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Response of sunflowers to *Phomopsis* culture filtrates

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Outline

- Introduction
- Rationale
- Research objectives
- Methodology
- Results
- Summary

Introduction

- Production of a phytotoxin “phomozin” by *Phomopsis helianthi* isolates have been demonstrated during infection on sunflowers

  (Mazars et al. 1990)

- Purified two phytotoxic metabolites (*cis*- and *trans*-4,6-dihydroxymellein) from cultures of French and Italian of *P. helianthi* isolates with varying degrees of virulence

  (Avantaggiato et al. 1999)

Rationale

- ✓ Production of phytotoxic compounds by U.S isolates of *Phomopsis* is not confirmed
- ✓ Sensitivity of sunflower to toxic metabolites (if present) remains poorly studied
- ✓ These compounds when characterized,
  - ✓ Could complement conventional inoculation methods
  - ✓ A tool for screening sunflower genotypes for resistance to *Phomopsis*

    Eg: *Rhizoctonia solani* and potato (Zhang et al. 2021)

Objectives

1. To determine the production of any phytotoxic metabolites by the U.S. isolates of *P. helianthi* and *P. gulyae*

2. To evaluate the sensitivity of sunflowers to the phytotoxic metabolites (if present) in the crude culture filtrates of *Phomopsis*

Objective 1

To determine the production of any phytotoxic metabolites by the U.S. isolates of *P. helianthi* and *P. gulyae*

Untargeted metabolomics

- Goal: To comprehensively analyze all detectable metabolites, both known and unknown in the culture filtrates of *Phomopsis*

- Three isolates each of *P. gulyae* and *P. helianthi*

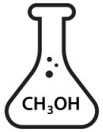
Sl. No.	Isolate	Species
1	18-OP-KOC-DIA-59 (DH1)	*P. helianthi*
2	18-OP-SF-DIA-131 (DH2)	*P. helianthi*
3	19-OP-SF-DIA-145 (DH3)	*P. helianthi*
4	16-OP-SF-DIA-66 (DG1)	*P. gulyae*
5	19-OP-SF-DIA-73 (DG2)	*P. gulyae*
6	16-OP-KOC-DIA-40 (DG3)	*P. gulyae*

Methodology

Potato dextrose Broth

1. Samples preparation

Ethyl acetate



Methanol

2. Metabolite extraction

AB Sciex API 4000 Triple Quad LC/MS instrument

3. Mass spectrometry

MZmine

4. Data acquisition

(Pang et al., 2021)

5. Feature selection

GNPS

gnps.ucsd.edu

SIRIUS

(Dührkop et al. 2020)

6. Identification of compounds

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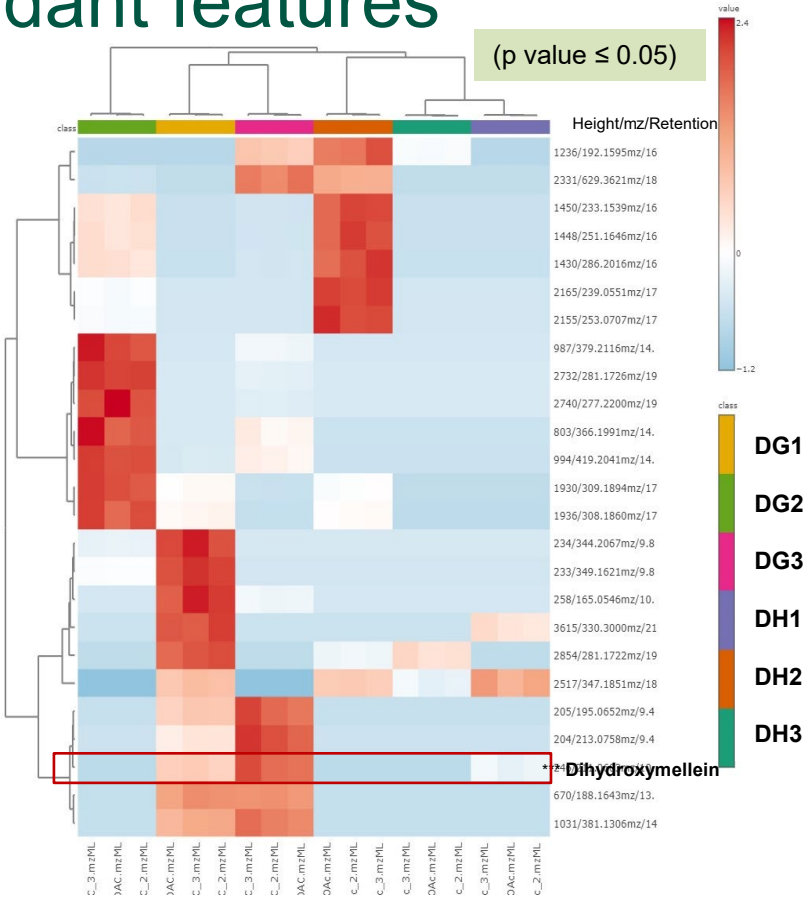
Heat map of top 25 most abundant features

- ✓ Rows - significantly expressed metabolites
- ✓ Columns – technical replicates of the six isolates
- ✓ Red-blue color scale indicates the log normalized intensity of the metabolites
- ✓ PDB without fungus was used as control

Inference:
4,6-dihydroxymellein significantly upregulated in the two isolates of *P. gulyae* (DG1 and DG 3) and downregulated in one *P. gulyae* isolate (DG2) and all *P. helianthi* isolates



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Objective 2

To evaluate the sensitivity of sunflowers to the phytotoxic metabolite present in the crude culture filtrates of *Phomopsis*

Greenhouse study

- Two experiments – different inoculation methods
  - **Mycelial contact method**
  - **Culture free filtrate inoculation**
- Isolates used:

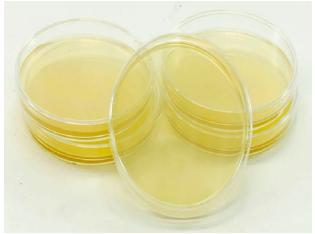
Sl. No.	Isolate	Species	State
1	18-OP-KOC-DIA-59	*P. helianthi*	MN
5	16-OP-SF-DIA-40	*P. gulyae*	SD

- Completely randomized design
- Three week old sunflower plants of a *Phomopsis*-susceptible variety N4HM354 (Nuseed genetics)
- Experiment conducted two times, six plants (replication) for each treatment

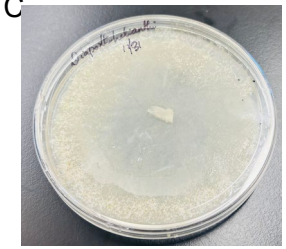
Inoculum preparation

Leaf inoculation using mycelial plugs

Potato Dextrose Agar



Incubate at 23° C for 10 days



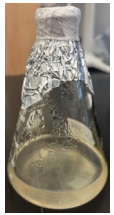
6 mm mycelial plugs

Culture free filtrate infiltration

10 mm mycelial plugs (10 days old)

Potato Dextrose Broth

Incubate as stationary cultures at 20° to 25° C for three weeks in dark



Whatman no. #1 Filter paper

Crude culture free filtrate

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Inoculation Methods

Mycelial contact method

Mycelial inoculation - Affixing mycelial plugs with tape

Culture free filtrate Infiltration

Culture-free filtrate inoculation - 200 µl filtrate delivered with a needle less syringe

- V3 growth stage
- On top most fully opened leaf

Greenhouse conditions

- Temperature regime - 23±2°C
- Light conditions - (16 h photoperiod)
- Relative humidity – 50 - 73%

Examination of symptoms

- After inoculation plants examined daily for necrosis

Necrosis refers to dead tissue visible after 3 to 4 days post-inoculation surrounding small, brown to black colored spot (Lamari and Bernier 1989)

Response of sunflower to *Phomopsis* filtrates

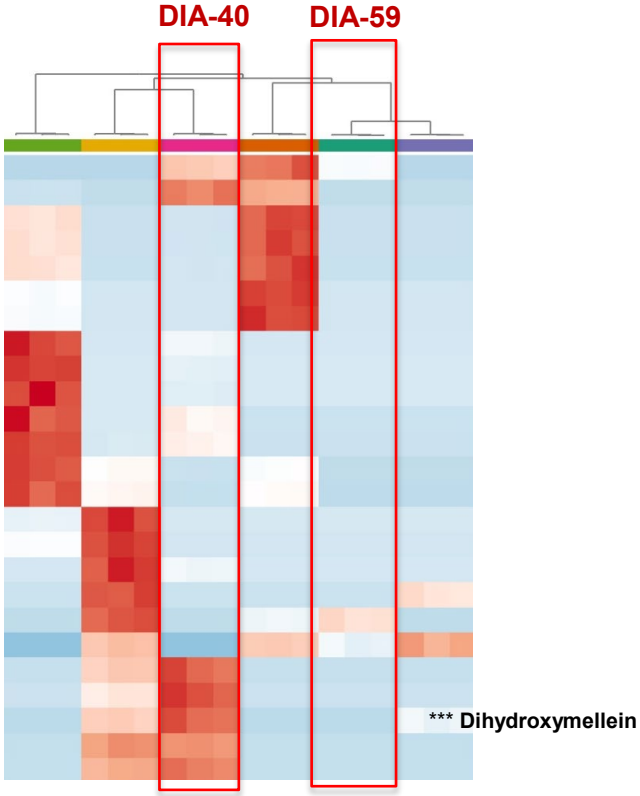
Culture free filtrate infiltration

Mycelial inoculation

16-OP-KOC-DIA-40

18-OP-KOC-DIA-59

Control



Culture filtrate of *Phomopsis* isolate (16-OP-KOC-DIA-40) with upregulation of 4, 6- dihydroxymellein produced larger necrotic lesion on the sunflower leaves

Summary

- Untargeted metabolomics
  - Top 25 significantly expressed metabolites in the culture free filtrates of *Phomopsis* included 4,6-dihydroxymellein
- Greenhouse study
  - Phomopsis isolate with upregulation of 4, 6- dihydroxymellein produced larger necrotic lesion on the sunflower leaves
  - Suggest possible role of 4, 6 – dihydroxymellein in the necrotic symptoms developed during stem canker development

(Avantaggiato et al. 1999)

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THANK YOU