Molecular interactions between *Medicago truncatula* and *Macrophomina phaseolina*

Andres Reyes Gaige*, Bin Shuai

Department of Biological Sciences, College of Liberal Arts and Sciences

Abstract. *Macrophomina phaseolina* is a soil-borne fungal pathogen that causes a disease commonly known as charcoal rot. Currently, there is not an effective method for controlling the disease, because knowledge about the pathogen, the development of the disease and how it interacts with the plant host is limited. Therefore, a study is proposed to investigate the interactions between *M. phaseolina* and the plant species *Medicago truncatula* using a molecular genetics approach where the host genes involved in the disease development will be identified. We will first conduct a genetic screen in a mutant population of *M. truncatula* and look for strains that have altered susceptibility to *M. phaseolina*. We will then identify the genes that are involved in host-pathogen interactions.

1. Introduction

Charcoal rot is a root disease caused by the soil borne fungus *Macrophomina phaseolina*. It is also called summer wilt or dry weather wilt because it often occurs during the summer, when plants are under heat and drought stresses. This disease has been an endemic problem in soybean growing areas where summers are dry, especially in the central part of the Midwest: Kansas, Nebraska and part of Missouri being the most affected regions [1]. When severe, the disease reduces yield by killing plants at early reproductive stages. Symptoms of charcoal rot appear during hot, dry weather when unfavorable environmental conditions stress the plant. It usually develops when soil temperatures are 80-95°F (27-35°C) for 2 to 3 weeks [2]. This fungus is highly variable with a wide host range and geographical distribution. Furthermore, it has the potential to infect more than 500 crops and weed species.

Results from controlled studies have demonstrated that *M. phaseolina* can reduce plant height, root volume, and root weight by more than 50% [3]. Damage to the root system is most evident during the pod formation and filling stages, when demand for water and nutrient absorption is high. Because diseased plants have smaller root systems, resulting seeds tend to be fewer and lighter. Diseased plants will mature several weeks earlier, which further contributes to yield loss. The charcoal rot pathogen survives from year to year as microsclerotia in soil and infected crop residue. Microsclerotia is a compact mass of hardened mycelium stored with reserve food material that becomes detached and remains dormant until a favorable opportunity for growth occurs.

A number of management measures have been applied to control *M. phaseolina*. Crop yield loss can be reduced by making rotations with small grains such as wheat and barley (poor hosts) for one or two years. Wheat and barley are considered poor hosts because they do not bloom and set pods during the normally dry months of July and August, so they are more likely to escape infection by charcoal rot. Also, increasing seeding rate is not recommended because high plant populations increase drought stress. Charcoal rot incidence can also be reduced through proper fertilization, weed control, and irrigation. However, no chemical treatments are available to control charcoal rot.

Considering the difficulties in managing charcoal rot disease with traditional methods, genetic engineering may be the best alternative. However, we know very little about the interaction between *M. phaseolina* and its hosts at the molecular level. Therefore, we propose to study the molecular mechanism that takes place when the plant species *Medicago truncatula* interacts with *M. phaseolina*.

In this investigation, we try to identify the host genes that are involved in the disease development using a molecular-genetic approach. This study will lead to a better understanding of the charcoal rot disease at the molecular level, and provide important information for crop improvement in the future.

2. Experiment, Results, Discussion, and Significance

First, the interaction between the plant species *Medicago truncatula* and the fungus *Macrophomina phaseolina* must be shown. This is demonstrated in both in-vivo and in-vitro assay. The in-vivo assay was performed by directly exposing the root of *M. truncatula* to the fungus. The in-vitro assay was done by exposing a detached leaf of *M. truncatula* to growing hyphae of the fungus. Both assays have shown that Medicago can be easily infected with *M.
phaseolina. Our next step is to screen a mutant population of *M. truncatula* plants to identify strains that have altered susceptibility to the fungus. These *M. truncatula* mutants lines were generated by using a Tnt1 retrotransposon. The Tnt1 retrotransposon was first identified in tobacco, and it is considered to be an efficient insertional mutagen in *M. truncatula* [4]. It has been estimated that approximately 14,000 – 16,000 lines would be sufficient to tag 90% Medicago genome. Therefore, by analyzing 150 different mutant plants in our initial screen, we will be covering ~0.3% of the genome.

By applying the in-vitro assay, we will identify the mutant lines that have altered susceptibility to *M. phaseolina*, and the interaction will be confirmed with in vivo assay. In case we find a plant with altered susceptibility, we will try to identify the genes that are involved in the host-pathogen interaction using molecular biology tools. The *Fabaceae* (legumes) are second only to the *Poaceae* (grasses) in importance to humans as a source of food, feed for livestock, and raw materials for industry [5]. Seeds and shoots of legumes are a rich source of dietary protein, oil, carbohydrates, fiber, minerals, vitamins, and other beneficial secondary compounds for humans and livestock [6]. In addition, legumes account for approximately one third of the world's primary crop production, human dietary protein, and processed vegetable oil. We can see that legumes are of great importance for an appropriate sustainability of human-beings on earth. Moreover, legumes are a lynch pin of sustainable agriculture because they supply their own nitrogen (N) by 'fixing' it in a symbiotic association with bacteria called rhizobia. This mutually beneficial association provides legumes and subsequent crops with a free and renewable source of usable nitrogen. It is estimated that between 40 million and 60 million tonnes of nitrogen are fixed annually by cultivated legumes [7], equivalent to about US$10 billion fertilizer [5]. The impact of charcoal rot disease on many legume species will become more devastating, as the dry and warm weather become more prominent in many parts of the world as a result of global warming. We hope that our research will be valuable for future development of much-needed disease-resistant crop plants.

3. Conclusions

We have created ideal conditions in the lab to establish the interaction between *Macrophomina phaseolina* and *Medicago truncatula*. We have also developed a protocol that will be applied in the mutant screening. We hope that our effort in identifying the genes that are involved in the host-pathogen interaction will generate the knowledge required for managing this fungus.