NATURAL PLANT IMMUNE RESPONSES AGAINST A GLOBAL CROP DESTROYER

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ABSTRACT

Macrophomina phaseolina (*Mp*) is a fungal plant root pathogen commonly found in soil. It grows in warm conditions and has a detrimental effect on crop plants, giving it the name "global crop destroyer". It works by infecting the roots and then invading the vascular system of the host plant. Because previous methods to control *Mp* utilize toxic chemicals that are harmful to the environment and current methods are inefficient, it is important to create alternative sustainable methods to control it. In order to do so, a better understanding of plant immunity in regard to *Mp* must be developed. While plant immune responses against many microbial plant pathogens are well understood, immunity against *Mp* is not well understood.

Several years ago, the lab of Thomas Eulgem at UCR began to use the model plant species *Arabidopsis thaliana* (Arabidopsis) to examine immune mechanisms against *Mp* (Schroeder et al., 2019). Arabidopsis is a well-developed model system in plant biology, and it allows for the application of a wide variety of experimental methods. I screened a collection of natural Arabidopsis accessions for lines that show high or low levels of immunity against *Mp* by quantifying the severity of disease symptoms. Among the wild-type accessions, I found Cvi-1 to be relatively resistant against *Mp*. In addition to identifying accessions with high or low levels of resistance, the mediation of immunity through the *TAT3* gene, which possibly contributes to the biosynthesis of a novel antimicrobial compound, was confirmed. Screening mutants of this gene, I identified more and less tolerant mutants to infection by *Mp*. I found a *TAT3* mutant line to be less tolerant to infection by *Mp*. My results will be used for future research in immunity against *Mp* and may lead to the development of new methods to protect crops against this detrimental root pathogen.

ACKNOWLEDGEMENTS

This project would not have been possible without the support and guidance from my mentors, lab members, peers, and family. I would first like to thank my faculty mentor and the principal investigator Dr. Thomas Eulgem for the academic, professional, and personal development he has provided me with throughout my time in the lab. Additionally, in the Eulgem lab, I would like to thank Ph.D. candidate, Ayat Riahi, for allowing me to learn from her through this project and providing me with the opportunity to conduct research independently in the lab. I want to also acknowledge additional lab members Dr. Jianqiang Wang, Miwa Shirai, and David Fiske from whom the help I have received has contributed to the completion of this project. I am also extremely grateful to the University Honors Program and related faculty members for the opportunity to conduct a Capstone project in an undergraduate setting. Specifically, I want to thank Dr. Stephanie Dingwall and Dr. Richard Cardullo for their guidance in navigating the logistics of completing the Capstone project. Lastly, I would like to thank my parents, sister, grandmother, and friends for their unwavering support. With their belief in my abilities, I have been able to push myself to accomplish my goals.

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INTRODUCTION

Macrophomina phaseolina (*Mp*) is a generalist soilborne fungal species, deemed as a "global crop destroyer" due to its capability to cause tremendous damage in agriculture. As a necrotroph, *Mp* has demonstrated the ability to cause disease in a large variety of crops such as corn, soybean, cowpea, sorghum, and many more (Su G, Suh SO, Schneider RW, Russin JS, 2001). In plants, it typically causes diseases such as charcoal root rot, stem rot, seedling blight, and damping off (Ghosh, T., Biswas, M. K., Guin, C., and Roy, P, 2018). As a result, it has caused massive losses in the global economy. In a particular example, among diseases causing soybean yield suppression in 2003, charcoal root rot caused the second highest suppression in the United States. This resulted in an estimated reduction of soybean yields by 1.975 million tonnes in 2003 across the entire country (Wrather JA, Koenning SR, 2006).

While *Mp* can be found globally, it tends to favor regions with warmer climates and lower soil moisture. It is particularly difficult to control because *Mp* microsclerotia can persist in soil for decades (Gupta, G., et al, 2012). Microsclerotia, which are dark brown aggregates of cells, grow hyphae upon germination in the aforementioned favorable conditions. The hyphae infect the plant through the roots, and then grow into the host plant's vascular tissue (Chowdhury, S., Basu, A., Ray Chaudhuri, T. et al., 2014). These hyphae secrete enzymes that assist the pathogen with degrading the cell wall of the host plant, such as oxidases and other hydrolytic enzymes (Islam, M.S., Haque, M.S., Islam, M.M. et al., 2012). As a result, *Mp* prevents the host plant from effectively distributing nutrients it requires to grow throughout its various parts. Because the plant is inefficiently distributing nutrients, plants infected with *Mp* typically exhibit growth inhibition. Other typical symptoms include chlorosis and necrosis, or yellowing of the leaves and death of leaf tissue, respectively. As the vascular tissues become

more infected over time, the transport of important nutrients is blocked. Eventually, this is what causes the plant to exhibit more severe disease symptoms.

Because *Mp* microsclerotia can remain dormant in soil depending on the environmental conditions prior to germination, this presents an issue for agriculture. This is problematic for farmers across the globe, especially due to the increasing prevalence of global warming. Rising temperatures allow *Mp* to thrive in warm soil conditions in farmland and, once *Mp* has already infected an area, it is extremely difficult to control it due to how persistent it is in soil. Therefore, it is essential to determine methods to eradicate *Mp*. Previous methods to control *Mp* utilize toxic chemicals that are harmful to the environment. For example, fungicides such as methyl bromide have previously been used as a proven effective method to kill this pathogen (Cal, A. D., et al, 2004). However, the use of this chemical in particular has been severely limited as it has been proven to be harmful for the environment and is theorized to be harmful to people (Sande D., et al, 2011, Park, M.G., et al, 2020). Many regions, including the United States, have subsequently restricted the use of methyl bromide in agriculture.

In addition to using fungicides, many farmers have turned to using agricultural methods of controlling *Mp*. Because these methods do not completely eradicate *Mp*, they mainly aim to reduce the disease severity experienced by the plant. One such example is the use of irrigation. As previously mentioned, *Mp* thrives in environments with lower soil moisture. While it does not prevent initial infection of *Mp* to the host plant, using irrigation to increase soil moisture has been observed to decrease the disease severity in the plant (Jordaan, Estiene, et al., 2019). However, this method is not very sustainable. Specifically, as available water resources are decreasing over time, it will be more difficult to use this method to control *Mp*. Another method of control is the use of varying crop systems. For example, it was found that growing sesame as

mixed cropped or intercropped showed a lower incidence of *Mp* stem rot and root rot in comparison to growing sesame alone (Rajpurohit, T. S., 2002). Mixed cropping and intercropping refer to different methods of using the same area of land for two different crops. While this method does not completely eliminate *Mp*, it also is effective in limiting disease severity. However, this solution is also not effective in the long term, as it does not target the main issue – the persistence of *Mp* microsclerotia in soil.

To create alternative methods to control it, a better understanding of plant immunity in regards to *Mp* must be developed. While plant immune responses against other microbial plant pathogens are well understood, immunity against *Mp* is not. Thus, the goal of this project is to examine infections caused by *Mp* in various accessions of Arabidopsis thaliana (Arabidopsis) in order to identify the resistant and susceptible wild-type lines. Arabidopsis is used as the host plant for *Mp* in this project due to its prominence as a model system in plant biology. It is relatively easily transformed due to its genetic stability. Because its genome has been fully sequenced, information regarding its and genetics is readily available – including accessibility to mutants. There has been extensive research conducted on this plant and information regarding the molecular biology and biochemistry of immune responses in Arabidopsis is readily available. In particular, it has been documented that immune responses present in Arabidopsis involve the plant hormones salicylic acid, ethylene, and jasmonic acid (Nishimura, M. T., & Dangl, J. L., 2010).

As the first lab to use the *Mp*-Arabidopsis infection plate assay, the Eulgem lab identified interactions between *Mp* and Arabidopsis and established a model system in a previous project (Schroeder et al., 2019). It was determined that Arabidopsis mutants deficient in ethylene/jasmonic acid-signaling were more susceptible to *Mp* in comparison to the wild-type

reference accession Col-0. This project aims to build upon this research by identifying more susceptible versus more resistant lines of Arabidopsis using similar methods with the wild-type line Col-0 as the reference accession. Col-0 is used as the reference accession because it is a well-characterized line of Arabidopsis in plant biology. This makes information regarding its genome and availability of mutants readily accessible.

In addition to using wild-type accessions of Arabidopsis, I examined Arabidopsis mutants of the *TAT3* gene. This gene codes for a tyrosine aminotransferase protein, which is suspected to be involved in the Arabidopsis immune response. Comparing these mutants to the wild-type reference accession Col-0, the same method of screening was used to quantify the tolerance of each mutant to *Mp*. As a loss of function mutant, this portion of the project examined tolerance to *Mp* based on the lack of the gene present. Overall, my data allows for further research to be conducted to determine any potential genetic differences between the most susceptible and resistant candidates of wild-type lines of Arabidopsis, and between the more tolerant and less tolerant mutants. Ultimately, this can be used to develop a better understanding of the mechanisms that determine immunity against *Mp*.

MATERIALS AND METHODS

Materials:

To prepare the ½ MS Agar plates, MS, MES, sucrose and agar were all used. The pH was adjusted to 5.7 by adding in potassium hydroxide (KOH). To sterilize the seeds for each accession, HCl and bleach were required. For the accessions, wild-type seeds were ordered and bulked accordingly, including those for the Arabidopsis *TAT* T-DNA mutants. The *TAT* mutants used in this project were tat3-1, tat2A, and tat2B. These were mutated using a T-DNA insertion to kill the *TAT* gene. The tat3-1 mutant contains an insertion at an exon, whereas tat2A and tat2B contain insertions at two separate introns. To grow the seeds, ½ MS Agar plates, a pipette with tips cut to fit seeds, and micropore tape were used. To grow *Mp* for the *Mp* plates, a 1,000 µL pipette, ½ MS Agar media, 50 mL tube, a mortar and pestle and a hot plate were needed. To transplant the seedlings, an alcohol burner and a beaker of EtOH were used to sterilize the materials, such as tweezers, to be used for the transferring process.

Methods:

In order to infect the Arabidopsis plants with *Mp*, Arabidopsis seedlings had to be transplanted onto plates with *Mp*. The accessions were divided into separate sets, each one with Col-0 as the reference accession.

½ MS Agar Plate Preparation:

½ MS Agar plates were utilized throughout the experiments. Around 15-20 plates are required to sow seeds for each set, with each accession requiring around 3-5 plates depending on the amount of seeds sterilized. Around 18-24 plates were required for each set to grow *Mp*, with each accession requiring 6 *Mp* plates. For each set, an additional 6-8 agar plates were required to use as the control without *Mp*, with each accession required 2 control plates.

Seed Sterilization:

Around 70 seeds of each accession were sterilized in tubes under the fume hood by combining hydrochloric acid (HCl) and bleach, with around 40 seeds in each 1.5 mL Eppendorf tube. These tubes remained open and were placed in a tub, along with a beaker that contained 3 mL of HCl and 75-100 mL of bleach. The seeds remained in the tubes in this manner for around 4-6 hours, allowing the chlorine gas to sterilize the seeds.

Seed Stratification and Growth:

After the seeds were sterilized, they could be sown into the agar plates and stratified. In each agar plate, a maximum of 15 seeds were sown into the plates. This was repeated, until all seeds that were sterilized had been plated. Upon completion, micropore tape was wrapped around each labeled plate. Additionally, the plates were stacked and wrapped in aluminum foil to keep the seeds in a dark environment, as this is crucial for the stratification process. These were then placed into the cold room or refrigerator for 3-6 days. After this time period has passed, the seeds were moved into the growth room at 23 ℃ for an additional 10 days. The plates were placed upright, against a flat surface, in order for the roots to grow down along the plate following gravity. The growth room was set for the long day condition with 16 hours of light and 8 hours of darkness to speed up the growing process of the Arabidopsis seedlings.

Plating *Mp* and Growth:

The base of each *Mp* plate consisted of ½ MS Agar plates of 35 mL. Prior to plating, a mortar and pestle, MilliQ water, pipette tips, tweezers, and forceps needed to be autoclaved. To plate *Mp*, dried *Mp* was grinded with a mortar and pestle and autoclaved MilliQ water was slowly added to liquify it. Additional ½ MS Agar media was made, but not plated. Once the media solution was cooled down enough, *Mp* was added to the media. To determine if the

concentration of *Mp* was appropriate, 100 µL of this *Mp* media was pipetted onto a microscope glass slide. Under the lowest magnification, the aim was to have around 70-80 microsclerotium per 100 µL. If there was too little of an amount of microsclerotia, more ground *Mp* was added into the media. If there was too high of an amount, more clean media was added.

Upon creating the *Mp* media, a total of 6 mL was pipetted into each ½ MS Agar plate. The plates were propped up on their lids, and 3 mL of the *Mp* media was pipetted onto the bottom portion of the plate. After this was set, the plates were turned around and propped up on the other side of the plate using the lids. Then, 3 mL of the *Mp* media was pipetted on the empty side of the plate. After repeating this process for the total amount of plates, each *Mp* plate was wrapped in micropore tape and incubated at 34 ℃ for about 6 days. The plates were stacked and wrapped in aluminum foil for the incubation period, as darkness is crucial for *Mp* growth. After these 6 days, *Mp* plates were moved into the growth room in the same long day conditions as the Arabidopsis accessions for 1 additional day.

Transferring Seedlings:

After the seedlings had grown for 10 days and the *Mp* plates had grown for 1 day in the growth room, the seedlings were ready to be transferred into the *Mp* plates. Ethanol (EtOH) was used along with an alcohol burner to sterilize the tweezers used to handle the seedlings. In each *Mp* plate, 6 seedlings were transferred: 3 seedlings were plated in the top half, and 3 seedlings were in the bottom half. This was repeated an appropriate amount of times so that each accession had a total of 5 *Mp* plates, or 30 seedlings to be infected in total.

Each accession also had 2 control plates. In each plate, 3 seedlings were transplanted onto clean ½ MS Agar media plates to demonstrate the healthy condition for each accession.

After all seeds were transferred, the plates were wrapped in micropore tape and placed in the growth room horizontally in the same long day conditions until scoring.

Scoring and Picturing of Infected Plants:

Scoring of each accession was completed at the time points day 7, day 11, and day 14 after infection with *Mp*. These time points were determined based on changes observed in the reference accession Col-0. When Col-0 is infected with *Mp*, larger physical changes can be observed at these time points, such as increased chlorosis.

The seedlings were quantified based on a disease index from 0 to 5. A score of 0 meant that the plant was 100% healthy. This score would only be used for the control plants, as they did not experience any infection from *Mp* from the clean media plates. A score of 1 meant that no chlorosis or necrosis was observed, but there was growth inhibition due to *Mp*. A score of 2 meant the seedling overall exhibited 25% chlorosis and/or necrosis. A score of 3 meant the seedling exhibited 50% chlorosis and/or necrosis. A score of 4 meant the seedling exhibited 75% chlorosis and/or necrosis. A score of 5 meant the seedling exhibited 100% chlorosis and/or necrosis. Additionally, seedlings could be given intermediary scores such as 0.5, 1.5, 2.5, 3.5, and 4.5 following this scoring system.

At each time point after scoring, the seedlings were pictured to retain a copy of what the plants looked like physically at those specific time points.

RESULTS AND DISCUSSION

In this project, various accessions of wild-type Arabidopsis and *TAT* mutants were examined for their tolerance to *Mp*. In all sets, Col-0 was examined as it is the reference control generally used for Arabidopsis lines. Among the wild-type Arabidopsis accessions, Col-0, Ksk-1, Shigu-1, Cvi-1, Bolin-1, Borsk-1, Ak-1, Rld-2, Mr-0, Galdo-1, Eds-9, Tdr-8, Quar-8, Hov-3, and Can-0 were screened.

In the first set of the wild-type Arabidopsis accessions, the following accessions were examined: Col-0 and Ksk-1. The data collected for this set is as follows:

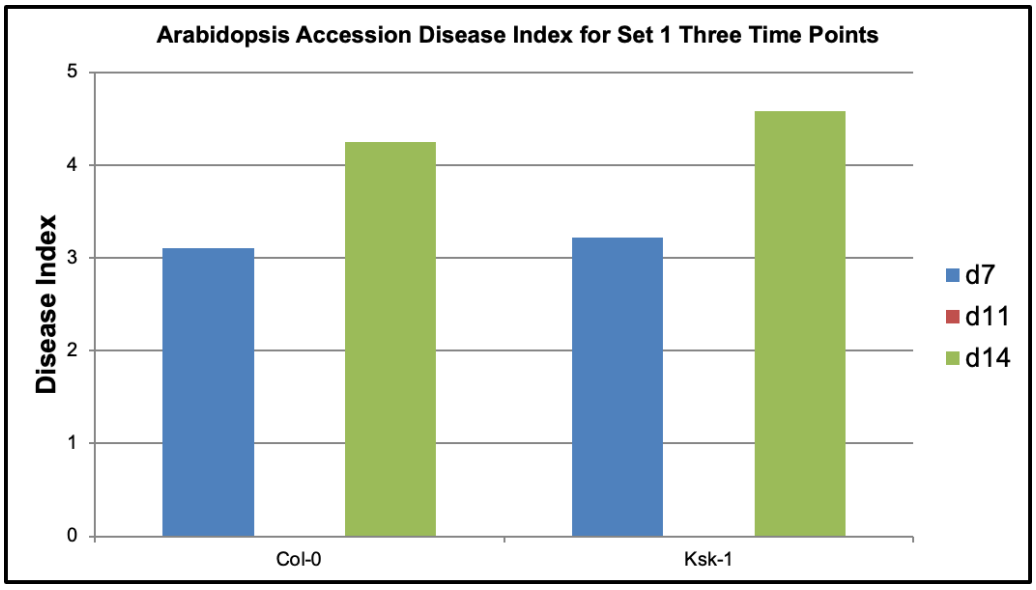

Figure 1: Disease index scores for Mp-infected Col-0 and Ksk1 lines. Plotted are averages of disease indices for Col-0 and Ksk-1 plants based on 30 tested plants per line.

In this set, screening at the day 11 time point was not conducted as this was the first set of data I had collected for this project. Yet, at the day 7 time point, both Col-0 and Ksk-1 display a similar disease index. However, at the day 14 time point, Ksk-1 shows slightly more susceptibility to *Mp* infection than Col-0 does.

In the second set, the accessions Shigu-1, Cvi-1, and Col-0 were examined. The data collected for Set #2 is as follows:

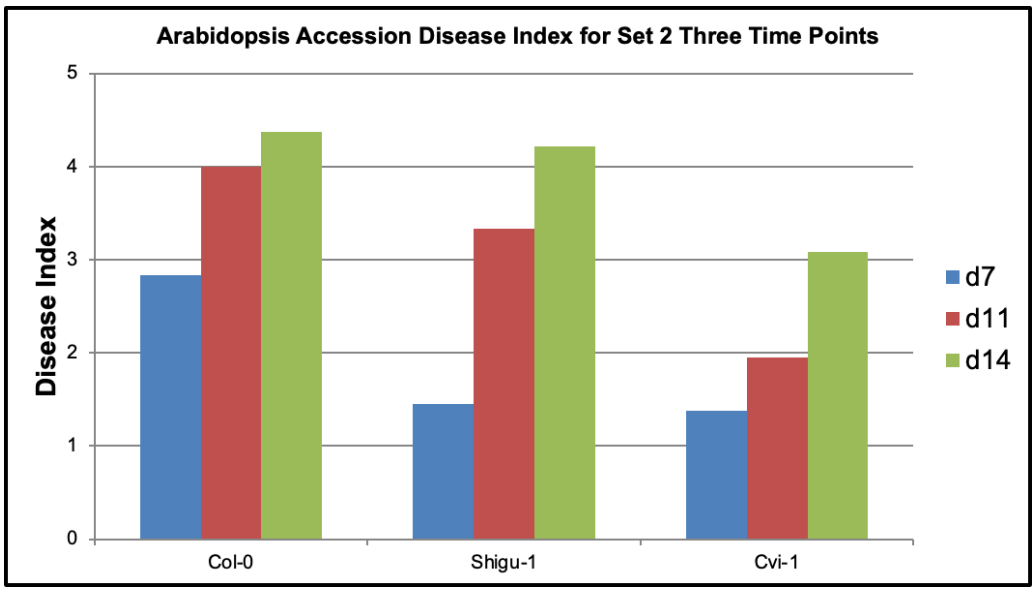

Figure 2: Disease index scores for Mp-infected Col-0, Shigu-1, and Cvi-1 lines. Plotted are averages of disease indices based on 30 tested plants per line.

In this set, both Shigu-1 and Cvi-1 show more resistance to *Mp* than the reference accession Col-0. However, for day 11 and day 14, Cvi-1 shows more resistance to *Mp* than Shigu-1 or Col-0. By day 14, while Shigu-1 is slightly more resistant to *Mp* than Col-0, it seems to be relatively similar to the disease index of Col-0.

In the third set, Cvi-1, Bolin-1, and Col-0 were examined. The data collected for set #3 is as follows:

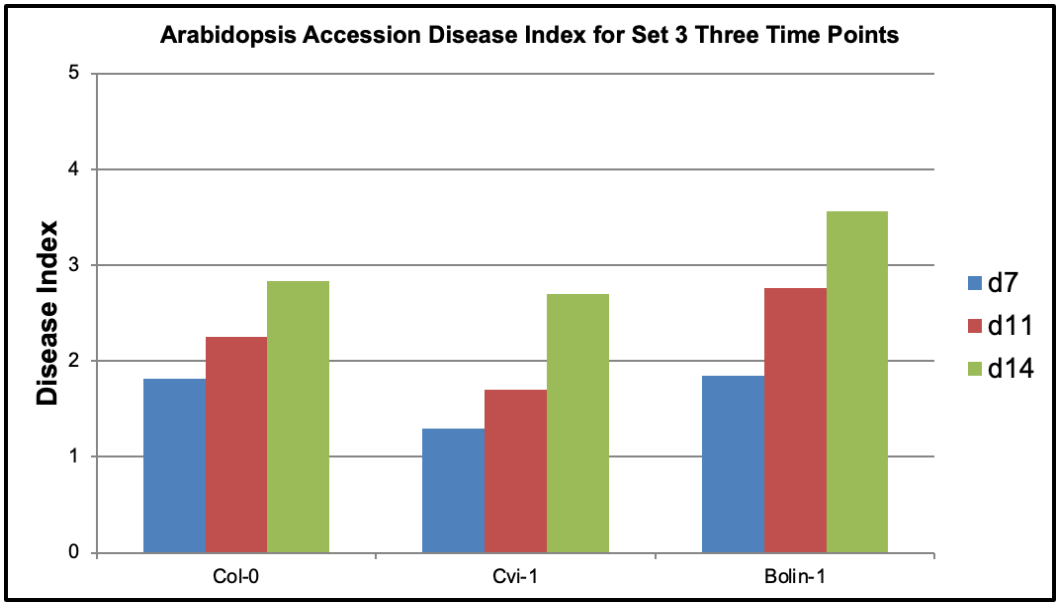

Figure 3: Disease index scores for Mp-infected Col-0, Cvi-1, and Bolin-1 lines. Plotted are averages of disease indices based on 30 tested plants per line.

In this set, Cvi-1 shows more resistance to *Mp* in comparison to Col-0. While the difference is more apparent through day 7, the disease index for Cvi-1 and Col-0 seem to be closer at day 14. In the previous set, the difference between Cvi-1 and Col-0 was more obviously drastic at all 3 time points. This discrepancy could be due to differences in the media plates used for these two accessions between the sets. However, Cvi-1 still seems to remain the most resistant among the accessions used in this set. Additionally, Bolin-1 shows slightly more susceptibility to *Mp* in comparison to Col-0. At the day 7 time point, Bolin-1 and Col-0 display a similar disease index. At day 11 and day 14, the difference between the indexes for these two accessions seems to grow.

In the fourth set, Borsk-1, Col-0, and Rld-2 were examined. The data collected for set #4 is as follows:

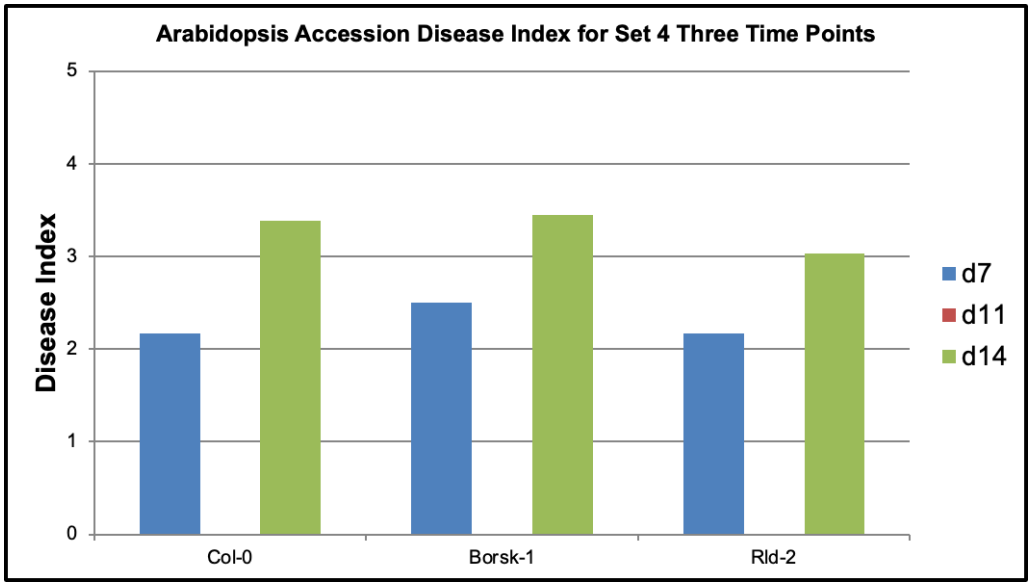

Figure 4: Disease index scores for Mp-infected Col-0, Borsk-1, and Rld-2 lines. Plotted are averages of disease indices based on 30 tested plants per line.

In this set, scoring at the day 11 time point was not conducted due to scheduling conflicts. However, Borsk-1 seems to be slightly more susceptible than Col-0 at both time points, and Rld-2 seems to be slightly more resistant at both time points. Interestingly, Borsk-1 seems to be similar to Col-0 at the day 14 time point, while Rld-2 seems to be similar to Col-0 at the day 7 time point.

In the fifth set, Mr-0, Galdo-1, Eds-9, and Col-0 were examined. The data collected for set #5 is as follows:

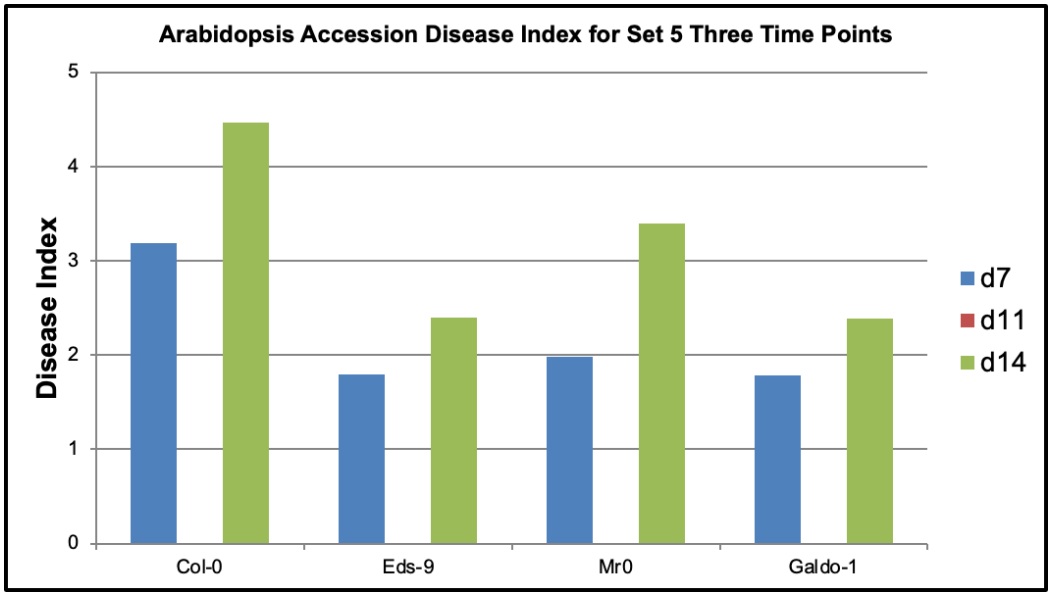

Figure 5: Disease index scores for Mp-infected Col-0, Eds-9, Mr0, and Galdo-1 lines. Plotted are averages of disease indices based on 30 tested plants per line.

In this set, scoring at the day 11 time point was not conducted. All accessions in this set seem to be more resistant to *Mp* than Col-0. However, of these, Mr-0 seems to be more susceptible in relation to Eds-9 and Galdo-1 upon comparison to Col-0.

In the sixth set, Tdr-8, Cvi-1, Ak-1 and Col-0 were examined. The data collected for set #6 is as follows:

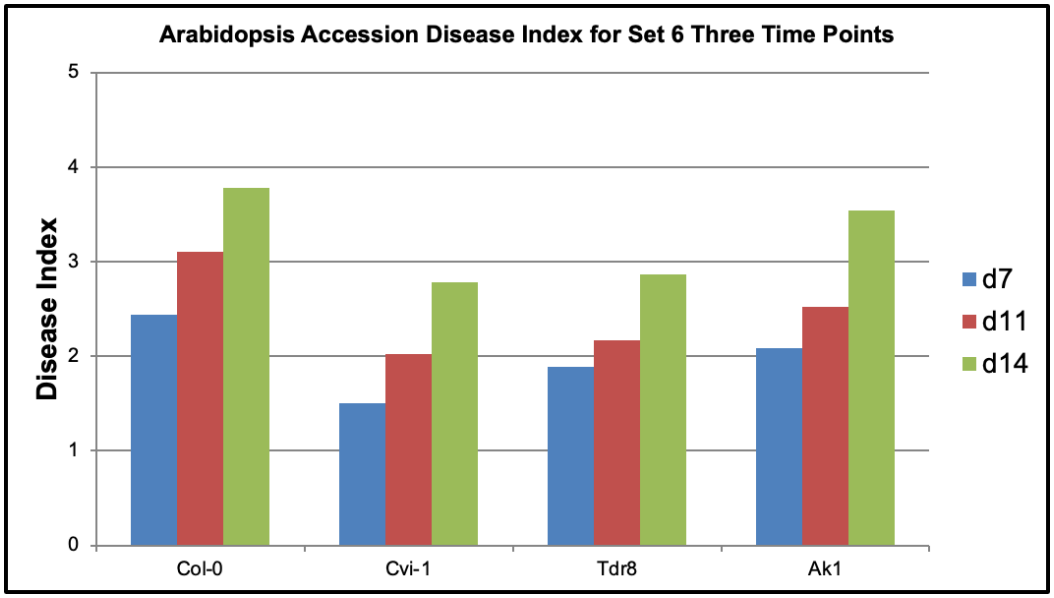

Figure 6: Disease index scores for Mp-infected Col-0, Cvi-1, Tdr-8, and Ak-1 lines. Plotted are averages of disease indices based on 30 tested plants per line.

In this set, all accessions seem to be more resistant to *Mp* than Col-0 is at each time point. Interestingly, both Cvi-1 and Tdr-8 seem to behave similarly at the day 14 time point. Among the additional three accessions, it seems that Ak-1 is more susceptible to *Mp* than Cvi-1 and Tdr-8 are upon comparison with Col-0. Additionally, out of all of the accessions in this set, Cvi-1 seems to display the most resistance to *Mp*.

In the seventh set, Quar-8, Hov-3, Col-0, and Can-0 were examined. The data collected for set #7 is as follows:

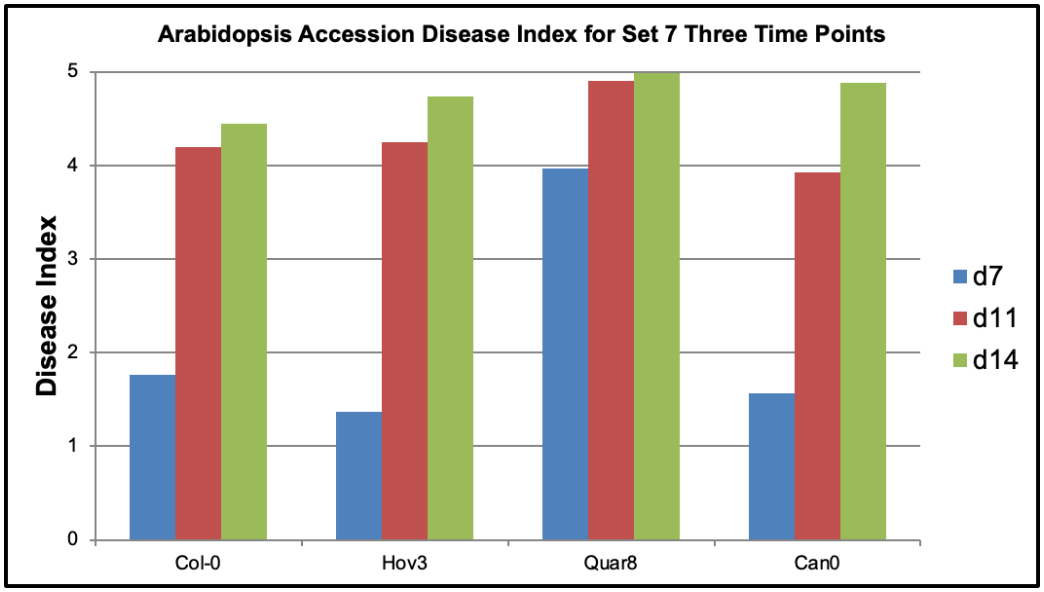

Figure 7: Disease index scores for Mp-infected Col-0, Hov-3, Quar-8, and Can-0 lines. Plotted are averages of disease indices based on 30 tested plants per line.

In this set, all three accessions seem to be more susceptible to *Mp* in comparison to Col-0 at the day 14 time point. In particular, Quar-8 seems to be the most susceptible in this set. At the day 11 time point for this accession, the mean disease index is close to a score of 5. For Hov-3 and Can-0, though, these accessions seem to be more resistant to *Mp* than Col-0 is up until the day 7 time point. At the day 14 time point, they begin to differ. Can-0 remains slightly more resistant to *Mp*, while Hov-3 behaves relatively similarly to Col-0.

Among the wild-type accessions, the most resistant and the most susceptible candidates were selected. A higher score on the disease index meant that the line was more susceptible, while a lower score meant it was more resistant. For the more resistant candidates, the data shows that Cvi-1 tends to have more tolerance to *Mp*. For the more susceptible candidates, the data shows that Bolin-1 and Quar-8 tend to have less tolerance to *Mp*. It should be noted that a large portion of the accessions screened for this project were tested only once against *Mp*. Further experiments should be conducted in order to determine the accuracy of this data.

In addition to screening the wild-type accessions, the mutants for two *TAT* genes, *TAT2* and *TAT3* were tested for their tolerance to *Mp*. The following mutants were compared to Col-0: *tat3-1*, *tat2A*, and *tat2B*. The data for this set is as follows:

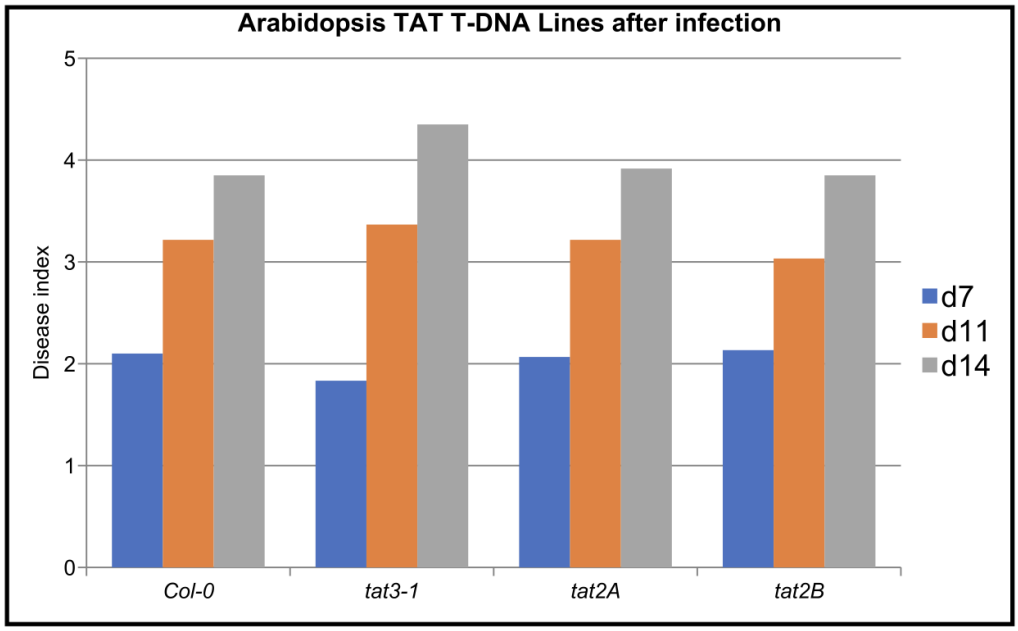

Figure 8: Disease index scores for Mp-infected Col-0, *tat3-1*, *tat2A*, and *tat2B*. Plotted are averages of disease indices based on 30 tested plants per line.

In this set, *tat3-1* seems to exhibit slightly less tolerance to *Mp*, while *tat2A* and *tat2B* seem to behave similarly to each other and to Col-0. Thus, *TAT2* is unlikely to play a role in immunity against *Mp*. However, *TAT3* clearly affects the outcome of *Mp* infections and contributes to immunity against *Mp*. It should be noted that this set was also only conducted once. Further experiments should be conducted to determine the accuracy of this data.

CONCLUSION

Ultimately, the candidate from the wild-type accessions found to be more resistant to infection by *Mp* was Cvi-1. Across multiple sets of experiments, it was consistently one of the more resistant candidates. Additionally, it typically displayed little chlorosis and hardly any necrosis. The main disease symptom observed from Cvi-1 upon *Mp* infection in this project was growth inhibition. The candidates that were more susceptible to *Mp* infection in the wild-type accessions were Bolin-1 and Quar-8. While these were only measured once across all of the sets, these two lines showed a larger difference in comparison to the disease index Col-0 displayed in the respective sets in comparison to other lines that showed susceptibility to *Mp*. Bolin-1 mainly displayed chlorosis, with some plants exhibiting necrotic characteristics upon *Mp* infection. Quar-8 exhibited chlorosis earlier in the infection process and necrosis as *Mp* infection persisted in the later time points.

Among the *TAT* mutants, *tat3-1* displayed more tolerance to *Mp*. As a loss of function mutant, this implies that the lack of the wild-type function of *TAT3* may make the Arabidopsis accession Col-0 less tolerant to infection from *Mp*. Additionally, the T-DNA insertion for this mutant is in an exon. Because this mutation becomes encoded in the mRNA transcript, the final tyrosine aminotransferase that is translated would not be a properly functioning enzyme. This is what is hypothesized that may alter the immune response in Arabidopsis. It would be interesting to further examine the role *TAT3* plays in the immune response in this way, since its loss seems to cause a difference in the disease index in comparison to Col-0.

The mutants tat2A and tat2B displayed disease indexes similar to each other and to Col-0. While these are also loss of function mutants, these mutants contain T-DNA insertions in two different introns. This implies that inserting sequences in noncoding regions causes the plant

to display similar behavior to the wild-type accession Col-0. These mutations are not encoded into the final mRNA transcript, but it can disrupt mRNA processing and splicing. This would eventually cause the translated protein to improperly function.

Overall, this data can be used for further research on Arabidopsis immune responses against *Mp* infection by examining the more and less tolerant wild-type accessions and *TAT3* mutants.

FUTURE RESEARCH

Due to time constraints, replicates of each set were not able to be completed. As a result, many wild-type accessions and the *TAT3* mutants were only tested once across the entirety of this project. Future research could include executing multiple replicates of the sets listed in this project, as well as comparing the more resistant lines and more susceptible lines within their groups in comparison to the reference accession Col-0. Additionally, because certain sets and accessions were lacking the day 11 time point, repeating the sets for multiple replicates can solidify the existing data for those accessions. Using the same screening method, more data points can be collected to obtain more accurate data.

Another point for future research is to examine production of biochemical molecules that signify an immune response in Arabidopsis between the more resistant lines and the more susceptible lines. The Eulgem lab previously found that Arabidopsis mutants deficient in jasmonic acid and ethylene signaling were more susceptible to *Mp* infection (Schroeder et al., 2019). Therefore, examining the signaling pathway of jasmonic acid and ethylene in the identified resistant and susceptible candidates among the wild-type accessions could provide useful insight into the immune response.

This could also be applied towards the *TAT3* mutants. Examining this signaling pathway in the mutants and comparing it to the reference accession Col-0 could provide insight into the impact of the *TAT* gene and tyrosine aminotransferase in the immune response. If the *tat3-1* mutant, which shows less tolerance to *Mp* infection, shows limited signaling in the jasmonic acid and ethylene pathway, this could confirm that *tat3-1* is more susceptible to *Mp* than Col-0.

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