

Molecular Biology and Etiology of FOV in Cotton

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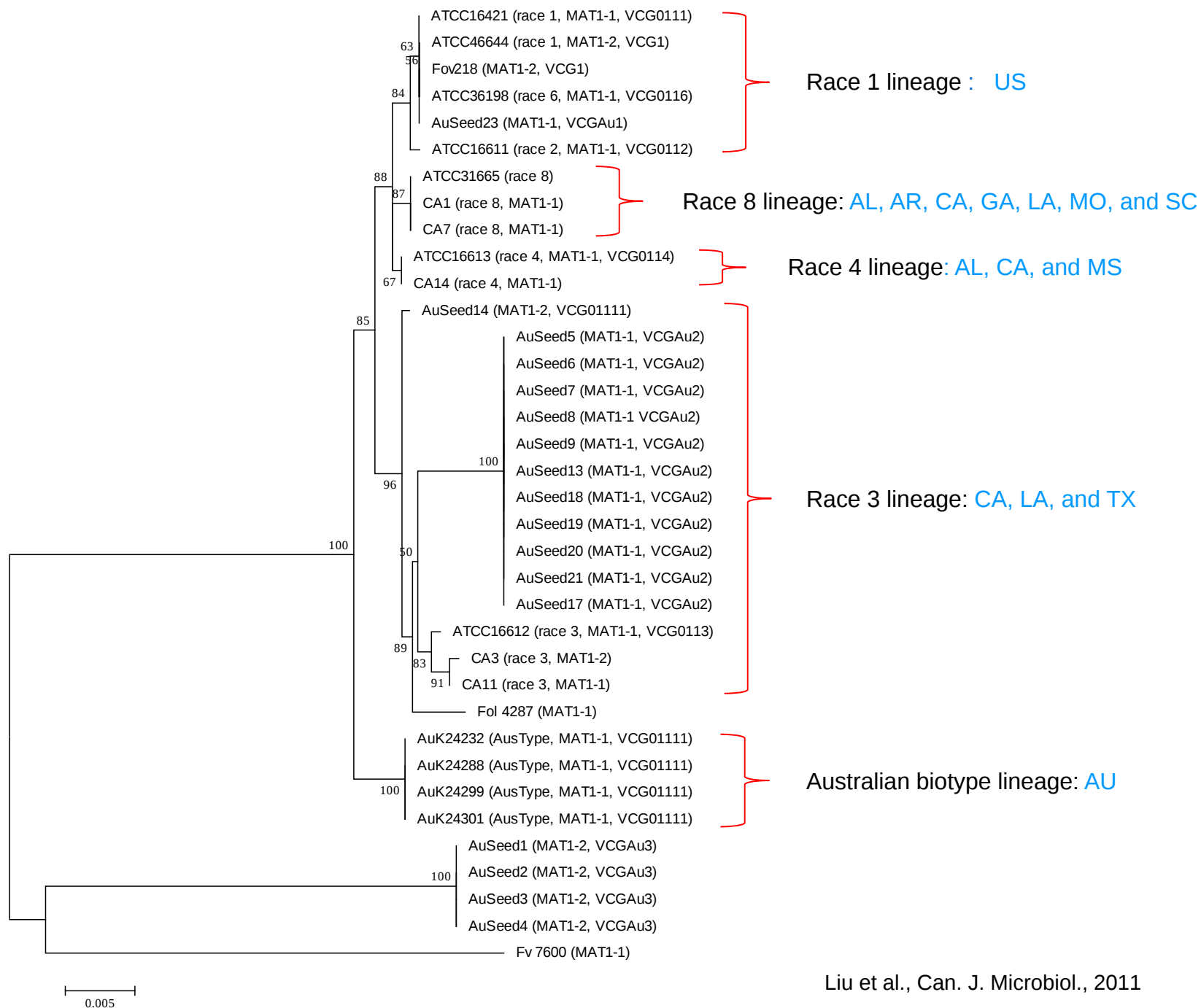
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Differences Between U.S. and Australian Biotypes, Race 4

Australian Biotype and Race 4

1. Attacks cotton in heavy clay soil
2. Nematodes are not required for infection

Greater than 50% of U.S. (from Texas to California) cotton is grown on heavy clay soil currently not infested with nematodes

3. Attacks at the seedling stage
4. Stem puncture inoculation is ineffective for disease expression (primarily rots the roots)
5. >70% of the isolates produce >1500mg/L of **fusaric acid** in defined media

U.S. race (Races 1, 2, & 6)

1. Attacks cotton in neutral or acidic sandy soil
 2. Severe wilt usually occurs in soils heavily infested with nematodes
-
3. Attacks seedlings, but usually attacks plants later in the season
 4. Stem puncture inoculation is effective for disease expression (Primarily invades the vascular system)
 5. >50% the isolates produce <420mg/L of **fusaric acid** in defined media

Inoculation Stem Puncture vs. Root Dip

Australian Biotype

U.S. Race 1 Lineage

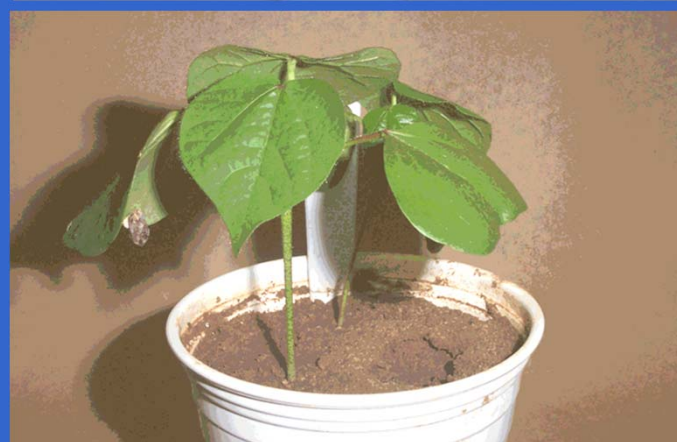
Stem
Puncture
Inoculated



Root
Dip
Inoculated

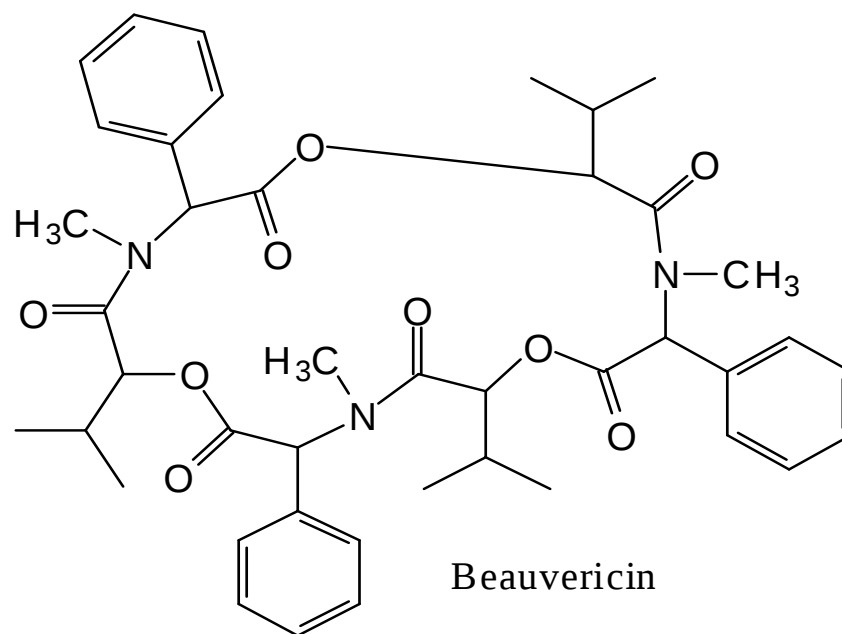


Root Rot



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 maize 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.

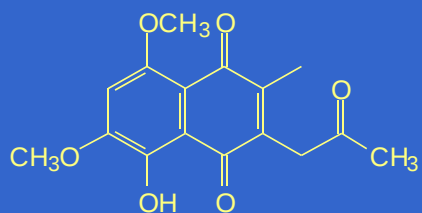
- Free radical generator.
- Cation chelating agent.
- Phytotoxic to peas and citrus.

Triketide: fusaric acid.

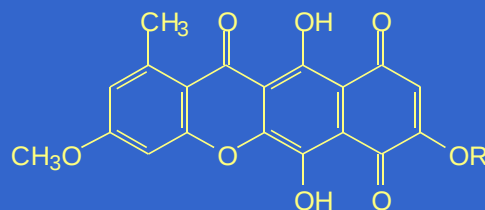
- Potent phytotoxin for many plant species. Five minute root-dip in 70 ppm fusaric acid causes severe wilt of cotton.

Polyketide Phytotoxins from *Fov*

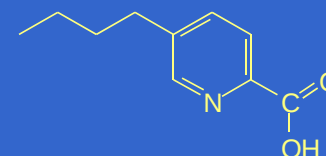
Produced by U.S.
Fov race 1 lineage



5-O-Methyljavanicin



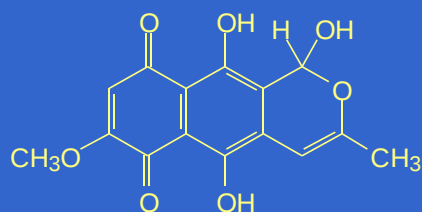
Bikaverin: R = -CH₃
Norbikaverin: R = -H



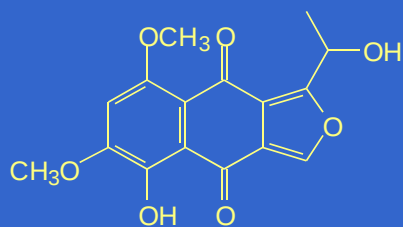
Fusaric Acid

Nonaketide

Triketide



Anhydrofusarubin Lactol



Nectriafurone

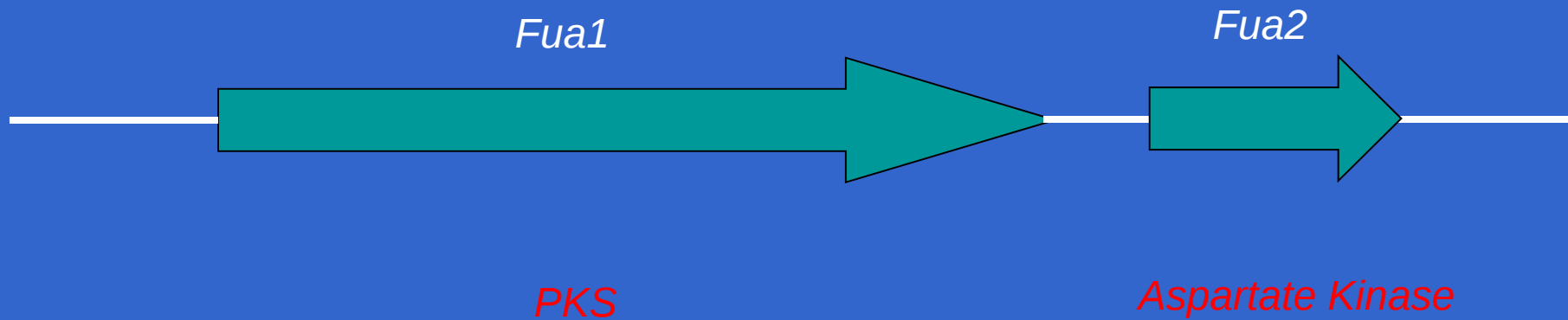
Heptaketide

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

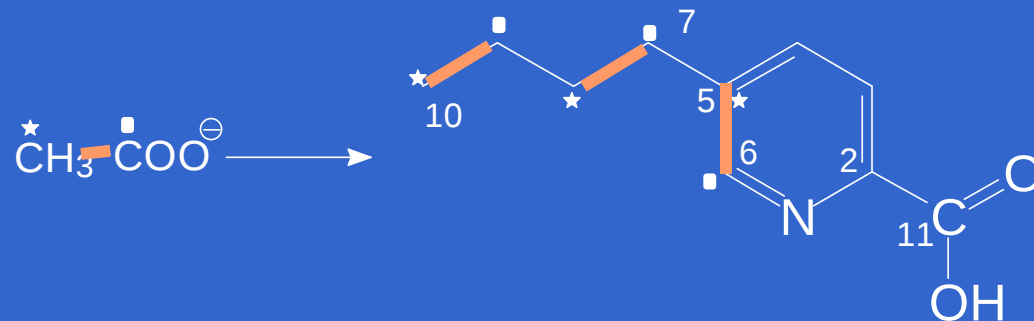
FA production by different *Fov* race lineage group isolates

Lineage group	Number of isolates	FA production (mg/L of culture)	
		Range	Median
Race A	18	0 - 2158	415
Race 3	6	1054 - 4020	1531
Race 4	5	653 - 2101	1783
Race 8	3	635 - 1119	1000
Aust Bio	4	2192 - 3781	2566

- 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.



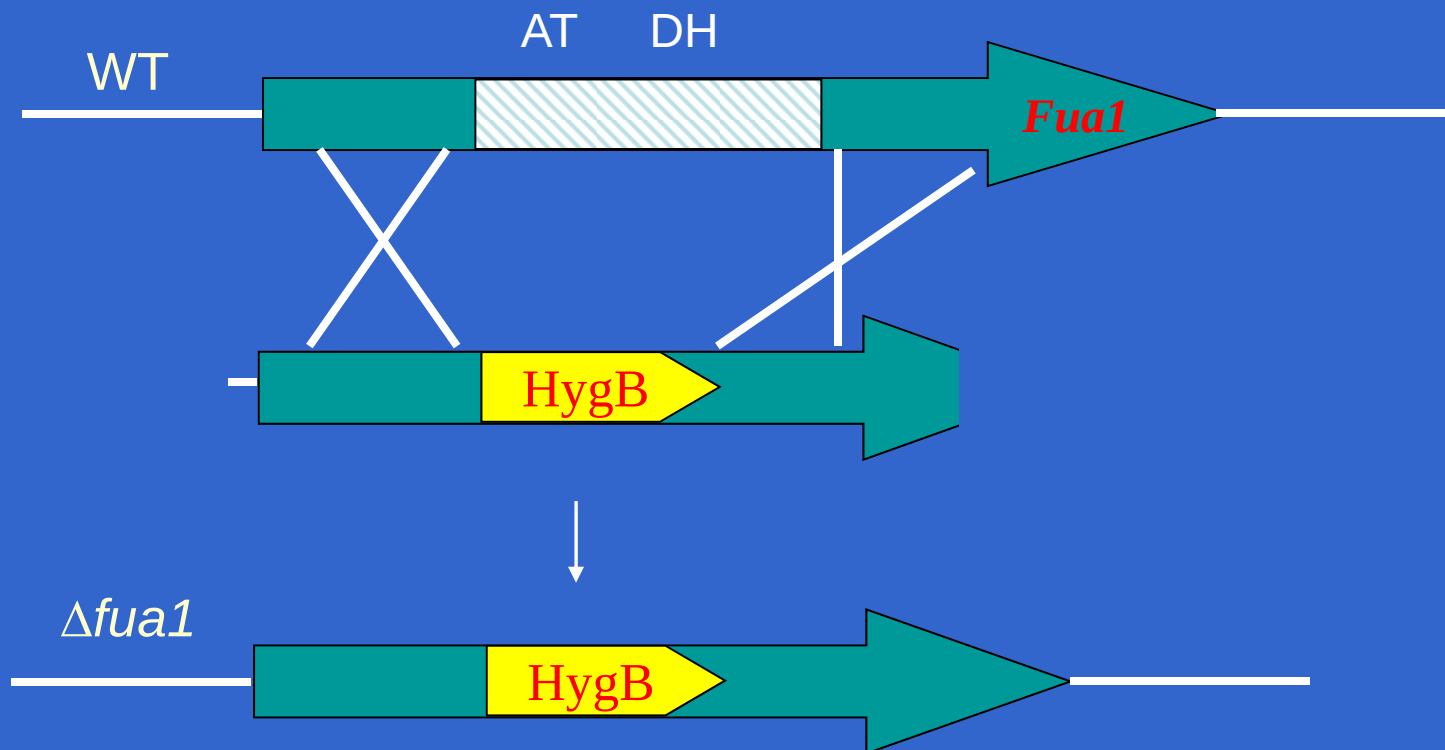
Fua1 Domain Structure (2410 aa)



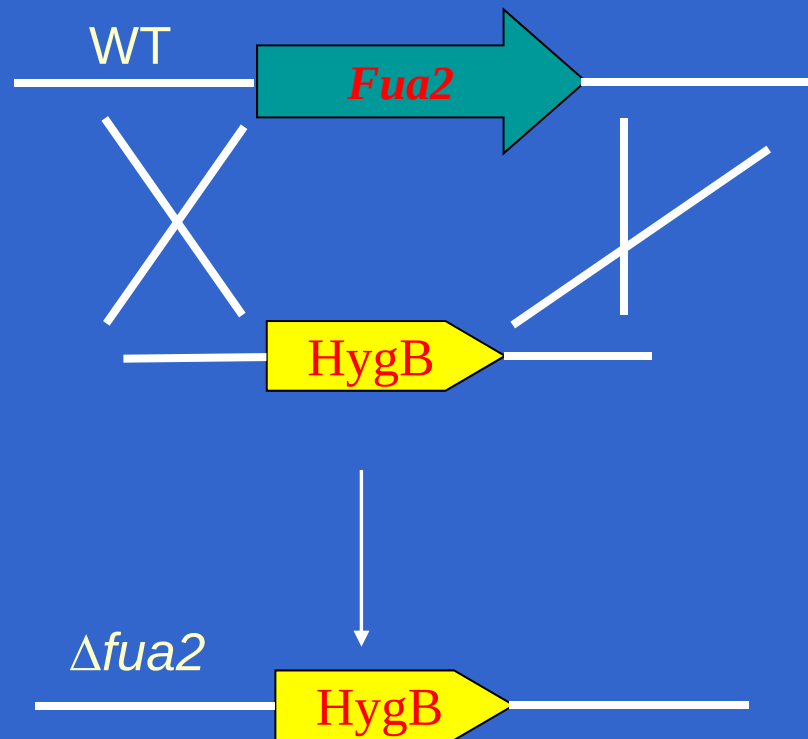
Targeted Gene Disruption

- Knock-out mutants for fusaric acid synthesis genes were generated by targeted gene disruption.
- A PEG mediated fungal transformation procedure was used.

Fua1 Disruption



Fua2 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: 5×10^4 conidia/plate.
- Five tomato (Rutgers) seeds/plate.
- 28°C (day)/24°C (night) for 13 days.



Wild-type — Back



Wild-type — Front



Fusaric acid knock out mutant — Back



Fusaric acid knock out mutant — Front

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	

1 x 2.5 inch
50 g

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.



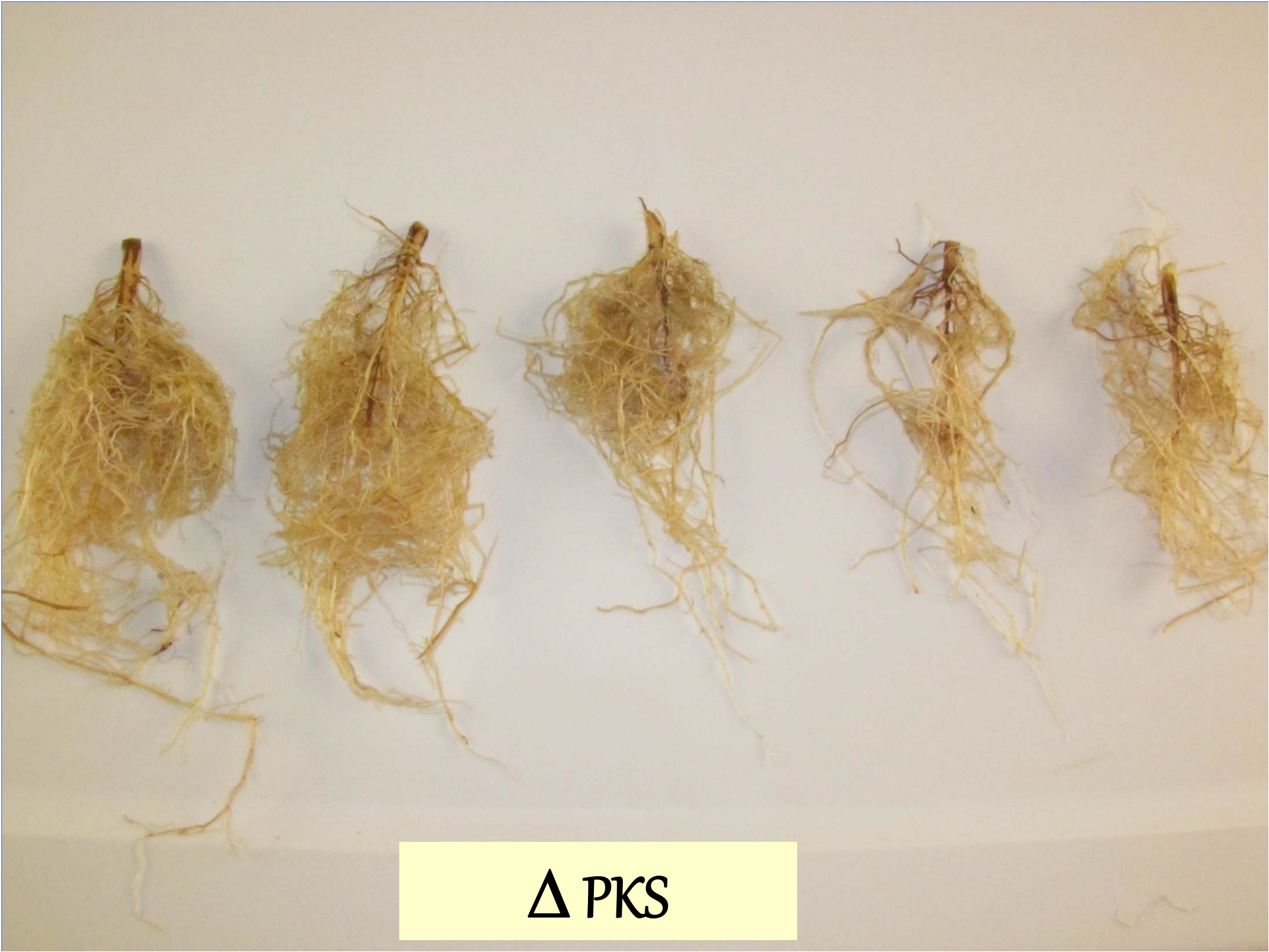












The image displays five plant root samples arranged horizontally on a light-colored surface. From left to right, the roots show a progression of decay. The first two roots on the left are relatively healthy, with a dense, fibrous structure and a yellowish-tan color. The third root shows more extensive decay, with a more irregular, clumped shape and some darker, brownish areas. The fourth and fifth roots on the right are severely affected, appearing much more sparse and fragmented, with many roots broken or missing, leaving only a few thin, remaining strands. This visual progression likely represents the stages of root rot or a similar fungal disease.

Δ PKS



△ Asp

1089



R-4



CA 9



CA 14

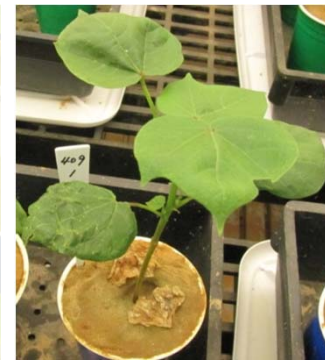


Pathogenicity Assay with Coker 312

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.

**Fov108
9
(PDB)**



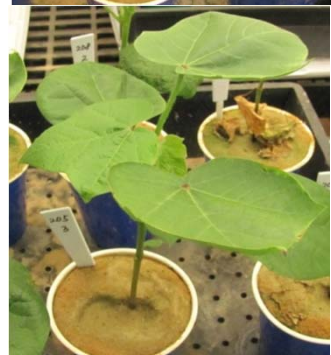
1/5

**Fov108
9
 ΔPks
(PDB)**



0/5

**Fov108
9
 ΔAsp
(PDB)**



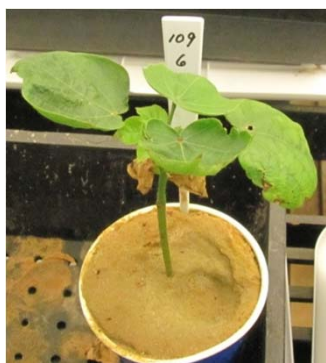
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**H₂O
Control**



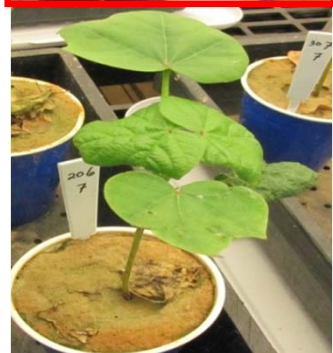
0/5

**Fov108
9
(PDA)**



1/5

**Fov108
9
 ΔPks
(PDA)**



0/5

**Fov108
9
 ΔAsp
(PDA)**



0/5

**H₂O
Control**



0/5

Disease symptom development after inoculation by root-dipping (5 min)

1

2

3

4

**Fov11
(PDB)**



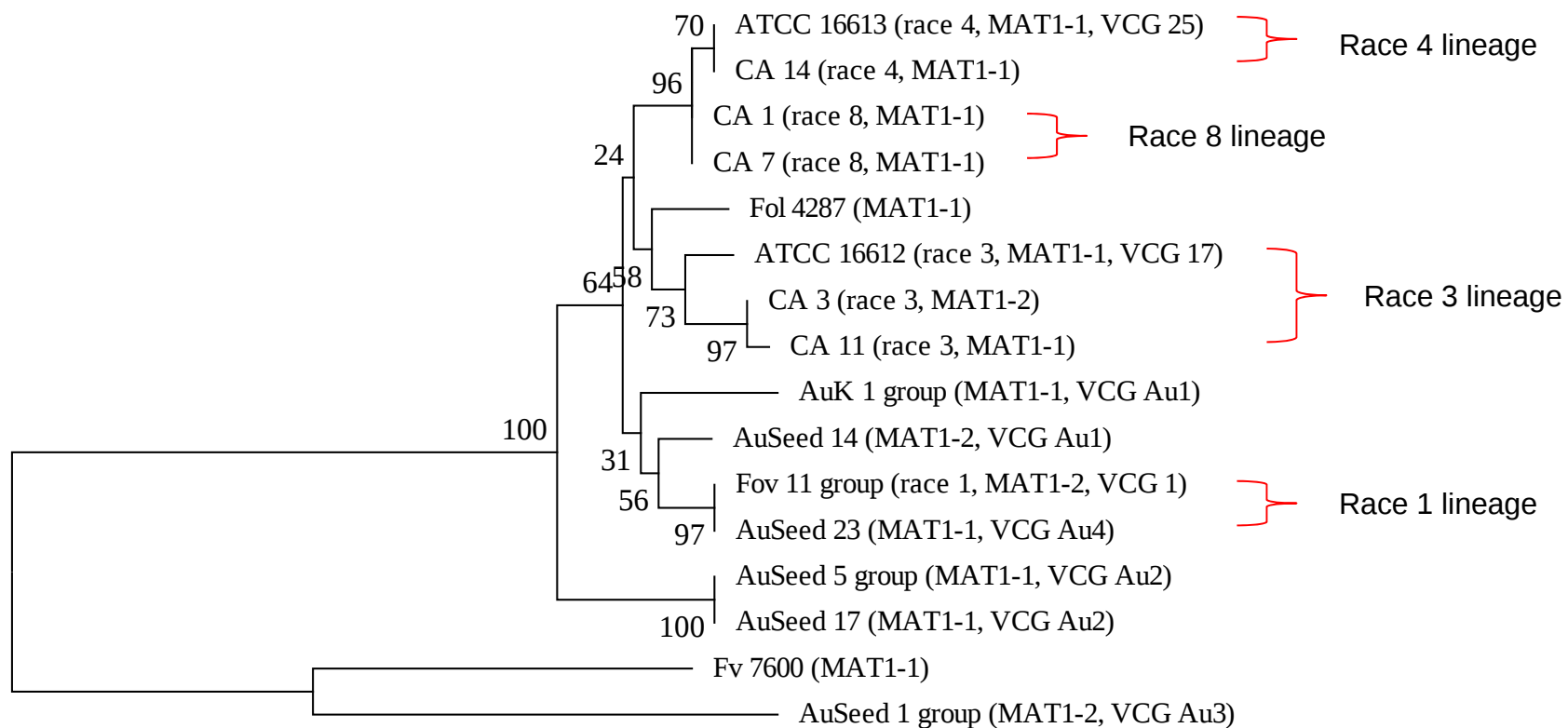
4/5

**Fov11
(PDA)**

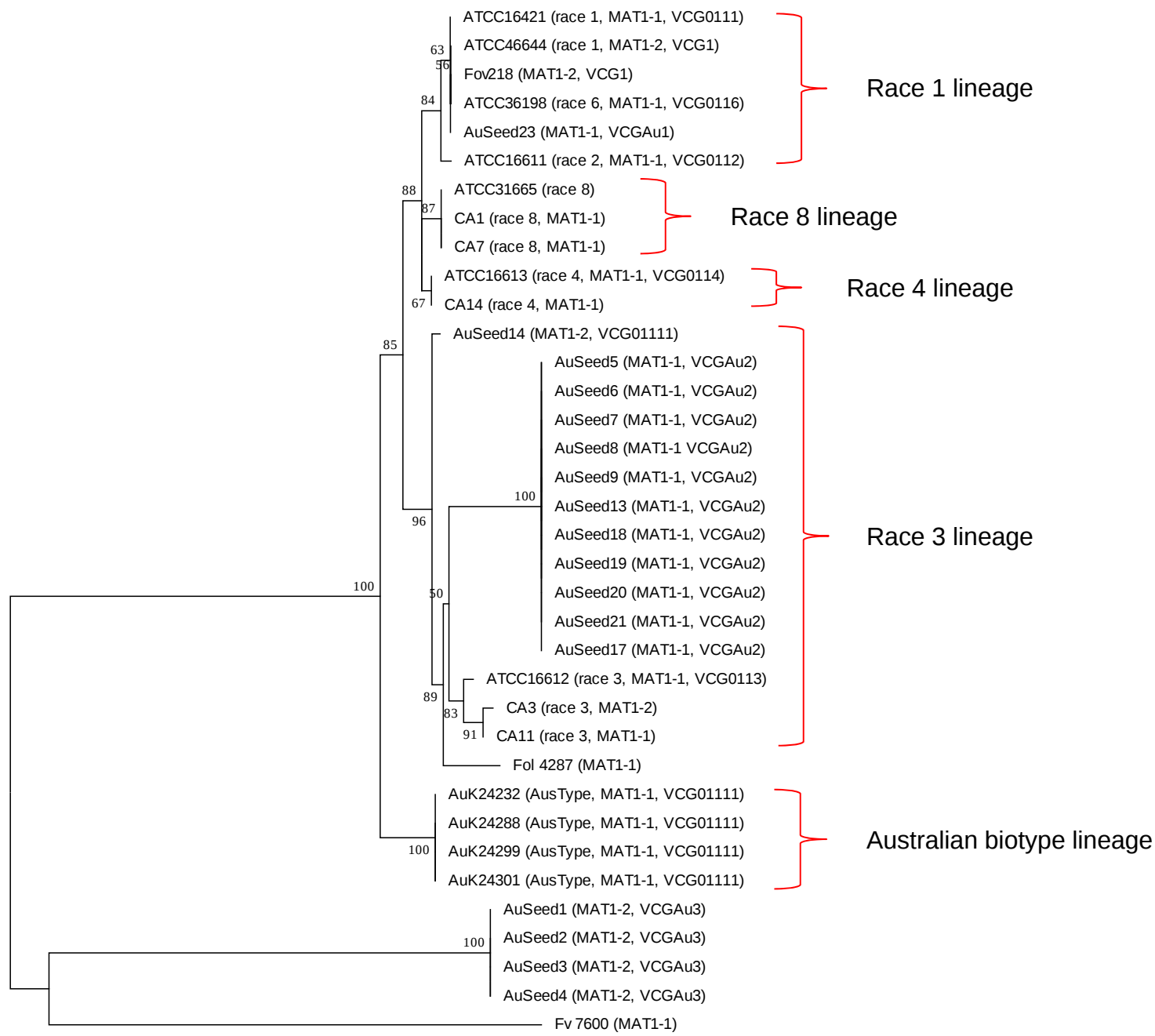


4/5

35 Days after inoculation (tmt # 4 & 9)



0.01



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.

Acknowledgements

We thank Cotton Incorporated for their support of this research.