Study of Charcoal Rot Disease Using Model Plant *Arabidopsis thaliana*

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**Abstract.** To understand the disease mechanisms of charcoal rot caused by *Macrophomina phaseolina* at the molecular level, we established a pathosystem using the model plant *Arabidopsis thaliana*. The cellular infection process and pathogen propagation within the host system during the early and late stages of infection were examined by microscopy. Studies have shown that phytohormones play crucial role in the induction of defense signaling pathways. In this study, we use a genetics approach to understand the mechanisms of plant immunity against *Macrophomina* mediated by plant hormones.

1. **Introduction**

*Macrophomina phaseolina* a necrotrophic fungus which is responsible for charcoal rot disease that affects over 500 species of plants including crops such as soybean [1]. Necrotroph proceeds via secretion of disease causing agents such as phytotoxins, cell wall degrading enzymes and other extracellular enzymes that result in extensive necrosis, tissue maceration, and plant rots [2]. These fungi mainly act by disrupting the defense signaling by influencing the host phytohormone level [3] or by suppressing defenses by manipulating the host cellular machinery [2]. *M. phaseolina* microsclerotia are the primary source of infection, and produced in the infected stem and roots of host tissues [4]. High temperatures, repeated freezing and thawing of soil [5], low C:N soil ratios [6] and low moisture content [7] are favorable conditions for the pathogen. Although population levels of the pathogen can be lowered using some management strategies, these approaches are not always effective [8]. Therefore in order to develop novel strategies to control charcoal rot and develop disease-resistant plants, understanding the fungal infection strategies and molecular processes occurring during infection is important. In this study we use genetics approach to understand the host-pathogen interactions at the molecular level using *Arabidopsis thaliana* as a model plant.

Among the important roles of plant hormones is their involvement in biotic or abiotic stress responses [9]. The naturally occurring plant hormones including gibberellins, auxins, cytokinins, ethylene (ET), and abscisic acid and the more recently discovered brassinosteroids, jasmonic acid (JA), salicylic acid and various other small peptides play important roles in regulating plant growth and development [10]. Although it was noted that several of these hormones are involved in plant disease responses, the involvement of a particular hormone in mediating resistance or susceptibility to a specific pathogen depends on kind of pathogen itself [11][9]. There is often a complex negative or positive regulatory cross talk between these hormones to ensure an effective defense response. Previously in our laboratory, microarray was used to determine the change in gene expression in the model legume *M. truncatula* due to *M. phaseolina* infection. From this microarray study, it was concluded that JA, ET and auxin were involved in the disease development. To further investigate the roles of auxin as well as other plant hormones in disease resistance against *M. phaseolina*, we used *Arabidopsis thaliana* to establish a pathosystem in this study. Here we hypothesized that the necrotrophic fungus *M. phaseolina* invades the host by affecting the biosynthesis and/or induction of the plant hormones (mainly JA, ET and auxin) that are directly or indirectly involved in mounting a defense response against the pathogen. If these hormone signaling pathways are in fact disrupted by the pathogen then we should be able to see a variation in survival rates of the different mutant plants that have either compromised or overexpressed genes involved in these signaling pathways when compared to the wild-type. We hope that understanding the molecular interactions between the pathogen and *A. thaliana* will allow us to devise a better management approach or even engineer disease resistant plants in the future.

2. **Experiment, Results, Discussion, and Significance**

In order to assess which of the genetic component are involved in host susceptibility, it was first determined that *Arabidopsis thaliana* is a susceptible host for the pathogen by inoculating the wild type plants by root-dipping method. The symptoms were monitored on a daily basis to look for chlorosis or necrosis in leaves and formation of sclerotia in the roots by performing microscopic analysis every 12 hours.
Multiple hormone pathways are found to be involved in *Arabidopsis* resistance to necrotrophic fungi. In the second part of experimental analysis, we studied the role of different signaling molecules including JA, ET, ABA, SA and Auxin in development and regulation of defense response against *M. phaseolina*. To study the roles of these phytohormones, we obtained mutants that have defects in one of the hormone response pathways. 24 plants from each mutant line and 24 wild-type control were inoculated at 5 weeks with *M. phaseolina* and disease symptoms were compared with that in wild type plants to determine if any of the affected genes play a role in the host-pathogen interactions. Symptoms were monitored from 1 DPI to 7 DPI using a scoring matrix in the scale of 0-6.

3. **Conclusion.** Results from our study have shown that *A. thaliana* is a susceptible host of *M. phaseolina*. Although we expected auxin, JA and ET mutant lines to show a differential response against the pathogen, there was no noticeable difference in development and progression of disease symptoms when compared to wild-type in most of the tested lines. The mutant line *axr2-1* did show a slightly higher survival rate than the wild type even after day 8 post inoculation, indicating some of the auxin genes may have a potential role in imparting defense against *M. phaseolina*.

Table 1: Tabular Representation of Experimental Results of Tested Mutant Lines

<table>
<thead>
<tr>
<th>Mutant Name</th>
<th>Gene ID</th>
<th>Pathway involved</th>
<th>Experimental Results</th>
<th>Mutant Name</th>
<th>Gene ID</th>
<th>Pathway involved</th>
<th>Experimental Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ein2</td>
<td>AT5G03280</td>
<td>ET</td>
<td>Death rate same as that of wildtype</td>
<td><em>axr2-1</em></td>
<td>AT3G23050</td>
<td>Auxin</td>
<td>Mutant plants survive longer</td>
</tr>
<tr>
<td><em>etr1-1</em></td>
<td>AT1G66340</td>
<td>ET</td>
<td>Death rate same as that of wildtype</td>
<td><em>axr4 axr1</em></td>
<td>AT1G05180</td>
<td>Auxin</td>
<td>Death rate same as that of wildtype</td>
</tr>
<tr>
<td>aos</td>
<td>AT5G42650</td>
<td>ET</td>
<td>Death rate same as that of wildtype</td>
<td><em>tir1-1</em></td>
<td>AT3G62980</td>
<td>Auxin</td>
<td>Death rate same as that of wildtype</td>
</tr>
<tr>
<td><em>npr1-2</em></td>
<td>AT1G64280</td>
<td>Salicylic acid</td>
<td>Death rate same as that of wildtype</td>
<td><em>axr1-3</em></td>
<td>AT1G05180</td>
<td>Auxin</td>
<td>Death rate same as that of wildtype</td>
</tr>
<tr>
<td><em>abi1-1</em></td>
<td>AT4G26080</td>
<td>ABA</td>
<td>Death rate same as that of wildtype</td>
<td><em>coli;35s::erf1-2</em></td>
<td>AT3G23240</td>
<td>Ethylene</td>
<td>Death rate same as that of wildtype</td>
</tr>
<tr>
<td><em>aba2-1</em></td>
<td>AT1G52340</td>
<td>ABA</td>
<td>Death rate same as that of wildtype</td>
<td><em>coli;35s::erf1-2</em></td>
<td>AT3G23240</td>
<td>JA and Et</td>
<td>Death rate same as that of wildtype</td>
</tr>
</tbody>
</table>

References