The role of plant hormone auxin on the interaction between *Macrophomina phaseolina* and *Medicago truncatula*

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Abstract. *Macrophomina phaseolina* is a soil-borne fungal pathogen that causes the charcoal rot disease in many plant species. This disease causes many problems to the soy industry as no consistently effective control has been found. Using Medicago as a model, we study the molecular interactions between the pathogen and its plant host. A microarray chip was used to measure the changes in the expression levels of *M. truncatula* genes when the plant was under attack by the pathogen at the 24-hour, 36-hour and 48-hour intervals. Analysis of the data showed that genes in the auxin transport and response pathways were differentially expressed. Auxin is a phytohormone that is commonly associated with plant growth, but recent discoveries have turned up a possible link between auxin and plant defense. We will be testing the effect of auxin on the plant's response to *M. phaseolina*. With the results obtained, we hope to better understand the molecular interactions between the pathogen and its host, which can then allow us to determine how best to control this disease biologically.

1. Introduction

Fungi among viruses, bacteria and nematodes, are the source of most plant diseases. More than 8,000 of the 70,000 known fungi are known plant pathogens and many more diseases caused by fungi are being discovered every year. The fungus *Macrophomina phaseolina* (Tassi) Goid causes the charcoal rot disease in over 500 plant species worldwide, including agronomically important plants such as soybean, corn, sorghum, and cotton [1]. Symptoms of the disease (also known as dry-weather wilt and summer wilt) arise during dry and hot conditions where discoloration of the root and death of seedlings is common. Microsclerotia are often produced in the pith of the stem in such numbers that they give an illusion of finely sprinkled charcoal on the stem hence the name of the disease, charcoal rot. These microsclerotia can survive in dry soil or embedded in its host residue for 2 years or longer. In wet soils however, the microsclerotia do not survive longer than 7-8 weeks and the mycelia cannot survive longer than 7 days. Microsclerotia in soil will continue to propagate as long as soybean or other hosts are continuously grown in the same plots with the disease escalating in severity with successive soybean crops. There have also been cases where infection was observed through an infected seed. Among the many diseases that plague the soybean industry, charcoal rot is among the top ten most important disease affecting world production of soybean [2]. The United States is the leading producer of soybean, producing 2.96 billion bushels of soybean in 2009. The estimated yield loss attributable to charcoal rot in 2005 was 495,753 tons valued at $117 million.

There have been several efforts in controlling the disease in soybean. Rotation of crops is of little benefit because of the vast variety of hosts that the fungus can infect. But, it has been proven that the fungus is found in lower densities when soybean is planted in rotation with cotton or corn [3]. Control of the disease has been expanded to tillage; however it has not been shown to have an effect on *Macrophomina* incidence or severity [4]. A fluorescent *Pseudomonas* was found to reduce the charcoal rot in peanut by inhibiting the growth of the fungus [5]. A study of 4 fungi, *Trichoderma hamatum, T. harzianum,T. polysporum* and *T. viride* revealed that these fungi were able to inhibit the growth of *Macrophomina* by a small percentage [6]. To explore the possibility of engineering a resistant strain, we establish a pathosystem using *Medicago truncatula* to study the molecular interactions between the pathogen, *M. phaseolina* and its plant hosts.

Medicago truncatula, a relative of the alfalfa family, has a small diploid genome, is self-fertilized and has a rapid reproductive cycle allowing it to be an excellent model plant for studying mycorrhizal interactions such as the infection of the root pathogen, *Macrophomina phaseolina*. Medicago is also related to many important crop legumes such as pea, faba bean, chickpea, lentil and clover. Therefore, Medicago exhibits a high level of gene conservation and similar genetic organization with these plants. It is possible then for us to transfer genome information found in Medicago to these crop legumes.

A gene expression profiling study on *Medicago truncatula* infected with root rot, *Phymatotrichopsis omnivore* revealed the roles of jasmonic acid, ethylene and the flavonoid pathway in disease development [7]. By analyzing the gene expression profile of Medicago infected with *M. phaseolina,* we were able to isolate genes in different biological pathways that are differentially expressed during the pathogen attack. One of pathways involves auxin signaling and response. Auxin is a hormone in plants that is commonly associated with plant growth by regulating cell elongation, cell division, phototropism, gravitropism, root initiation, and apical dominance. However, there have been new discovery linking auxin to regulation of plant defense such as auxin signaling affecting resistance and susceptibility to separate pathogen groups as well as affecting disease outcome through effects on development.With this mind, we thought of testing the effects of auxin application in Medicago plants that are infected with *M. phaseolina,* to see whether it would increase the susceptibility or resistance to the pathogen.

2. Experiment

Microarrays were used to monitor the expression profiles and molecular processes associated with infection of the

initial entry (24 hours-post-inoculation, hpi) and colonization (36, 48 and 72 hpi). The arrays were done using Affymetrix's GeneChip Medicago Genome Array for *Medicago truncatula*.

During statistical analyses, it was found that 96 genes were upregulated at 24hpi, 473 genes were upregulated at 36hpi, and 718 genes were upregulated at 48hpi. As for downregulation, 36 genes were downregulated at 24hpi, 311 at 36hpi and 173 at 48hpi. The genes that were up or downregulated at 72hpi were discounted as there were several factors that could have attributed to the differential expression of these genes such as cellular processes due to death of the plant rather than the effect of the pathogen infection. After using the MAPMAN program, it was noted that some of the differentially expressed genes are found in the auxin synthesis and response pathway. To confirm the results of the microarray, real-time quantitative PCR will be done on the RNA samples of Medicago infected with *M. phaseolina* for the genes in the auxin pathway. This is then followed by the assay in which the plants are supplemented with auxin. The auxin supplied will be in the form of indole-acetic acid (IAA), a major form of auxin in most plants. The assay involves planting Medicago in IAA-supplemented phytagel in Magenta boxes, followed by inoculating with *M. phaseolina* and disease monitoring.

The planting of Medicago starts by treating the *A17* seeds with concentrated sulfuric acid, and followed by surface sterilization with bleach. The seeds were then germinated on Murashige and Skoog (MS) agar at room temperature in the dark for 3 days. Seedlings were transplanted into Magenta boxes containing IAA-supplemented phytagel. When the plants were 2 weeks old, they were inoculated with the fungal pathogen. The in-vitro assay inoculation involves the use of a single wheat seed that is colonized with the fungus with the control being a sterilized wheat seed.

3. Results and Conclusions

Preliminary results from the first run of this experiment demonstrated that IAA contributes to increased resistance in Medicago. The IAA in low concentrations such as 5 nM and 50nM led to healthier plants, and exhibited slight resistance to the infection of the fungus (Figure 1). This slight resistance is quantified by observation of how the plant fared (whether the leaves had started to yellow, detachment of leaves, and eventually death of the plant when no green tissue is present). The experiment will be repeated to confirm the results found.

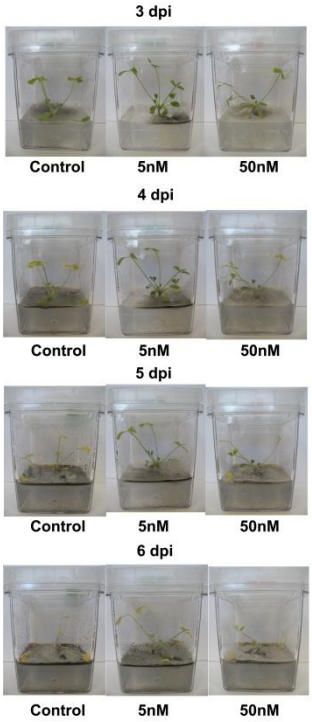

Figure 1: Medicago plants treated with auxin show partial resistance to *M. phaseolina*. Plants were grown in MS media supplied with 5 nM or 50 nM IAA or no IAA (control). Two-week-old plants were inoculated with a single wheat seed covered with M. phaseolina sclerotia. Pictures were taken at 3 day-post-inoculation (dpi) to 6 dpi.

4. References

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