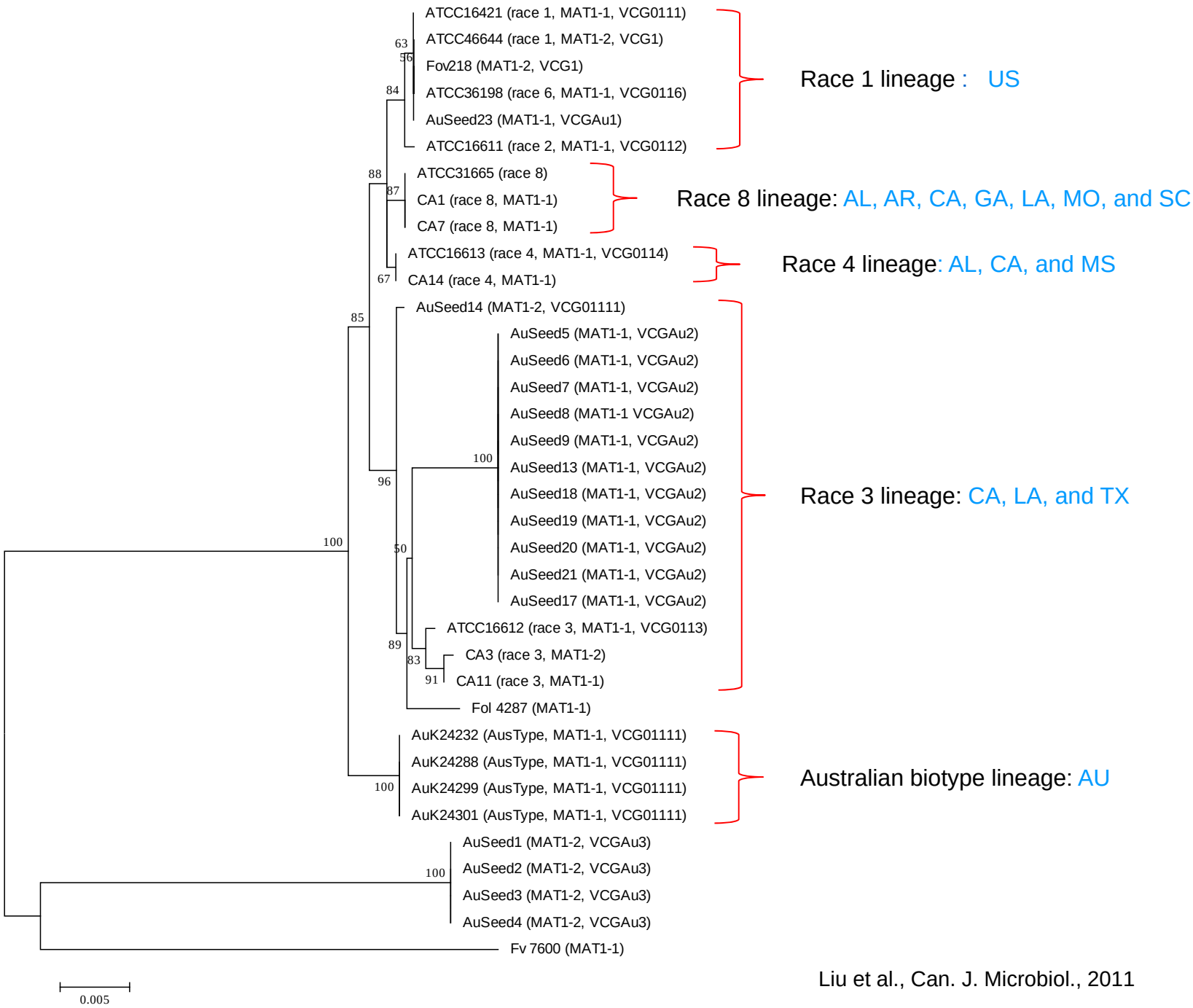
Molecular Biology and Etiology of FOV in Cotton

Jinggao Liu
Alois A. Bell
Jaemin Cho
Robert D. Stipanovic
Lorraine Puckhaber
Jose Quintana

Southern Plains Agricultural Research Center
Agricultural Research Service
U. S. Department of Agriculture
2765 F & B Road, College Station, TX USA

Dallas, TX, April 3-4, 2012



Liu et al., Can. J. Microbiol., 2011

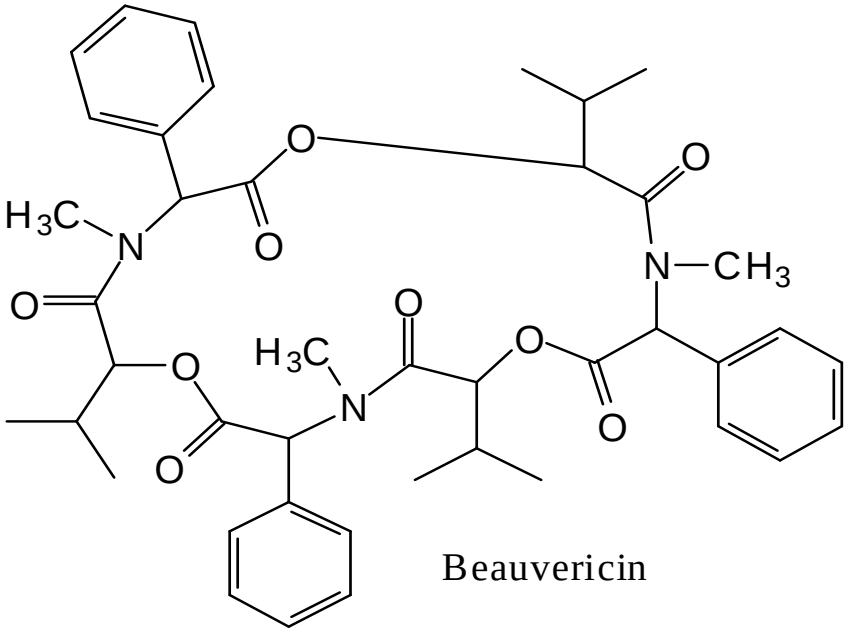
Differences Between U.S. and Australian Biotypes, Race 4

Australian Biotype and Race 4	U.S. race (Races 1, 2, & 6)
1. Attacks cotton in heavy clay soil	1. Attacks cotton in neutral or acidic sandy soil
2. Nematodes are not required for infection	2. Severe wilt usually occurs in soils heavily infested with nematodes
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	3. Attacks seedlings, but usually attacks plants later in the season
4. Stem puncture inoculation is ineffective for disease expression (primarily rots the roots)	4. Stem puncture inoculation is effective for disease expression (Primarily invades the vascular system)
5. >70% of the isolates produce >1500mg/L of **fusaric acid** in defined media	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



Beauvericin

- 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.

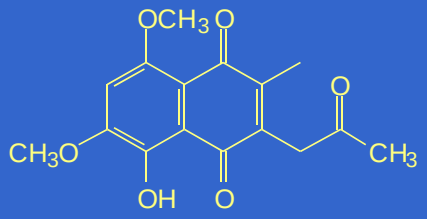
- Free radical generator.
- Cation chelating agent.
- Phytotoxic to peas and citrus.

Triketide: fusaric acid.

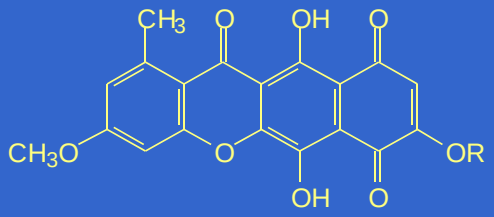
- Potent phytotoxin for many plant species. Five minute root-dip in 70 ppm fusaric acid causes severe wilt of cotton.

Polyketide Phytotoxins from *Fov*

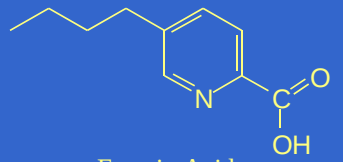
Produced by U.S.
Fov race 1 lineage



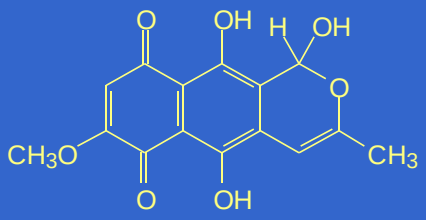
5-*O*-Methyljavanicin



Bikaverin: R = -CH_3
Norbikaverin: R = -H



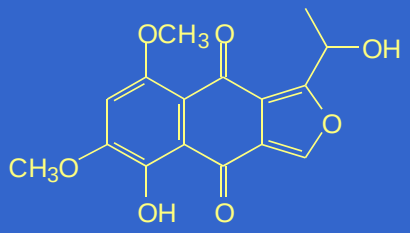
Fusaric Acid



Anhydrofusarubin Lactol

Nonaketide

Triketide



Nectriafurone

Heptaketide

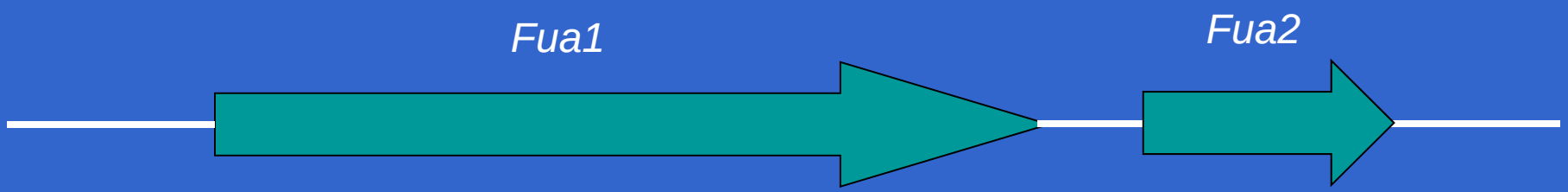
Produced by Australian
Fov biotype and race 3
and race 4 lineages

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.

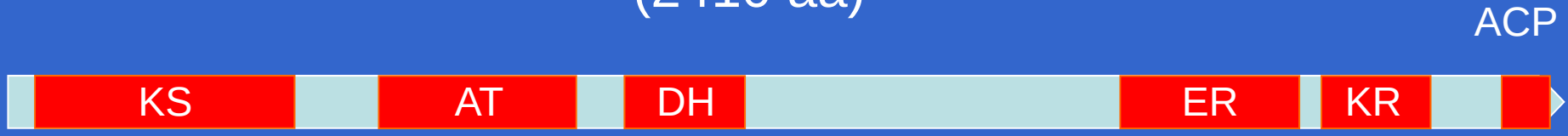
- To unequivocally prove the role of fusaric acid in pathogenicity, we identified and cloned a polyketide synthase gene (PKS) as well as an amino acid kinase gene involved in the biosynthesis of fusaric acid.

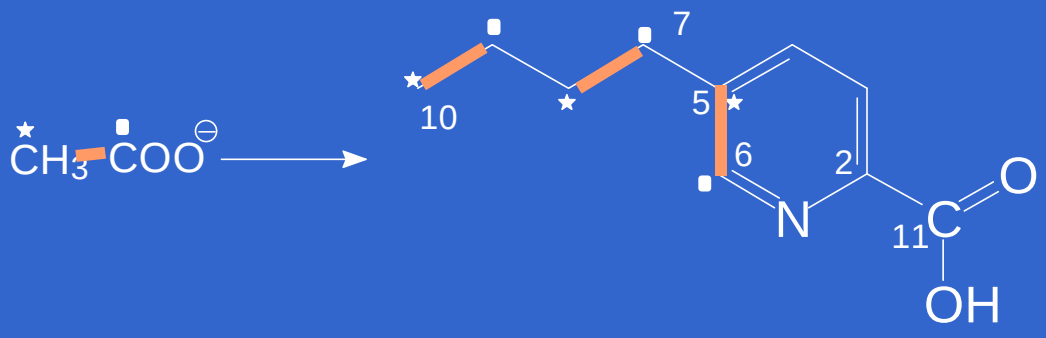


PKS

Aspartate Kinase

Fua1 Domain Structure (2410 aa)



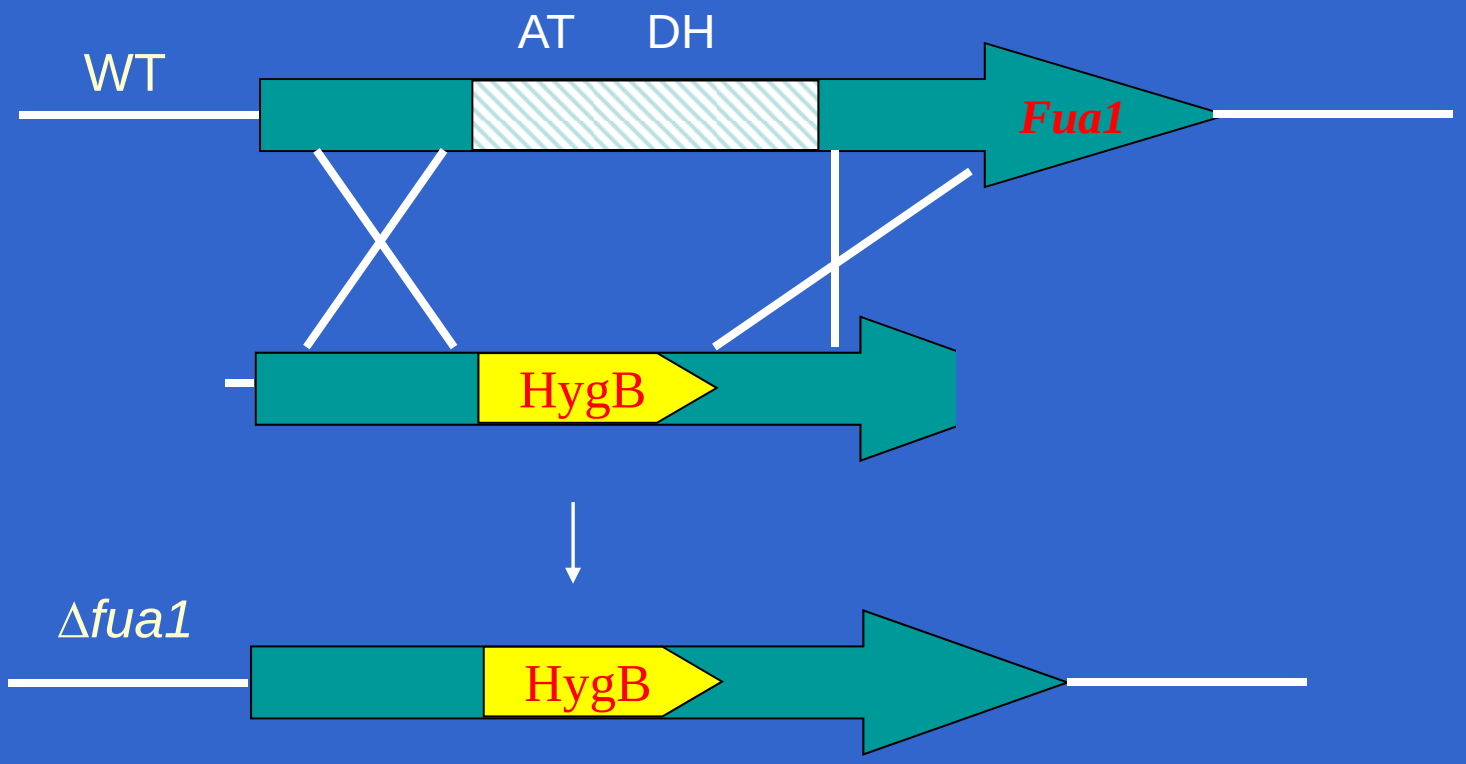


Targeted Gene Disruption

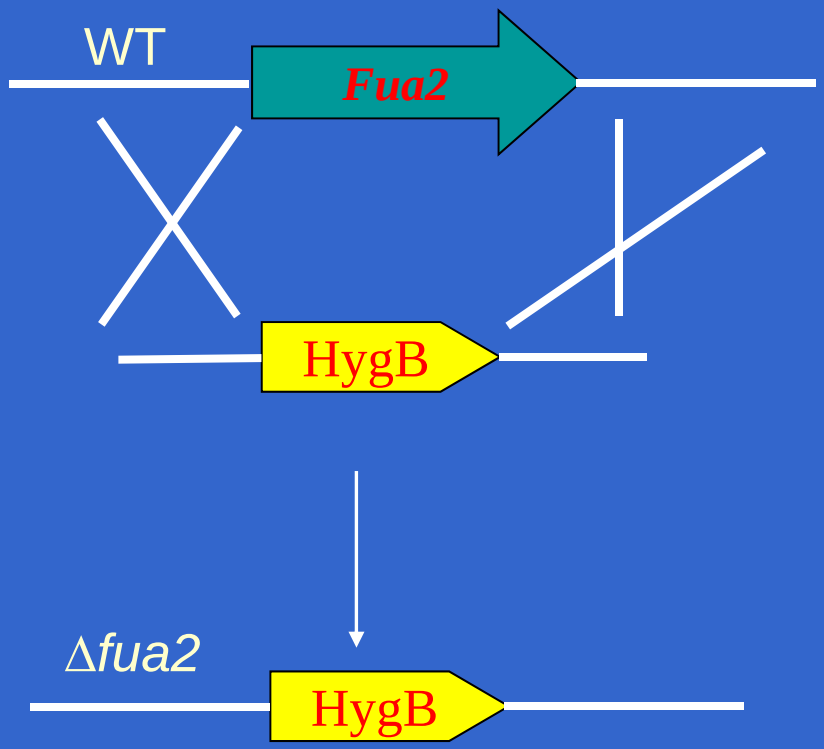
•Knock-out mutants for fusaric acid synthesis genes were generated by targeted gene disruption.

•A PEG mediated fungal transformation procedure was used.

Fua1 Disruption



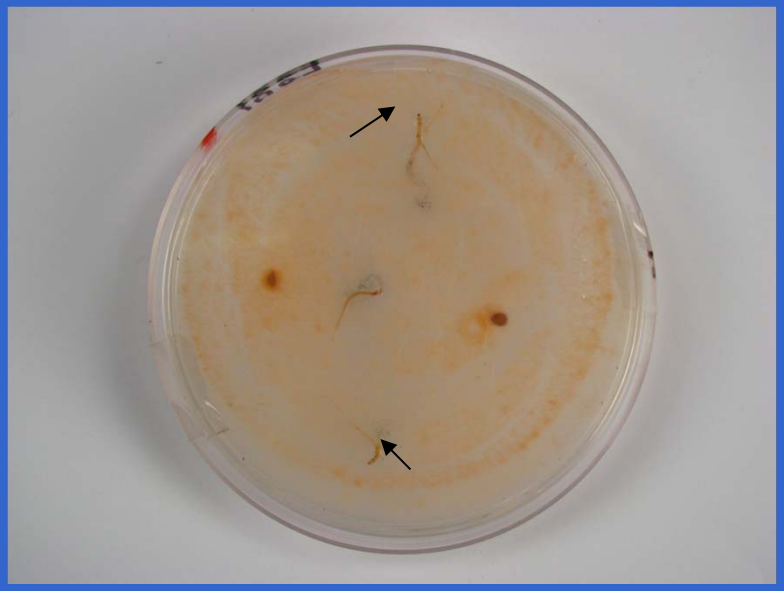
Fua2 Disruption



We generated both *ΔFua1* (PKS-disruption) and one *ΔFua2* (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 x 10^4 conidia/plate.
- Five tomato (Rutgers) seeds/plate.
- 28^{o}C (day)/24^{o}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^{o}C (day)/24^{o}C (night) for 5 weeks.


FoV
1089

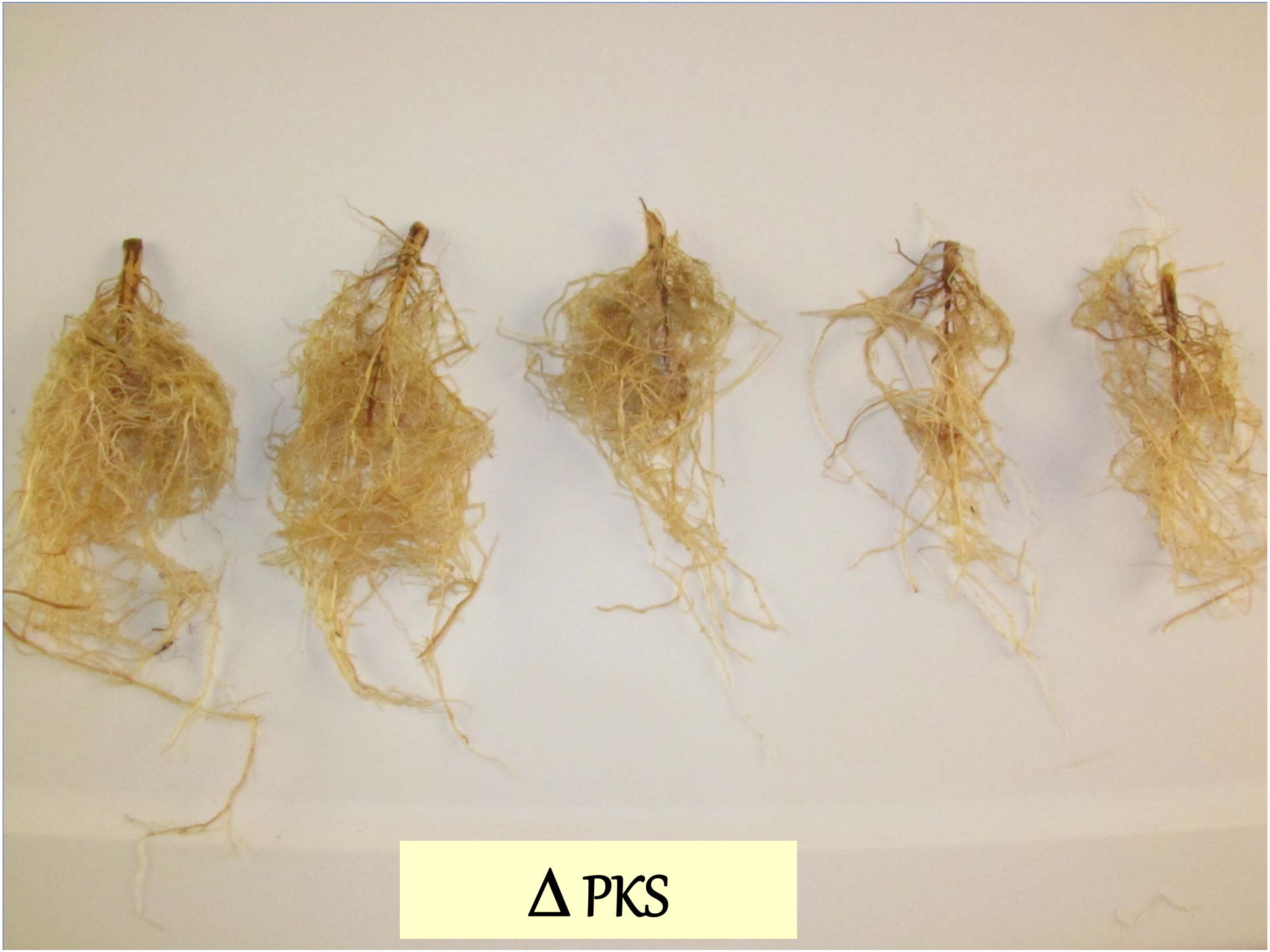

ΔASP
1089
ΔASP


Δ PKS
1089
Δ PKS


Δ Asp
1089
Δ PKS


1089


Δ Asp


Δ PKS


Δ Asp
1089

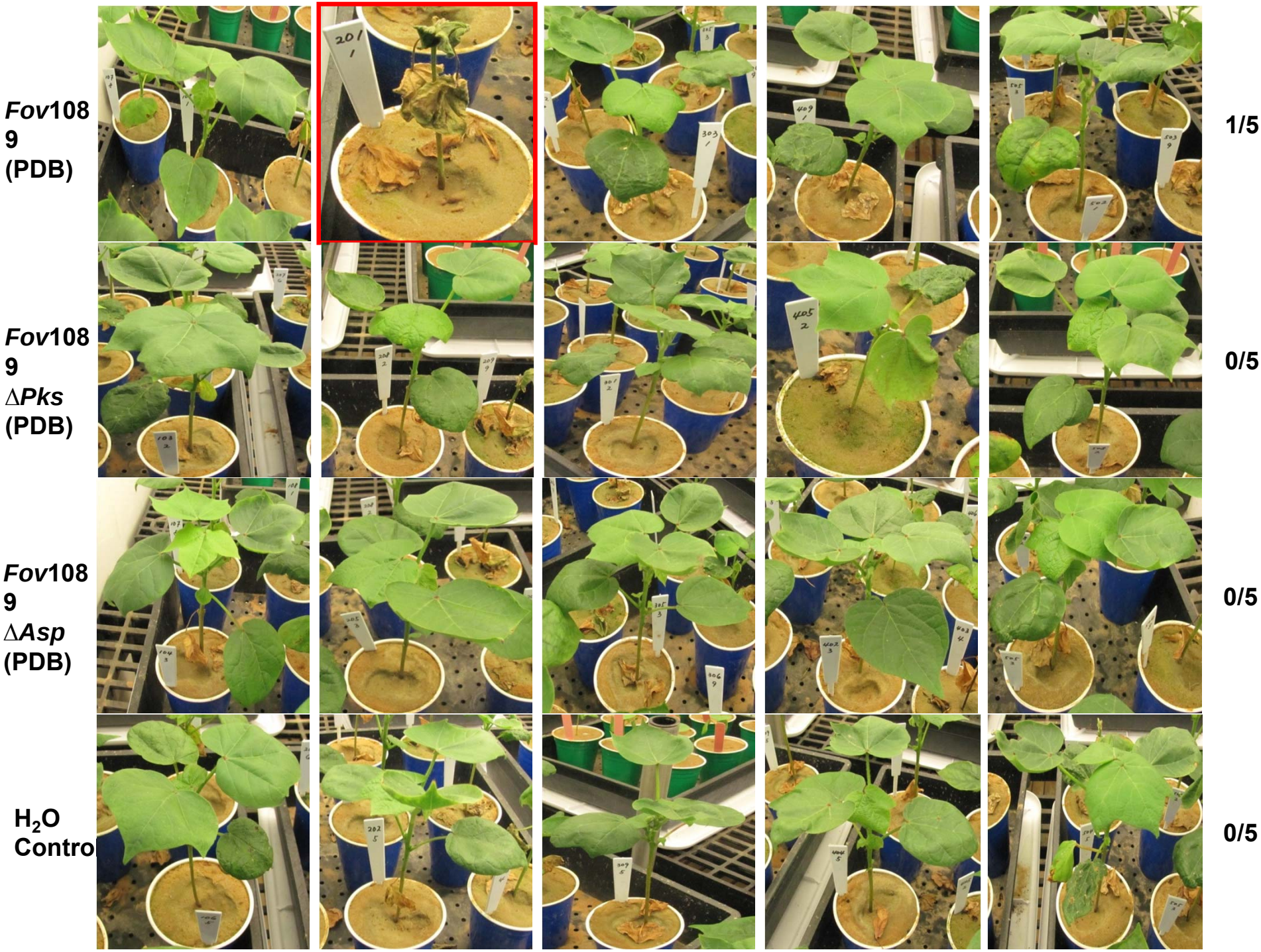

CA14-1
CA9
Fov 9
CA-12

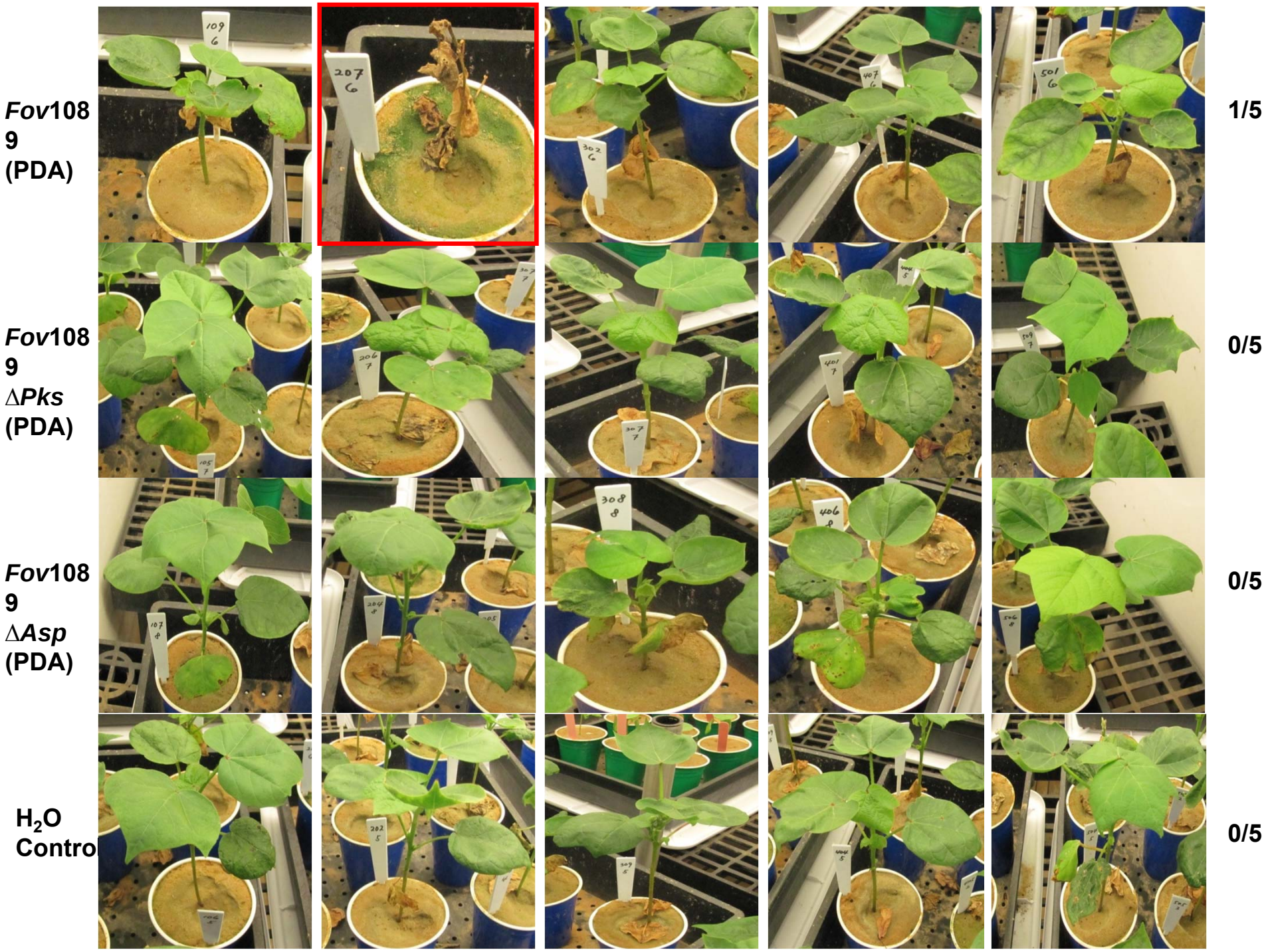

R-4
CA 9
CA 14

Pathogenicity Assay with Coker 312

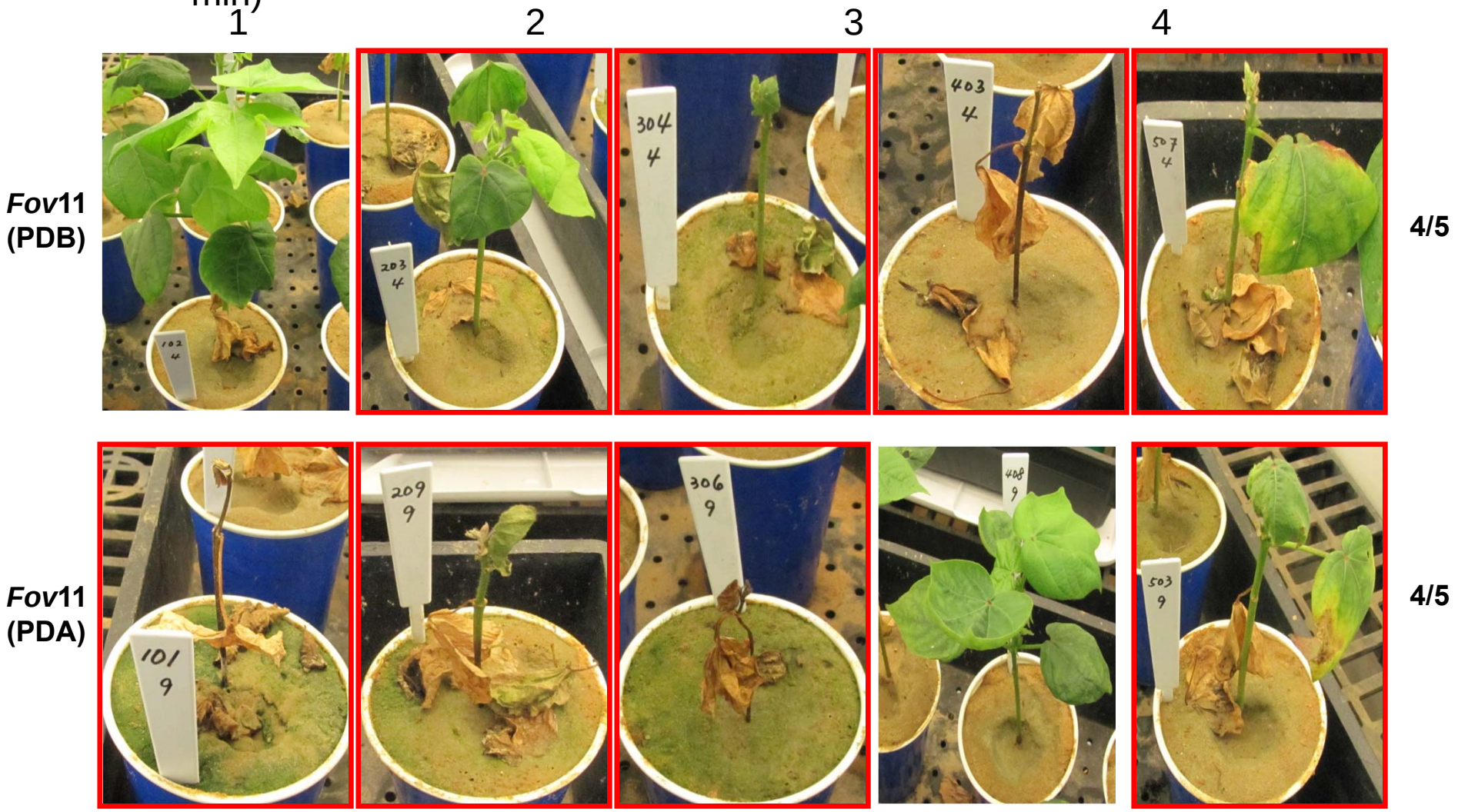
Root Dip Assay (Jaemin Cho)

- Roots of two-week-old Coker 312 seedlings dipped in 106 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^{o}C (day)/24^{o}C (night) for 5 weeks.

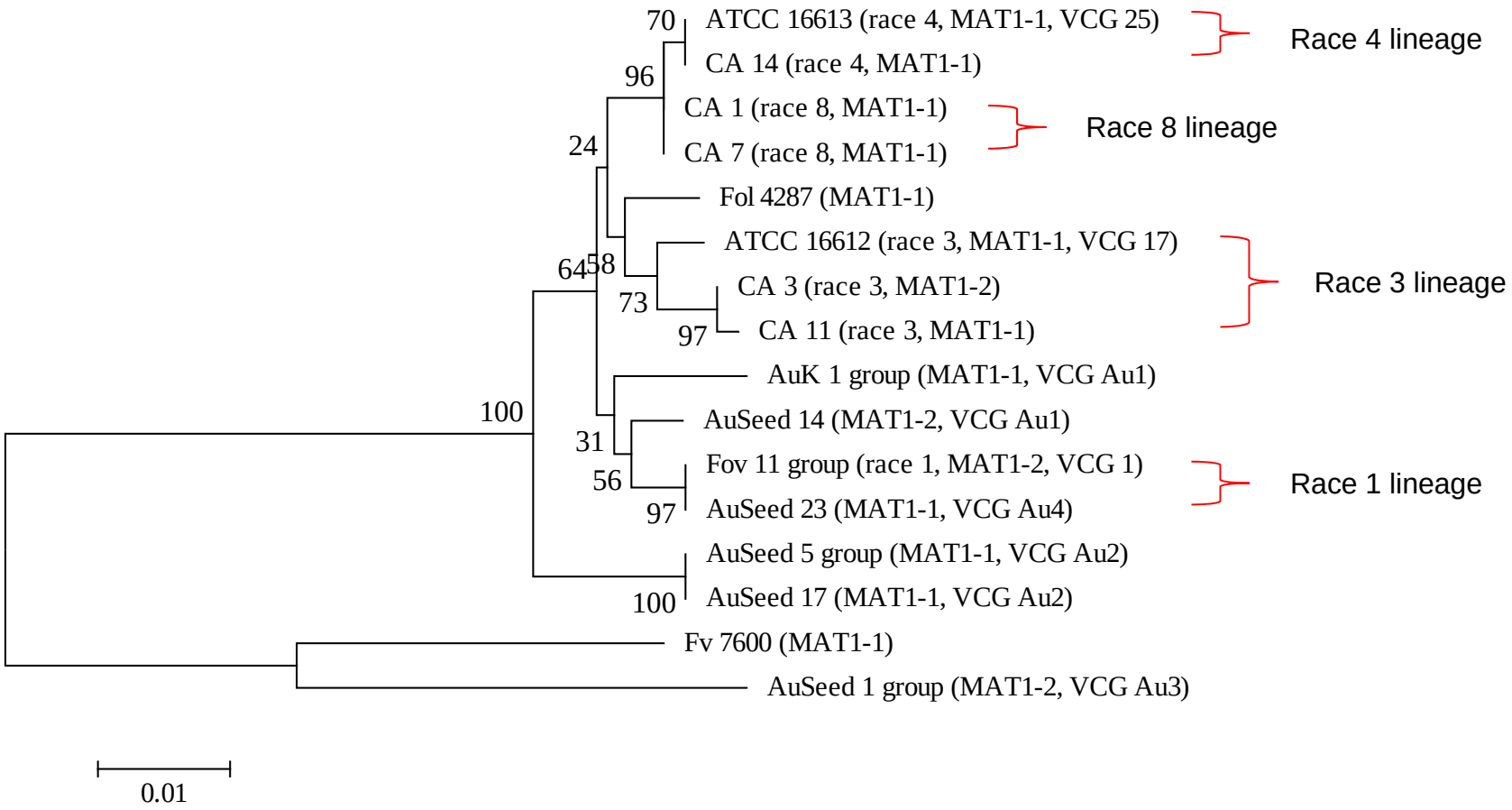

*Fov*1089 (PDB)
1/5
*Fov*1089 ΔPks (PDB)
0/5
*Fov*1089 ΔAsp (PDB)
0/5
H_2O Contro
0/5

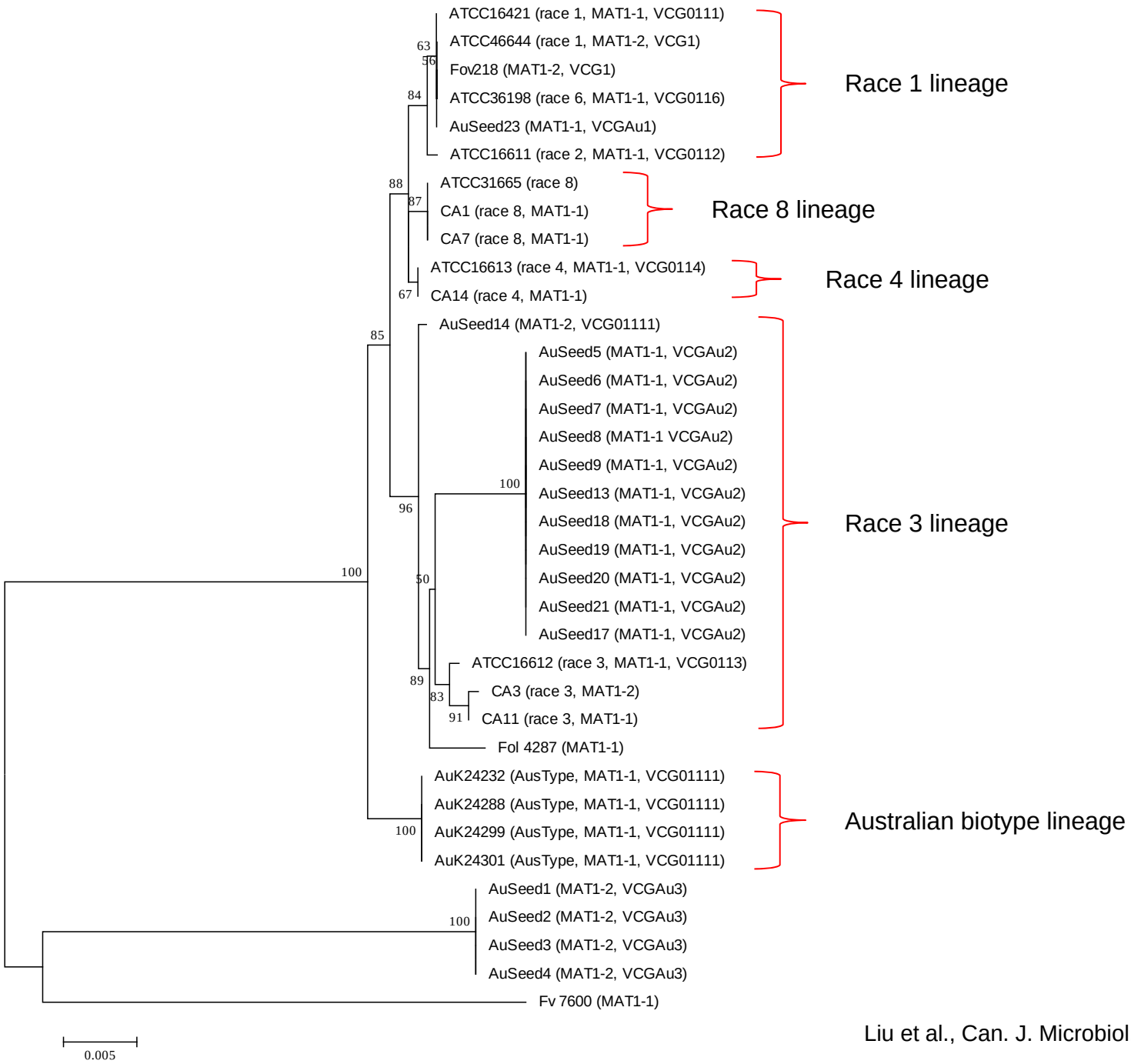

*Fov*1089 (PDA)
1/5
*Fov*1089 Δ*Pks* (PDA)
0/5
*Fov*1089 Δ*Asp* (PDA)
0/5
H_2O Contro
0/5

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



35 Days after inoculation (tmt # 4 & 9)


70
ATCC 16613 (race 4, MAT1-1, VCG 25)
CA 14 (race 4, MAT1-1)
Race 4 lineage
96
CA 1 (race 8, MAT1-1)
CA 7 (race 8, MAT1-1)
Race 8 lineage
24
Fol 4287 (MAT1-1)
ATCC 16612 (race 3, MAT1-1, VCG 17)
64
58
CA 3 (race 3, MAT1-2)
73
97
CA 11 (race 3, MAT1-1)
Race 3 lineage
AuK 1 group (MAT1-1, VCG Au1)
100
AuSeed 14 (MAT1-2, VCG Au1)
31
Fov 11 group (race 1, MAT1-2, VCG 1)
56
97
AuSeed 23 (MAT1-1, VCG Au4)
Race 1 lineage
AuSeed 5 group (MAT1-1, VCG Au2)
100
AuSeed 17 (MAT1-1, VCG Au2)
Fv 7600 (MAT1-1)
AuSeed 1 group (MAT1-2, VCG Au3)
0.01



Liu et al., Can. J. Microbiol., 2011

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.