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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 fiber1.

Chia field trials 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
- Sequencing of the fungi DNA with SSU rRNA primers showed two distinct sequencing groups
  - Sequence 1 had 6 matches to *Macrophomina phaseolina* 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 phaseolina* strain CBS 227.33, but a 100% match to *Cladosporium*

**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 phaseolina*

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**References**