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Characterization of Macrophomina phaseolina Infecting Chia Plants

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Introduction

Salvia Hispanica L., commonly known as chia, is a rising agricultural crop because of its seeds' high concentration of α-linolenic acid. α-linolenic acid provides several different health benefits, in addition to being a rich source of protein and fiber[1].

Chia field trails conducted by the Atamian lab during summer 2018, experienced high levels of disease incidence characterized by root rot, plant wilting, and eventual death of three-month old chia plants, which was identified as *Macrophomina phaseolina* based on morphological analysis on Potato Dextrose Agar plates.

Macrophomina phaseolina is a widespread fungus that causes a high mortality rate in nursery plants as well as in agricultural crops such as soybean, maize, sorghum, and cotton. The fungus damages the root system of the plant host, resulting in the inability of the root to obtain the required nutrients and water for plant's proper growth.

Methods

- The fungi were cultured on Potatoes Dextrose Agar (PDA) plates
- DNA extraction was completed using cetyl trimethylammonium bromide (CTAB)
- DNA was amplified through Polymerase Chain Reaction (PCR) using 3 sets of primers ITS 4 and 5, SSU rRNA, and MPK-1
- Ligation of the A tailing product of *Macrophomina phaseolina* PCR DNA fragment into pGEM T-Easy Vector
- Escherichia coli (E. coli) bacteria were used for the transformation of the pGEM T-Easy plasmid
- Transformed E. coli was grown on Lysogen Broth (LB) and Ampicillin (AMP) and LB + Carbenicillin (CARB) liquid media cultures and on agar plates
- The plasmids were extracted from the E. coli cells by an alkaline lysis method and sent for sequencing to the Eurofins lab
- Bioinformatics of the resulting DNA sequence of *Macrophomina phaseolina* was completed using the following applications multi align, genious, and the NCBI database

Morphological evidence of *Macrophomina phaseolina*

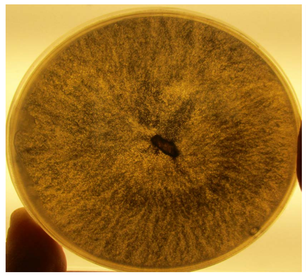

Figure 1: Fungus growth on Potato Dextrose Agar (PDA) plates

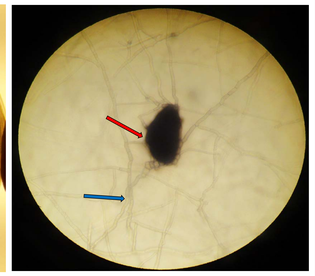

Figure 2: Microscopic view of mycelia (blue arrow) and sclerotia (red arrow) on PDA plates

Symptoms of *Macrophomina phaseolina* Infection in Chia

Figure 3: Charcoal root rot, a symptoms in two months old chia plants

Figure 4: Vertical cut in stem of infected plant showing dead vesicular tissue

Amplification of sequences using PCR

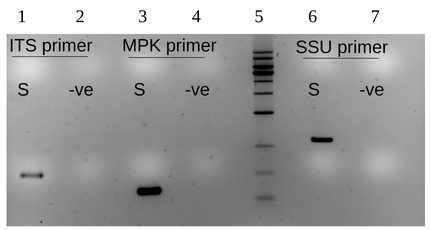

Figure 5: Gel electrophoresis of PCR amplified products on 1% gel. Lanes 1 and 2 used ITS 4 and 5 primers. Lanes 3 and 4 used MPK 1 and 2 primers. Lane 5 is ladder. Lane 6 and 7 used SSU rRNA primers. S: PCR reaction using fungal DNA as template; -ve: PCR reaction using water instead of fungal DNA (negative control)

Multiple sequence alignment

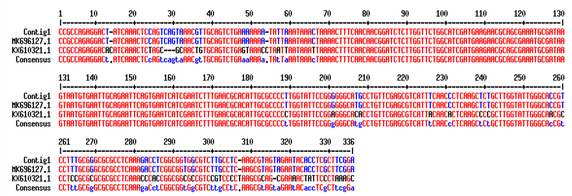

Figure 6: Multiple sequence alignment of the ITS sequence generated in this project (contig 1) with that of *Macrophomina phaseolina* (MK696127.1) and Cladosporium (KX610321) sequences. Red color indicates identity among the three sequences. Blue color represents identity between 2 sequences.

Phylogenetic tree

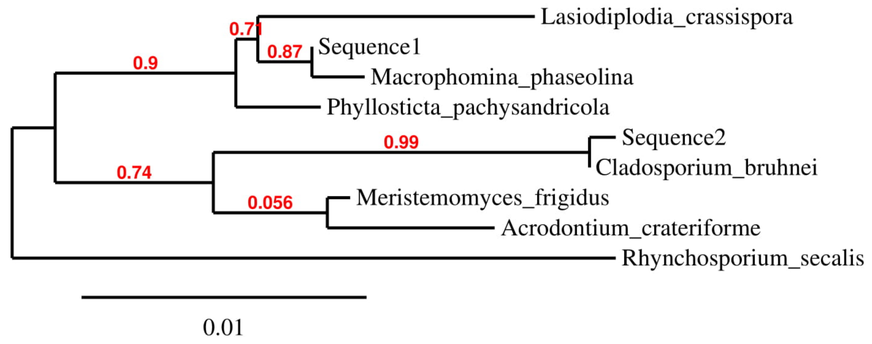

Figure 7: Maximum-likelihood tree based on the SSU sequences generated in this project with closely related fungal species. Bootstrap values for 100 replicates are indicated in red. Scale bar: number of substitution per site.

Key findings

The gene sequence of the fungi DNA that was amplified using the two set of primers; ITS 4 and 5 and MPK 1 and 2 both showed over a hundreds of matches in the NCBI database with a 100% query cover and identity cover to *Macrophomina paseolina*

Sequencing of the fungi DNA with SSU rRNA primers showed two distinct sequencing groups

- Sequence1 had 6 matches to *Macrophomina paseolina* in the NCBI database , with the highest match at a 99.2% identity to strain CBS 227.33
- Sequence2 had a 97% identity to Macrophomina paseolina strain CBS 227.33, but a 100% match to *Cladosporium*

Overall, our sequencing results confirmed the identity of *Macrophomina paseolina* and showed that it is a common strain. In addition, we identified the presence of *Cladosporium*, a fungi commonly found on dead leaf tissue, to be present in our infected chia samples.

Further directions

Testing the antibacterial potency of essential oil extracted from chia seeds against *Macrophomina phaseolina.*

Acknowledgements

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References

1. Muñoz, L. A., Cobos, A., Diaz, O., & Aguilera, J. M. (2013). Chia seed (Salvia hispanica): an ancient grain and a new functional food. *Food reviews international*, *29*(4), 394-408.

2. Elshafie, H. S., Aliberti, L., Amato, M., De Feo, V., & Camele, I. (2018). Chemical composition and antimicrobial activity of chia (Salvia hispanica L.) essential oil. *European Food Research and Technology*, *244*(9), 1675-1682. (about chia essential oil)