ABSTRACT: 241

METABOLOMIC STUDIES FOR THE INTERACTION GLYCINE MAX-
FUSARIUM TUCUMANIAE

SCANDIANI M M1,2, LUQUE A2, O’DONNELL K3, AOKI T4, SPAMPINATO C5 &
CERVIGNI G5

1Laboratorio Agrícola Río Paraná, Ruiz Moreno 225, San Pedro, 2930, Argentina,
2CEREMIC, Rosario, 2000, Argentina, 3Microbial Genomics and Bioprocessing Research
Unit, NCAUR, USDA, ARS, Peoria, Illinois 61604, U.S.A. 4NIAS, 2-1-2 Kannondai, Tsukuba,
Ibaraki, 305-8602, Japan, 5CEFOBI-Fac. Ciencias Bioquímicas y Farmacéuticas, UNR,
Rosario, 2000, Argentina
E-mail: labagricola@sanpedro.com.ar

Sudden death syndrome (SDS) of soybean can be caused in Argentina by 4 different
Fusarium species: F. brasiilense, F. crassistipitatum, F. tucumaniae and F. virguliforme. Fusarium tucumaniae and F. virguliforme are the primary etiological agents of soybean SDS
in Argentina and United States, respectively. The resistance mechanism to these pathogens in
soybean is complex. Metabolomic technology was explored to phenotype resistance to F.
tucumaniae. Two soybean cultivars (CV) with contrasting levels of resistance to SDS were
inoculated with an isolate of F. tucumaniae grown on sorghum grain, using the layer method.
Uninoculated controls were included for both genotypes. Plants were grown for 7, 10, 14 and
25 days. Four independent biological replicates were harvested at indicated times.
Foliar disease, root rot incidence and severity, shoot height, shoot and root weights were rated at
each time. Areas under disease severity progress curves were also calculated 25 days after
inoculation (DAI). All data were subjected to statistical analysis. Means were compared by
least significant differences (p<0.05). The resistant cultivar showed lower foliar disease and
root rot incidence and severity, higher plant height, and root and shoot weights, than the
susceptible cultivar. Uninoculated controls remained healthy. Experiments were extended to
root metabolite profiling by gas chromatography mass spectrometry (GC-MS). Metabolite
levels were normalized to the ribitol internal standard. Compounds were putatively identified
by comparison of their retention index and mass spectrum with those present in the
commercial mass spectra library NIST. Data from two biological replicates from tolerant and
susceptible CVs obtained at 7, 10, 14 and 25 DAI were evaluated by principal components
(PC) and principal coordinates analysis (ACoP). Analyses were either performed for
individuals or pools. Results obtained allow us to identify and monitor the relative levels of
30 metabolites, including amino acids, organic acids, soluble sugars, secondary metabolites,
inorganic and nitrogen compounds. These metabolites were more abundant at 7 and 10 DAI.
The first two PCs (PC1 and PC2) explained 76.97 %, 84.88% and 79.11% of the variance of
the susceptible, tolerant and pooled CV profiles, respectively. As these values are lower than
expected (> 90%), the first four PCs had to be considered. In the susceptible CV, inorganic
phosphate and sucrose showed the highest weight in PC1 and PC2, respectively. However, in
the tolerant CV, inositol and sucrose were the most important variables in PC1 and malonic
acid, citric acid, inorganic phosphate and myoinositol heavily contributed to PC2. When these
data were pooled and analyzed, inorganic phosphate and sucrose were associated with PC2
but no variables could be identified in PC1. In accordance with PC analysis, the first two
ACoP factors (F1 and F2) explained the 63.90%, 67% and 43.34% of the variance of the
susceptible, tolerant and pooled CV profiles, respectively. Four DAI metabolic profiles could
be clearly separated in the tolerant CV, but 7 and 10 DAI remained close together in the
susceptible CV and in the pool analyses.
METABOLOMIC STUDIES FOR THE INTERACTION *Glycine max*-
*Fusarium tucumaniae*

M. Mercedes SCANDIANI¹,², Alicia LUQUE², Kerry O DONNELL³,
Takayuki AOKI⁴, Claudia SPAMPINATO⁵, Gerardo CERVIGNI⁵

¹Laboratorio Agrícola Río Paraná, Ruiz Moreno 225, San Pedro, 2930, Argentina,
²CEREMIC, Rosario, 2000, Argentina, ³Microbial Genomics and Bioprocessing Research Unit, NCAUR, USDA, ARS, Peoria, Illinois 61604, U.S.A. ⁴NIAS, 2-1-2 Kannondai, Tsukuba, Ibaraki, 305-8602, Japan, ⁵CEFOBI-Fac. Ciencias Bioquímicas y Farmacéuticas, UNR, Rosario, 2000, Argentina,
E-mail: labagricola@sanpedro.com.ar
Introduction

Sudden death syndrome causal agents

- *F. tucumaniae*
- *F. virguliforme*
- *F. brasiliense*
- *F. crassistipitatum*

SDS has increased in prevalence and severity resulting in significant yield losses to Argentinean soybean farmers.

Yield losses vary considerably depending on several factors, but yield losses up to 41% were estimated in a trial conducted during the 2010/2011 growing season.

The use of resistant cultivars is one of the main measures for managing SDS. The resistance mechanism to these pathogens in soybean is complex.

Screening for SDS resistance has been conducted under field conditions, both in natural and artificially infested soil. Even when cultivars are screened in artificially infested soil, disease development is unpredictable due to the sensitivity of symptomology to environmental factors.
Isolating and identification of sudden death syndrome-causing *Fusarium* species

Soybean plant exhibiting typical SDS symptoms

Isolating (two protocols):
- Desinfecting tissues
- Direct macroconidia transferring

Determine growth rate on PDAS:
1) Fast growing → Discard those colonies
2) Slow growing (≤ 2 cm in 4 days) → Keep those colonies

**Identification**

* Colour
* Conidia

**F. solani**

(F. solani (clade 3), Macroconidia and microconidia abundant on long conidiophores (x1000).

**F. crassistipitatum**

1) yellow

**F. tucumaniae**

2) blue-green

**F. virguliforme**

**F. brasiliense**

* Microconidia are rarely observed

**F. tucumaniae** produces macroconidia longer and slender than **F. virguliforme**, this produces comma-shaped microconidia.

**F. brasiliense**

Macroconidia shorter and wider than **F. tucumaniae**.

Pathogenicity test
Symptoms of Sudden Death Syndrome

Foliar symptoms of SDS.
©Lisandro Lenzi INTA-EEA Marcos Juárez (Córdoba).

Root rot with blue sporulation.
Field symptoms of Sudden Death Syndrome
Main objective

Metabolomic technology was explored to phenotype resistance to *F. tucumaniae*.

Specific objectives

1. To identify which metabolites are produced during the host-pathogen interaction *Glycine max-F. tucumaniae*, in susceptible and resistant genotypes;

2. To determine the starting point of this response and the occurrence of metabolic changes during the experiment, on roots and shoots;

3. To compare the metabolic profiles in these tissues and identify the metabolic pathway of each one.
This study was conducted to detect and quantify metabolomic changes and identify potential biomarkers associated with this interaction.

Materials and methods

Disease rating

Root and shoot metabolite profiling by GC-MS (gas chromatography mass spectrometry)

Sorghum grain

Layer method

Resistant

Susceptible

5 seeds/pot
4 biological replicates (BR, each one of 5 plants)

Foliar disease, root rot incidence and severity, shoot height, shoot and root weights were rated at each time. Areas under disease severity progress curves were also calculated 25 days after inoculation. All data were subjected to statistical analysis. Means were compared by least significant differences (p<0.05).

Analysis by GC-MS (4 technical replicates)
Root metabolite profiling by GC-MS. Metabolite levels were normalized to the ribitol internal standard. Data from two BR were evaluated by principal components and principal coordinates analysis.
Compounds were identified by comparison of their retention index and mass spectrum with those present in the commercial mass spectra library NIST.
The resistant cultivar showed lower incidence than the susceptible cultivar.

Incidence (% plants with foliar symptoms)
The resistant cultivar showed lower foliar disease severity than the susceptible cultivar.

Foliar disease severity

AUDPCs Susceptible 492
Resistant 158
10 days after-inoculation

Resistant

Susceptible

Control

Treatment
The resistant cultivar showed lower root rot severity than the susceptible cultivar. Uninoculated controls remained healthy.
**Plant height**

**Susceptible CV**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Resistant CV**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (days)</td>
<td>Treatment (g)</td>
<td>Control (g)</td>
</tr>
<tr>
<td>------------</td>
<td>---------------</td>
<td>-------------</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Susceptible CV**

**Resistant CV**

**Shoot weight**

![Graph showing shoot weight over time for susceptible and resistant CVs.](image)
Root weight

<table>
<thead>
<tr>
<th>Time after inoculation (days)</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Susceptible CV</td>
<td>10</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Resistant CV</td>
<td>10</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>
30 metabolites were identified and quantified

- Sugars (xilulose, glucose, fructose and sucrose)
- Alcohols (arabitol, glycerol, inositol and myoinositol)
- Organic and inorganic acids (lactic, malonic, butanoic, fumaric, succinic, galactaric, citric and phosphoric acids)
- Lipids (palmitic and estearic acids and monoesters with glycerol)
- Aminoacids (Leu, Pro, Gly, Val, Ala, Ser, Asp and Asn)
- Nitrogen compounds (cadaverine, putreanine and urea)

Major differences in sugar, aminoacid and organic and inorganic acid levels were observed at 7 and 10 DAI.
Metabolite Profiling - roots

Galactaric acid

Susceptible CV

- Treatment
- Control

Tolerant CV

- Treatment
- Control

Resistant CV

- Treatment
- Control

Arabitol

Susceptible CV

- Treatment
- Control

Resistant CV

- Treatment
- Control

<table>
<thead>
<tr>
<th>Time after inoculation (days)</th>
<th>7</th>
<th>10</th>
<th>14</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Susceptible CV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tolerant CV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Resistant CV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time after inoculation (days)</th>
<th>7</th>
<th>10</th>
<th>14</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Susceptible CV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tolerant CV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Resistant CV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>Treatment</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-----------</td>
<td>---------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.020</td>
<td>0.020</td>
<td>0.020</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leu</th>
<th>Treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**Susceptible CV**
- Treatment
- Control

**Resistant CV**
- Treatment
- Control

### Time after inoculation (days)
- 7
- 10
- 14
- 25

### Relative expression
- Treatment
- Control

**Pro**

<table>
<thead>
<tr>
<th>Time after inoculation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>14</td>
</tr>
<tr>
<td>25</td>
</tr>
</tbody>
</table>

**Leu**

<table>
<thead>
<tr>
<th>Time after inoculation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>14</td>
</tr>
<tr>
<td>25</td>
</tr>
</tbody>
</table>
Conclusions

- The resistant cv differed from the susceptible one in all the disease parameters evaluated.

- Metabolomics studies were initiated to identify potential early markers of sensitivity to *F. tucumaniae*.

- There were no variations in the levels of organic acids, sugars and second messengers in roots due to treatment.

- However, higher levels of nitrogen compounds, phosphate and glycerol were observed in roots of the susceptible cultivar at the 7th day post-inoculation.
Further works

- Metabolic changes in shoots will be also studied.

- Considering the observations in roots, we will extend our studies to include the metabolomic changes occurring at earlier stages of the disease (<7 days) and other plant-pathogen interactions.
Thanks!

Ma. Valeria Razori
Lucila Ciancio
Claudio Cervigni
Claudia Spampinato
Mónica Hourcade

Takayuki Aoki
Donnell

Mercedes Scandiani
Lab Agrícola Río Paraná
San Pedro

E-mail: labagrícula@sanpedro.com.ar