Molecular Biology and Etiology of FOV in Cotton

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Race 1 lineage: US

Race 8 lineage: AL, AR, CA, GA, LA, MO, and SC

Race 4 lineage: AL, CA, and MS

Race 3 lineage: CA, LA, and TX

Australian biotype lineage: AU

Liu et al., Can. J. Microbiol., 2011
<table>
<thead>
<tr>
<th>Australian Biotype and Race 4</th>
<th>U.S. race (Races 1, 2, &amp; 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Attacks cotton in heavy clay soil</td>
<td>1. Attacks cotton in neutral or acidic sandy soil</td>
</tr>
<tr>
<td>2. Nematodes are not required for infection</td>
<td>2. Severe wilt usually occurs in soils heavily infested with nematodes</td>
</tr>
<tr>
<td></td>
<td>Greater than 50% of U.S. (from Texas to California) cotton is grown on heavy clay soil currently not infested with nematodes</td>
</tr>
<tr>
<td>3. Attacks at the seedling stage</td>
<td>3. Attacks seedlings, but usually attacks plants later in the season</td>
</tr>
<tr>
<td>4. Stem puncture inoculation is ineffective for disease expression (primarily rots the roots)</td>
<td>4. Stem puncture inoculation is effective for disease expression (Primarily invades the vascular system)</td>
</tr>
<tr>
<td>5. &gt;70% of the isolates produce &gt;1500mg/L of <strong>fusaric acid</strong> in defined media</td>
<td>5. &gt;50% the isolates produce &lt;420mg/L of <strong>fusaric acid</strong> in defined media</td>
</tr>
</tbody>
</table>
Inoculation
Stem Puncture vs. Root Dip

Australian Biotype

U.S. Race 1 Lineage

Stem Puncture Inoculated

Root Dip Inoculated

Root Rot

Vascular Invasion
Depsipeptide Phytotoxins from Fov

- Cation chelating agent
- Ionophore
- Antibiotic
- Toxic to tomato protoplasts at 50 and 100 μM, but no effect were observed for the germinating maze seedling at concentration up to 100 μM. Causes wilting in banana plantlets.
- Produced by both the vascular incompetent and competent groups, but rarely in avirulent isolates.
Polyketide Phytotoxins from *Fov*

Nonaketide: bikaverin.
- Free radical generator.
- Oxidative phosphorylation uncoupling.
- General antibiotic.
- Effect on plants?

Heptaketides: nectriafurone, 5-O-methyljavanicin, and anhydrofusarubin lactol.
- Free radical generator.
- Cation chelating agent.
- Phytotoxic to peas and citrus.

Triketide: fusaric acid.
- Potent phytotoxin for many plant species. Five minute root-dip in 70 ppm fusaric acid causes severe wilt of cotton.
Polyketide Phytotoxins from *Fov*

Produced by U.S. *Fov* race 1 lineage

- 5-O-Methyljavanicin
- Anhydrofusarubin Lactol
- Nectriafurone

Produced by Australian *Fov* biotype and race 3 and race 4 lineages

- Bikaverin: $R = -\text{CH}_3$
  - Norbikaverin: $R = -\text{H}$
- Fusaric Acid
- Heptaketide
- Nonaketide
- Triketide
## FA production by different *Fov* race lineage group isolates

<table>
<thead>
<tr>
<th>Lineage group</th>
<th>Number of isolates</th>
<th>FA production (mg/L of culture)</th>
<th>Range</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race A</td>
<td>18</td>
<td></td>
<td>0 - 2158</td>
<td>415</td>
</tr>
<tr>
<td>Race 3</td>
<td>6</td>
<td></td>
<td>1054 - 4020</td>
<td>1531</td>
</tr>
<tr>
<td>Race 4</td>
<td>5</td>
<td></td>
<td>653 - 2101</td>
<td>1783</td>
</tr>
<tr>
<td>Race 8</td>
<td>3</td>
<td></td>
<td>635 - 1119</td>
<td>1000</td>
</tr>
<tr>
<td>Aust Bio</td>
<td>4</td>
<td></td>
<td>2192 - 3781</td>
<td>2566</td>
</tr>
</tbody>
</table>
• Fusaric acid has been implicated in the pathogenesis of Fusarium wilt for a number of other plant species including tomato, banana, watermelon, and flax. Nevertheless, controversies exist regarding the role of fusaric acid in pathogenesis.

• To unequivocally prove the role of fusaric acid in pathogenicity, we identified and cloned a polyketide synthase gene (PKS) as well as an amino acid kinase gene involved in the biosynthesis of fusaric acid.
**PKS Aspartate Kinase**

**Fua1 Domain Structure**

(Fua1 2410 aa)

Diagram showing the domain structure of Fua1 and Fua2 proteins with PKS and Aspartate Kinase domains labeled. The Fua1 domain structure includes KS, AT, DH, ER, and KR domains, with an ACP at the end. There is also a chemical structure diagram showing the interaction of a methyl group and a carboxylate ion.
Knock-out mutants for fusaric acid synthesis genes were generated by targeted gene disruption.

A PEG mediated fungal transformation procedure was used.

**Fua1 Disruption**
We generated both $\Delta Fua1$ (PKS-disruption) and one $\Delta Fua2$ (Aspartate kinase-disruption) mutants. Both of them lost the ability to produce fusaric acid as expected.
Pathogenicity Assay with Tomato Seedling

- Czapek-Dox agar media plated with WT and gene disrupted mutants of Australian *Fov* isolates: $5 \times 10^4$ conidia/plate.

- Five tomato (Rutgers) seeds/plate.

- $28^\circ C$ (day)/$24^\circ C$ (night) for 13 days.
Pathogenicity Assay with Coker 312

Inoculum with Cotton-Root Powder Assay (Al Bell)

- Inoculum

   Dry, fine sand: 50 g
   Dry cotton-root powder: 1.5 g
   Water: 10 ml
   Carrot juice: 5 ml
   Fov culture (agar plug): 4 mm
   One month incubation

3:1 mixture of sandy loam and fine sand soil
16 oz
Pathogenicity Assay with Coker 312

Assay A continued

- Coker 312 seeds pre-germinated on paper towel with 1-2 cm radical transplanted into inoculum core.

- 28°C (day)/24°C (night) for 5 weeks.
Pathogenicity Assay with Coker 312

Root Dip Assay (Jaemin Cho)

• Roots of two-week-old Coker 312 seedlings dipped in 10^6 conidia/ml in PDB culture filtrate or water (PDA plate flooded with water) for 5 min.

• Transplanted into 3:1 mixture of sandy loam and fine sand soil.

• 28°C (day)/24°C (night) for 5 weeks.
Disease symptom development after inoculation by root-dipping (5 min)

Fov11 (PDB)

35 Days after inoculation (tmt # 4 & 9)

Fov11 (PDA)
Mitigate the Effect of Fusaric Acid

• Identify and clone the fusaric acid transporter gene from the Australian biotype isolate and express it in cotton.

• Alternatively, identify and clone a fusaric acid-detoxifying gene from a microbial source and express it in cotton.
Fusaric Acid Transporter gene

1. We identified several major facilitator super-family (MFS) transporter genes in the fusaric acid biosynthetic gene cluster and flanking regions.

2. One or several of these transporters may be involved in the secretion of fusaric acid and thus may provide protection against the self-toxic effect of fusaric acid as well as excreting the phytotoxin into the environment, thereby reducing viability of competitors and aid in attacking hosts.
Develop Cotton Plants Resistant to Emerging Virulent Strains of Fov by Expressing a Fusaric Acid Detox-Gene

- Screen and identify microbial strains that can detoxify fusaric acid.

- Clone the fusaric acid-detoxifying genes.
  - A gene that detoxify fusaric acid in a single enzymatic step is highly desirable for easy introduction and expression in cotton.
  - Identified a non-phytotoxic fusaric acid analogue.

- Introduce the fusaric acid-detoxifying gene into cotton, test the resulting transformants for resistance against the newly emerging Fov isolates, and select the resistant plants.

\[ \text{Decarboxylase} \]

\[
\begin{align*}
\text{Decarboxylase} & \quad \text{Decarboxylase} \\
\text{Fusaric Acid} & \quad \text{Non-Phytotoxic Analogue}
\end{align*}
\]
Acknowledgements

We thank Cotton Incorporated for their support of this research.