(12), 1247–1257.

Harrington, G. N., Franceschi, V. R., Offler, C. E., Patrick, J. W., Tegeder, M., Frommer, W. B., Harper, J. F., & Hitz, W.D. (1997). Cell specific expression of three genes involved in plasma membrane sucrose transport in developing Vicia faba seed. *Protoplasma* 197, 160–173.

Harrison, M. J., Dewbre, G. R., & Liu, J. (2002) A phosphate transporter from Medicago truncatula involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell*, 14, 2413–2429.

Howe, G.A., Major, I. T., & Koo, A. J. (2018). Modularity in jasmonate signaling for multistress resilience. *Annual Review Plant Biology,* 69, 387-415.

Hu, Y., Jiang, L., Wang, F., & Yu, D. (2013). Jasmonate regulates the inducer of cbf expression-C-repeat binding factor/DRE binding factor1 cascade and freezing tolerance in Arabidopsis. *Plant Cell*, 8, 2907-2924.

Hyer, A. H., Jorgenson, E. C., Garber, R. H., & Smith, S. (1979). Resistance to Root-Knot Nematode in Control of Root-Knot Nematode-Fusarium Wilt Disease Complex in Cotton1. *Crop Science*, 19(6), 898-901.

Ikeda, M., Hinohara, M., Umami, K., Taguro, Y., Okada, Y., Wada, Y., Nakanishi, Y., & Maeshima, M. (2001) Expression of V-ATPase proteolipid subunit of Acetabularia acetabulum in a VMA3-deficient strain of Saccharomyces cerevisiae and its complementation study. *European Journal of Biochemistry*, 268(23), 6097-6104.

Ingle, R. A., Stoker, C., Stone, W., Adams, N., Smith, R., Grant, M., Carré, I., Roden, L., & Denby, K. (2015). Jasmonate signaling drives time-of- day differences in susceptibility of Arabidopsis to the fungal pathogen *Botrytis cinereal. The Plant Journal,* 84(5), 937-948.

Ishiguro, S., Kawai-Oda, A., Ueda, J., Nishida, I., & Okada, K. (2001). The DEFECTIVE IN ANTHER DEHISCIENCE gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in Arabidopsis. *Plant Cell*, 13(10), 2191-209.

Jiang, Y. & Yu, D. (2014). The WRKY57 Transcription Factor Affects the Expression of Jasmonate ZIM-Domain Genes Transcriptionally to Compromise *Botrytis cinerea* Resistance1. (2016). *Plant Physiology*, 171(4), 2771-2782.

Jones, J. T., Haegeman, A., Danchin, E. G., Gaur, H. S., Helder, J., Jones, M. G. K., Kikuchi, T., Manzanilla-Lopez, R., Palomares, J. E., Wesemael, W. M. L., & Perry, R. N. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Plant Pathology*, 14(9), 946-61.

Kepinski, S., & Leyser, O., (2005). The *Arabidopsis* F-box protein TIR1 is an auxin receptor. *Nature*, 435(7041), 446-451.

Marella, H. H., Nielsen, E., Schachtman, D. P., & Taylor, C. G. (2013). The Amino Acid Permeases AAP3 and AAP6 Are Involved in Root-Knot Nematode Parasitism of Arabidopsis. *Molecular Plant-Microbe Interactions*, 26(1), 44-54.

McClure, M. A. (1977). Meloidogyne incognita: A metabolic sink. *Journal of Nematology* 9, 88–90.

Mira, M. M., Wally, O. S., Elhiti, M., El-Shanshory, A., Reddy, D. S., Hill, R. D., & Stasolla, C. (2016). Jasmonic acid is a downstream component in the modulation of somatic embryogenesis by Arabidopsis Class 2 phytoglobin. *Journal of Experimental Biology*, 67(8), 2231-2246.

Moa, Y. B., Liu, Y. Q., Chen, D. Y., Chen, F. Y., Fang, X., Hong, G. J., Wang, L. J., Wang, J. W., & Chen, X. Y. (2017). Jasmonate response decay and defense metabolite accumulation contributes to age-regulated dynamics of plant insect resistance. *Nature Communications*, 8, 13925.

Nahar, K., Kyndt, T., De Vleesschauwer, D., Höfte, M., & Gheysen, G. (2011). The jasmonate pathway is a key player in systemically induced defense against root knot nematodes in rice. *Plant Physiology*, 157(1), 305 – 316.

Nicol, J. M., Turner, S. J., Coyne, D. L., Nijs, L., Hockland, S., & Maafi, Z.T. (2011) Current Nematode Threats to World Agriculture. In: Jones, J., Gheysen, G., & Fenoll, C. (eds) *Genomics and Molecular Genetics of Plant-Nematode Interactions*. Springer, Dordrecht.

Pauwels, L. & Goosens, A. (2011). The JAZ proteins: a crucial interface in the jasmonate signaling cascade. *Plant Cell*. 23(9), 3089–3100.

Rausch, C., Daram, P., Brunner, S., Jansa, J., Laloi, M., Leggewie, G., Nikolaus, A., & Bucher, M. (2001). A phosphate transporter expressed in arbuscule-containing cells in potato. *Nature*, 414(6862), 462-465.

Sasser, J. N. & Freckman, D. W. (1987). In: Veech, J.A. and Dickson, D.W. (eds) *Vistas in Nematology* 1, 7–14, Hyattsville, MD: Society of Nematologists.

Sasaki, A., Inagaki-Ohara, K., Yoshida, T., et al. (2003) The N-terminal truncated isoform of SOCS3 translated from an alternative initiation AUG codon under stress conditions is stable due to the lack of a major ubiquitination site, Lys-6. Journal of *Biological Chemistry* 278, 2432–2436.

Smith, A. (2017). JAZ Levels in Arabidopsis after Infection by Parasitic Root-Knot Nematode. In BSU Honors Program Theses and Projects. Item 239. Available at: https://vc.bridgew.edu/honors_proj/239.

Snyder, D. W., Opperman, C. H., & Bird, D. M. (2006). A method for generating *Meloidognye incognita* males. *Journal of Nematology*, 38, 192-194.

Tegeder, M., Wang, X. D., Frommer, W. B., Offler, C. E., & Patrick, J. W. (1999). Sucrose transport into developing seeds of Pisum sativum L. *Plant Journal*, 18, 151–161.

Thatcher, M., Carrie, C., Andersson, C., Sivasithamparam, K., Whelan, J., & Singth, K. (2007). Differential Gene Expression and Subcellular Targeting of Arabidopsis Glutathione S-Transferase F8 Is Achieved through Alternative Transcription Start Sites. *The Journal of Biological Chemistry*, 282(39), 28915-28928.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S.Y., Howe, G.A., and Browse, J. (2007). JAZ repressor proteins are targets of the SCF(^{COI1}) complex during jasmonate signalling. *Nature*, 448, 661–665.

Triantaphyllou, A. C. (1981). Oogenesis and the chromosomes of the parthenogenetic root-knot nematode *Meloidogyne incognita. Journal of Nematology*, 13, 95-104.

Triantaphyllou, A. C. (1985). Cytogenetics, cytotaxonomy and phylogeny of root-knot nematodes. In: Sasser, J.N and Carter, C.C. (eds) *An Advanced Treatise on* Meloidogyne. *Biology and Control* 1, 113-126. North Carolina State University Graphics, Raleigh, North Carolina.

Tyerman, S. D., Whitehead, L. F., Day, D. A. (1995) A channel-like transporter for NH4+ on the symbiotic interface of N2-fixing plants. *Nature* 378, 629–632.

Ulmasov, T., Hagen, G., & Guilfoyle, T. (1999). Activation and repression of transcription by auxin-response factors. *Proceedings of the National Academy of Sciences of the United States of America*, 96(10),5844-5849.

Valenzuela, C. E., Acevedo-Acevedo, O., Miranda, G. S., Vegara-Barros, P., Holuigue, L., Figueroa, C. R., & Figueroa, P. M. (2016). Salt stress response triggers activation of the jasmonate signaling pathway leading to inhibition of cell elongation in *Arabidopsis* primary root. *Journal of Experimental Botany*, 67(14), 4209-4220.

Vanholme, B., Van Thuyne, W., Vanhouteghem, K., De Meutter, Cannoot, B., & Gheysen, G. (2007). Molecular characterization and functional importance of pectate lyase secreted by the cyst nematode *Heterodera schachtii. Molecular Plant Pathology,* 8(3), 267-278.

Wager, A., & Browse, J. (2012). Social Network: JAZ Protein Interactions Expand Our Knowledge of Jasmonate Signaling. *Frontiers in Plant Science*, 3, 41.

Wang, H. L., Offler, C. E., & Patrick, J.W. (1994). Nucellar projection transfer cells in the developing wheat grain. *Protoplasma* 182, 39–52.

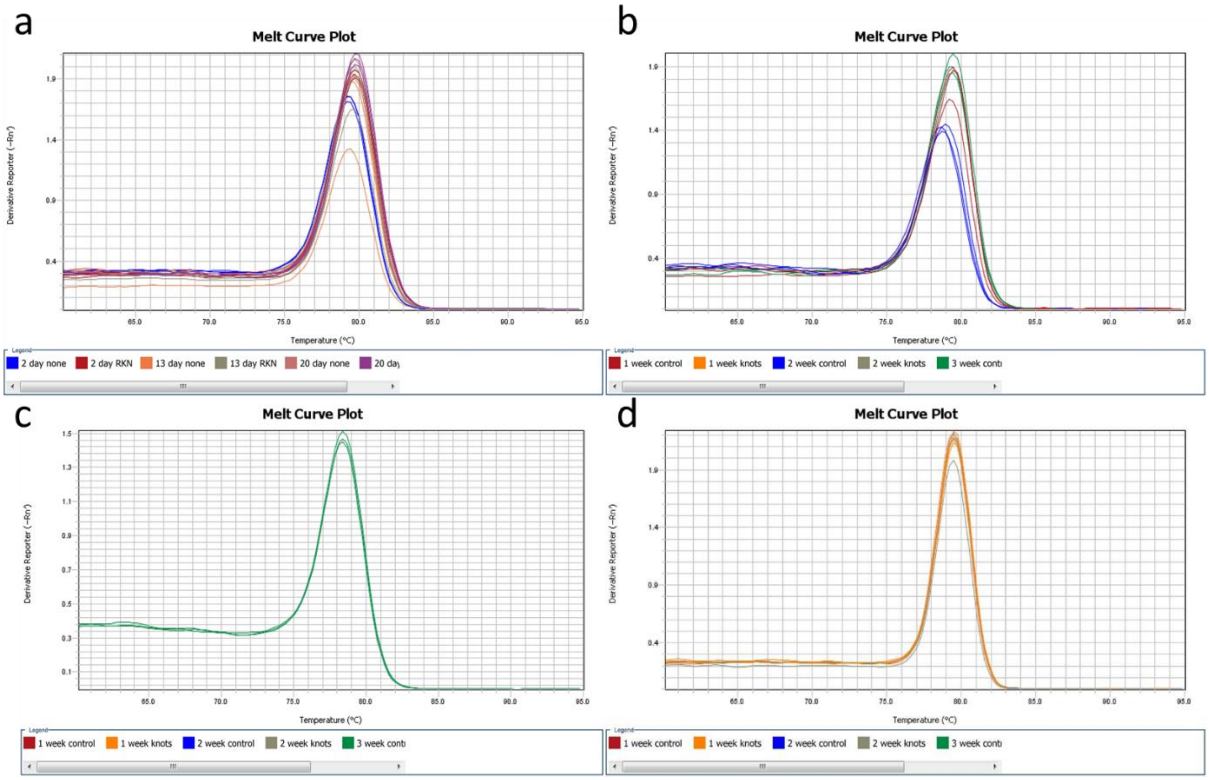
Wasternack, C. & Song, S. (2016). Jasmonates: biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. *Journal of Experimental Botany*, 68(6), 1303-1321.

Yan, Y., Stolz, S., Che´telat, A., Reymond, P., Pagni, M., Dubugnon, L., & Farmer, E. E. (2007). A downstream mediator in the growth repression limb of the jasmonate pathway. *Plant Cell*, 19, 2470–2483.

Yan, H., Yoo, M. J., Koh, J., Liu, L., Chen, Y., Acikgoz, D., Wang, Q., & Chen, S. (2014). Molecular Reprogramming of *Arabidopsis* in Response to Perturbation of Jasmonate Signaling. *Journal of Proteasome Research*, 13, 5751−5766.

Zhou, W., Lozano-Torres, J., Blilou, I., Zhang, X., Zhai, Q., Smant, G., Li, C., Scheres, B. (2019). A Jasmonate Signaling Network Activates Root Stem Cells and Promotes Regeneration. *Cell*, 177(4), 942-956.

Appendix 1



Appendix 1: Melt Curve Plots for JAZ1, JAZ12, and JAZ13
This figure demonstrates that each qPCR resulted in one amplicon. This indicates that only the target expressed gene is being amplified. a, b, c, and d show the melt curves of JAZ12 whole root cDNA, JAZ1 knot, JAZ12 knot, and JAZ13 knot, respectively.