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Research on Fusarium Wilt of Banana and its Management in China

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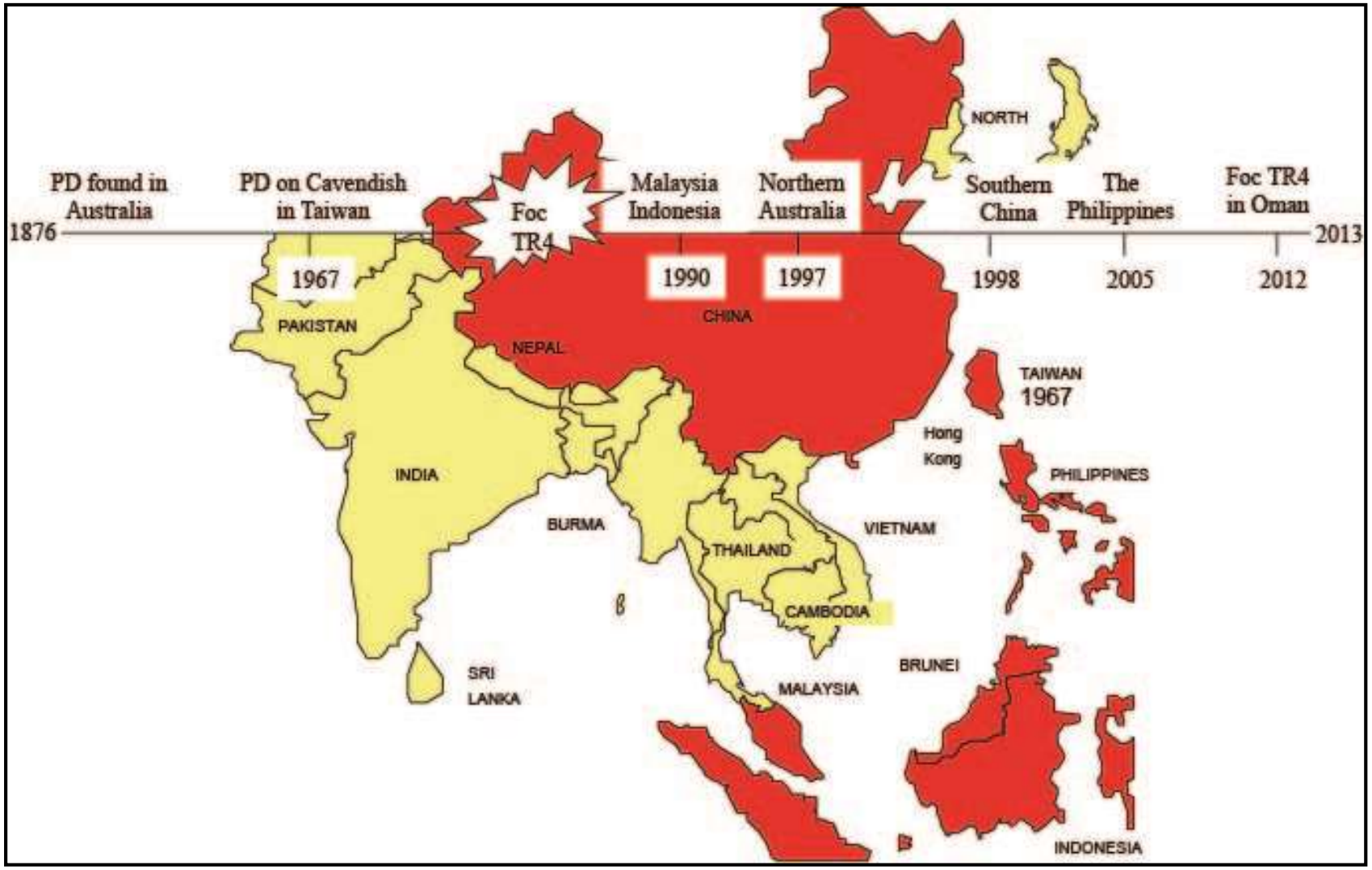
Outlines

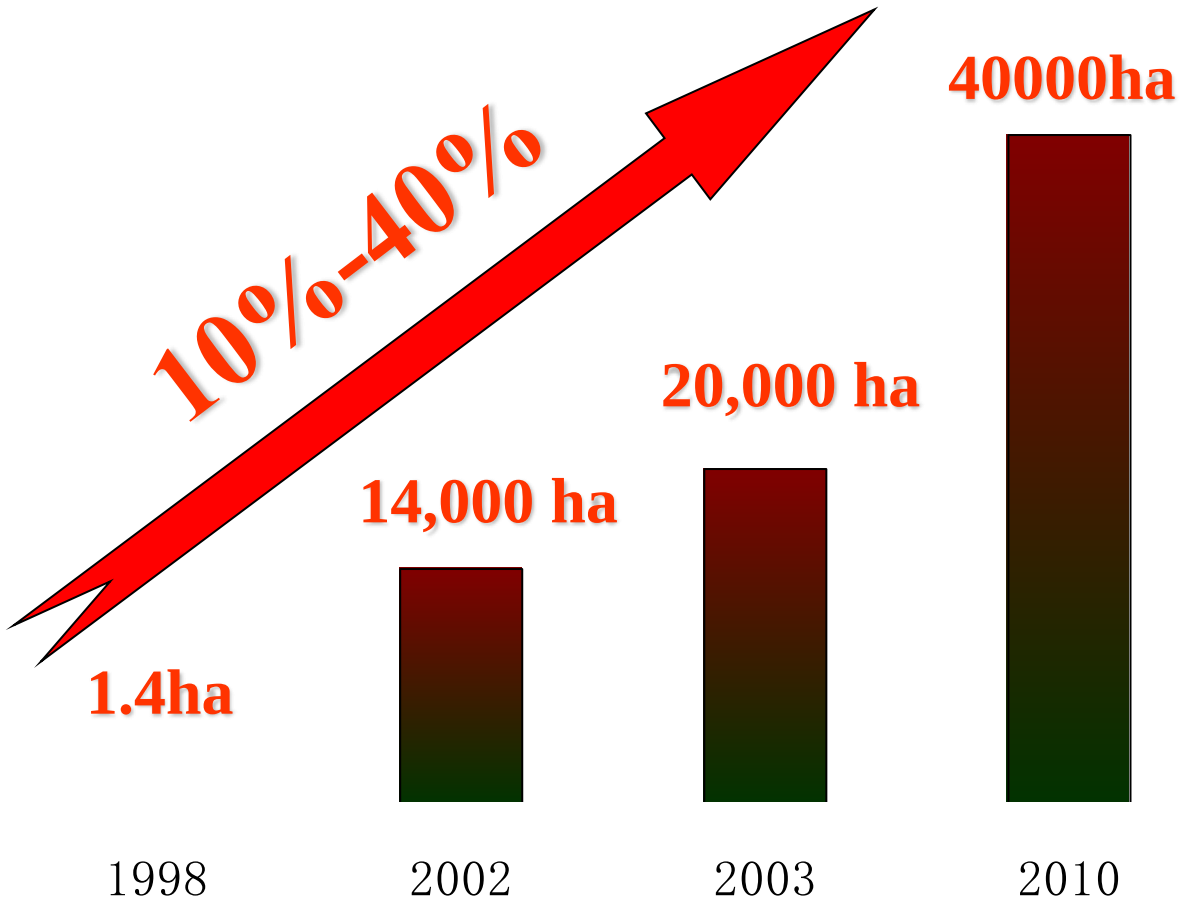
1. Symptoms
2. Threat or impacts on Chinese Banana industry
3. The diveristy of the Pathogens
4. Virulence of *Foc* TR4
5. Prevention measures
6. Developping Control measures
7. Conclusions
8. Acknowledgments

Symptoms

Fusarium Wilt of banana in Asia



Fusarium Wilt of Banana in China



Incidence Area in China

Producing center is moving from Guangdong province into Guangxi

Reasons for Fusarium wilt epidemics

1. Large scale monoculture of bananas
2. Small-scale farming by individual owners
3. Irregular production of tissue clture plantlets
4. River pollution and farming operations
5. Disregard of quarantine regulations
6. Movement of plants, people and equipment

Fusarium oxysporum f.sp. cubense

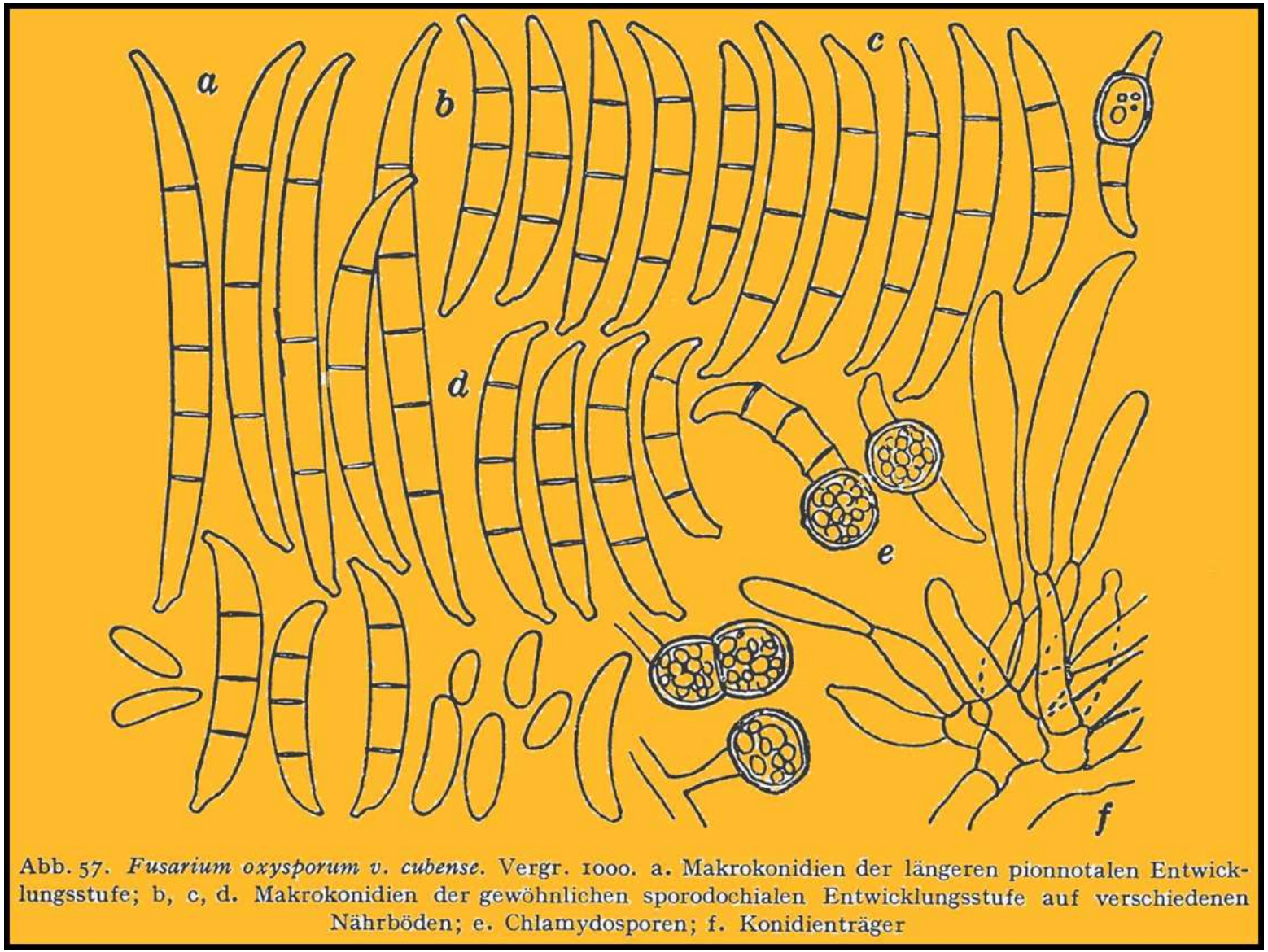


Abb. 57. *Fusarium oxysporum v. cubense.* Vergr. 1000. a. Makrokonidien der längeren pionnotalen Entwicklungsstufe; b, c, d. Makrokonidien der gewöhnlichen sporodochialen Entwicklungsstufe auf verschiedenen Nährböden; e. Chlamydosporen; f. Konidienträger

Races in Foc

Race 1 → **'Gros Michel' (AAA), 'Silk' (AAB), 'Pome' (AAB), 'Pisang Awak' (ABB), *Musa textilis***

Race 2 → **'Bluggoe' (ABB)**

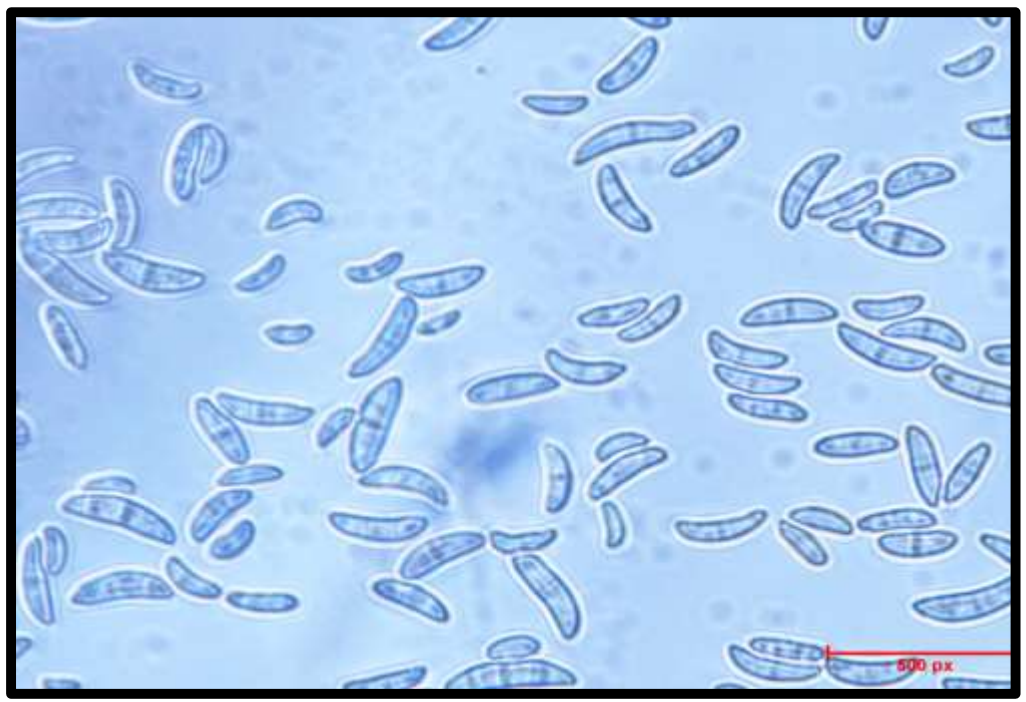
Race 3 → ***Heliconia* species**

Race 4 → **Cavendish (AAA), 'Pisang Mas' (AA), 'Pisang Berangen' (AAA), AAB plantains, plus cultivars susceptible to race 1 and race 2**

- **"tropical"**
- **"sub-tropical"**

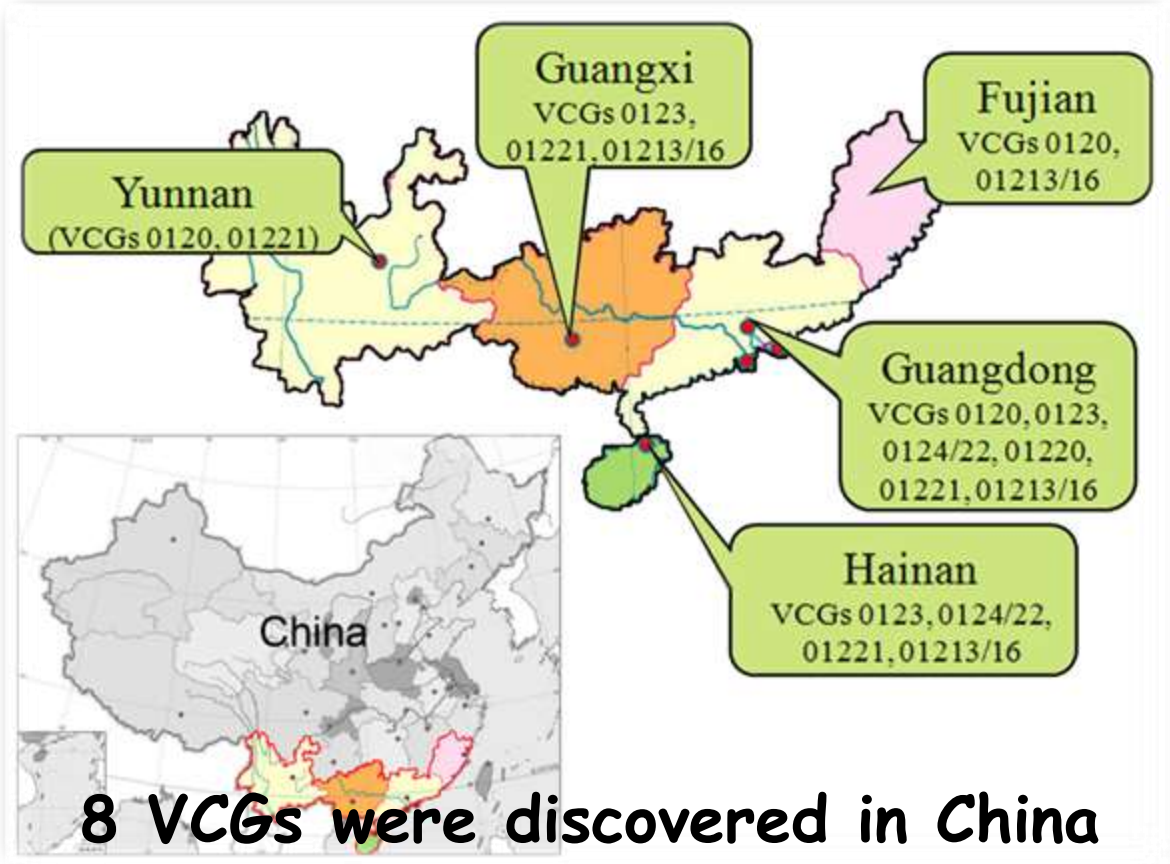
表 1 全球香蕉枯萎病菌 24 个 VCGs 菌株及大蕉枯萎病菌信息表

Number	VCG	Race	Host or cultivar	Origin
1	120	Subtropical Race 4	Cavendish	South Africa
2	121	Subtropical Race 4	Cavendish	China-Taiwan
3	122	Subtropical Race 4	Cavendish	Philippines
4	123	Race 1/2	Latundan	Philippines
5	124	Race 1/2	Lady finger	Australia
6	125	Race 1/2	Lady finger	Australia
7	126	Subtropical Race 4	Pisang Rubus	Indonesia
8	128	Race 2	Bluggoe	Australia
9	129	Subtropical Race 4	Lady finger	Australia
10	1210	Subtropical Race 4	Apple	United States
11	1211	Subtropical Race 4	SH3142	Australia
12	1212	Race 1/2	Ney poovan	Tanzania
13	1213	Tropical Race 4	Pisang Berangan	Indonesia
14	1214	Not included in Race identity system	Harare	Malawi
15	1215	Subtropical Race 4	Gros Michel	Costa Rica
16	1216	Tropical Race 4	Cavendish	Indonesia
17	1217	Race 1	Pisang Rastali	Malaysia
18	1218	Race 1	Pisang Siem	Indonesia
19	1219	Subtropical Race 4	Pisang Raja Sereh	Indonesia
20	1220	Race 4	Cavendish	Australia
21	1221	Race 1	Pisang Awak	Thailand
22	1222	Race 1	Pisang Awak Legor	Thailand
23	1223	Not included in Race identity system	Pisang Keling	Thailand
24	1224	Not included in Race identity system	Pisang Ambon	Thailand
25	Dajiao	Not included in Race identity system	Plantain	China Mainland



Diversity and distrubution of *Foc* In China

Cooperation: Dr Altus Viljoen
Stellenbosh Univeristy



8 VCGs were discovered in China

Chunyu Li and Ganjun Yi. 2014. Fungal Genomics and Biology

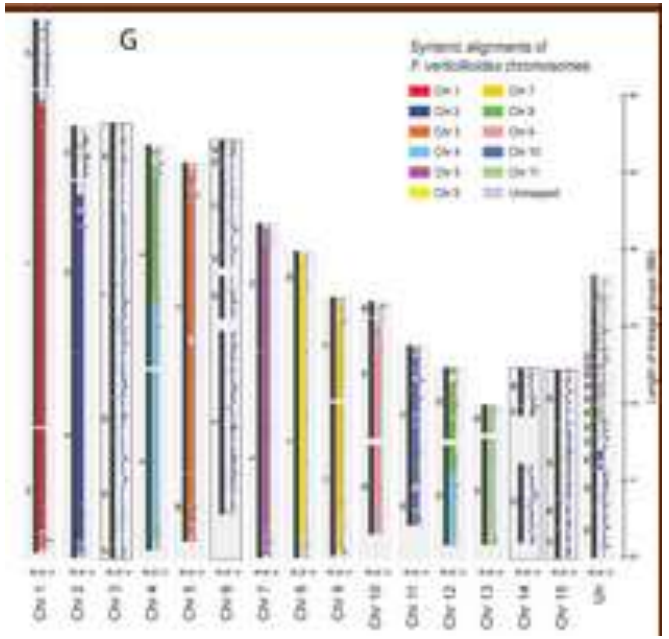
Evolution of *Foc* worldwide

1. **Genome re-sequencing**
   （1）56 Strains: From Dr Altus Viljoen, Randy and ourself
   (2) 4 Races、24VCGs and covering 35 contries worldwidely
2. **Co-operation :**
   Laboratory of fungal comparative genomics,Umass Amherst, USA

Altus Viljoen

Randy

Lijun Ma



Information of the reseqenced *Foc* isolates

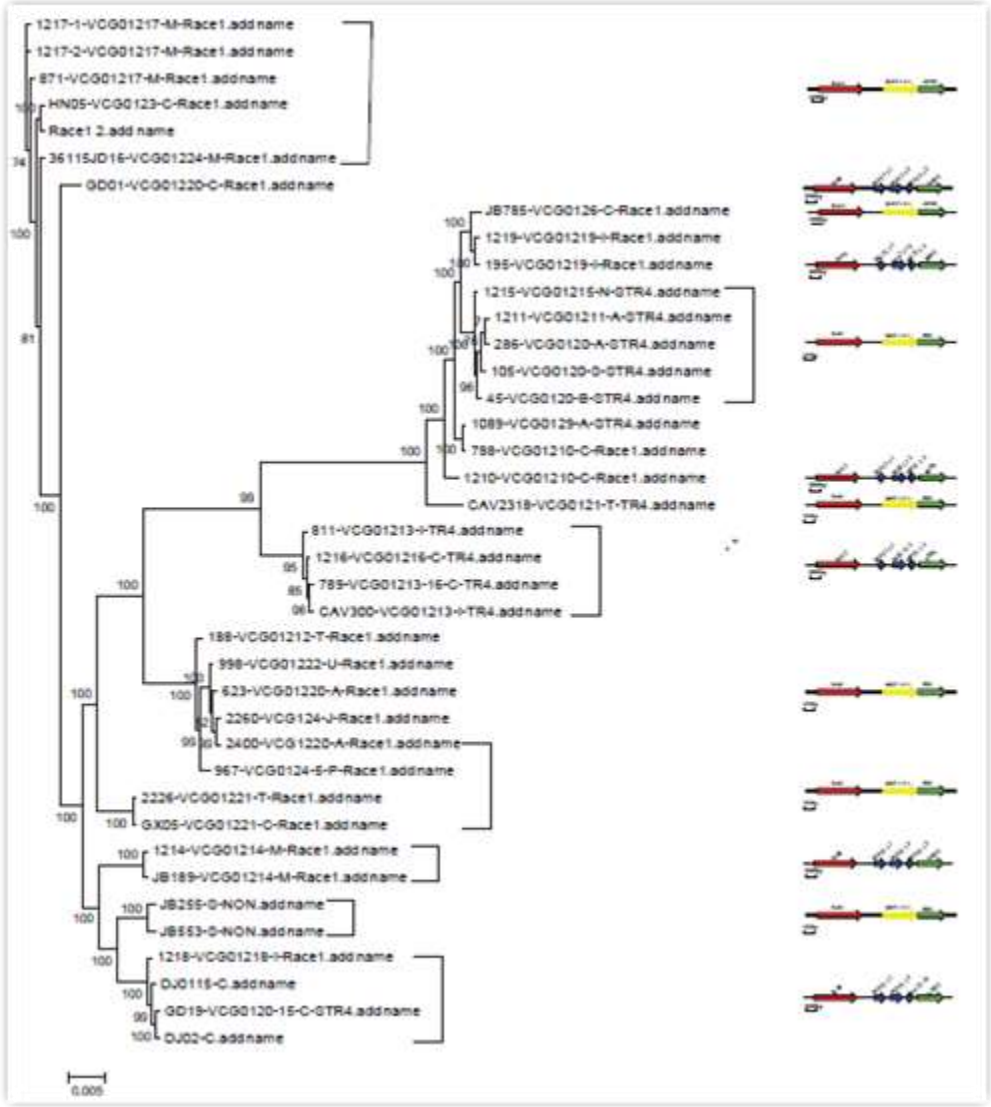
.	Strains	Host	Geological locations
TR4			
1.	CAV2318-VCG0121-T	Namwa (AAB)	Kuosin E, 2K Taiwan
2.	CAV300-VCG01213-I	Valery	Southeast Sumatra Indonesia
3.	811-VCG01213-I	Pisang Batan	Sumatra, Indonesia
4.	1216-VCG01216-C	Cavendish, Brizilian	Hainan, China
5.	604-VCG01216-M	Pisang Raja	MARDI, Selangor, Malaysia
6.	789-VCG01213/16-C	Cavendish, Brizilian	Guangdong, China
STR4			
1.	105-VCG0120-S	Cavendish	Kiepersol, South Africa
2.	286-VCG0120-A	Lady finger	Moorina, QLD, Australia
3.	612DJ12-VCG01215-C	Gros Michel	Costa Rica
4.	1089-VCG0129-A	Lady finger	Cooloolabin, QLD, Austrilia
5.	1211-VCG01211-A	SH3142	Queensland, Australia
6.	1215-VCG01215-N	Isolona, Costa Rica	Onne, Nigeria
7.	GD19-VCG0120/15-C	Cavendish, Brizilian	Guangdong, China
8.	45-VCG0120-B	Pacvan	Bahia, Brazil
RACE2			
1.	JB2013-VCG0128-V	Chuoi tay cao (AAB)	Van Giang town, Van giang
2.	128-VCG0128-A	Bluggoe	South johnstone, Queensland,
nonpathogenicity-isolates			
1.	JB255-S	.	Kiepersol, South Africa
2.	JB553-S	.	Kiepersol, South Africa
Djiao Isolates			
1.	DJ0115-C	Dajiao	Dongguan, China
2.	DJ02-C	Dajiao	Dongguan, China
RACE1			
1.	122-VCG0122-P	Saba	Philippines
2.	187-VCG01210-U	Apple	Florida, USA
3.	188-VCG01212-T	Nev Poovan	Tenguero Station, Tanzania

.	Strains	Host	Geological locations
4.	194-VCG01217-I	Pisang Siem	Indonesia
5.	195-VCG01219-I	Pisang Ambon	Indonesia
6.	618-VCG0122-P	Cavendish	Philippines
7.	623-VCG01220-A	Williams	Carnarvon, Western Australia
8.	788-VCG01210-C	Gros Michel	FHIA Villa Clara, Cuba
9.	847-VCG01219-I	Pisang Ambon	Indonesia
10.	848-VCG01219-I	Pisang Garing	Indonesia
11.	871-VCG01217-M	Pisang Rastali	Malaysia
12.	939-VCG0123-T	Kluai Namwa	Thailand
13.	967-VCG0124/5-P	Latundan	Philippines
14.	1210-VCG01210-C	Gros Michel	Sto. Domingo, Villa Clara
15.	1214-VCG01214-M	Harare	Misuku, Karonga, Malawi
16.	1217-1-VCG01217-M	Pisang Rastali	Kg. Taboh Naning, Negri
17.	1217-2-VCG01217-M	Pisang Rastali	Kota, Negri Sembilan,
18.	1219-VCG01219-I	Pisang raja garing	Cibinong Collection, Java
19.	1223-VCG0123-P	Latundan	Philippines
20.	1223-VCG0123-T	Kluai nam wa sai	Smoeng Hwy 1269, Chiang
21.	1224-VCG0124-H	Bluggoe	Honduras
22.	1983-VCG0123-T	Pisang Awak	Taiwan
23.	2226-VCG01221-T	Kluai nam wa	North of Chiang Rai, Hwy 1,
24.	2260-VCG124-J	Tetraploid 1242	Bodles, Jamaica
25.	2400-VCG1220-A	Williams	Carnarvon, West Australia
26.	36115JD16-VCG01224-M	Pisang Ambon	Kuching, Seman Matang,
27.	GD01-VCG01220-C	Pisang Awak	Guangdong, China
28.	HN05-VCG0123-C	Gros Michel	Hainan
29.	JB189-VCG01214-M	Harare	Misuku hills, Karonga,
30.	JB626-VCG0124/5-M	Harare	Chitipa, Karonga, Malawi
31.	GX05-VCG01221-C	Pisang Awak	Guangxi, China
32.	JB785-VCG0126-C	Pisang Awak	Guangdong, China
33.	1218-VCG01218-I	Pisang Siem	Indonesia
34.	998-VCG01222-U	Kisnia	Uganda
35.	941-VCG0125-T	Kluai Namwa	Thailand

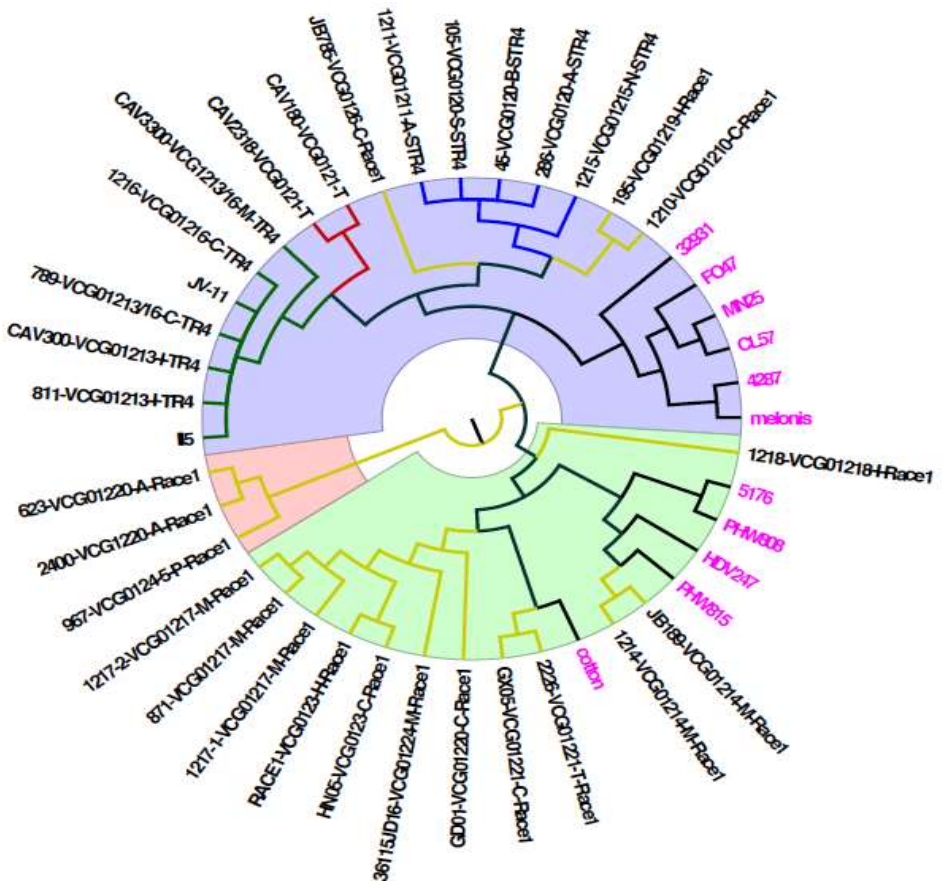
Quality of the data, and results of assembled contigs

Strains	MAT	Raw Reads	Raw Base (Gb)	Mapping reads	Contigs	N50	SNPs			Genome size (Mb)			Q20(%)	Q30(%)
							Total	Core	LS	Total	Core	LS		
TR4														
CAV2318-VCG0121	1	17932515	3.62	16797552	582	261394	97996	89632	8364	45.21	42.14	3.07	96.76	90.38
CAV300-VCG01213	1	7761474	1.55	7529068	721	309890	4534	1245	3289	46.44	42.03	4.42	93.3	91.49
811-VCG01213	1	19178728	3.87	30283950	721	309890	5223	1729	3494	46.47	42.13	4.34	98.18	93.84
1216-VCG01216	1	13454667	2.72	21721752	721	298116	5719	1739	3980	47.41	41.77	5.64	98.26	94.13
604-VCG01216	1	10546237	2.11	3351182	999	203352	13485	3986	9499	46.00	41.69	4.31	92.82	90.43
789-VCG01213/16	1	16337447	3.3	23838167	742	258654	4947	1410	3537	45.69	41.55	4.14	96.68	90.24
STR4														
105-VCG0120	2	16704459	3.37	26753840	686	303437	267139	239355	27784	45.02	42.88	2.14	98.21	93.97
286-VCG0120	2	14721588	2.94	13494085	854	235889	257854	231501	26353	44.6	41.17	3.43	92.49	79.75
612DJ12-VCG01215		19250024	3.89	19808024	798	257998	328457	277859	50598	43.5	41.09	2.41	96.87	90.99
1089-VCG0129		18959312	3.83	23042389	817	296741	283068	252371	30697	44.1	42	2.1	98.23	94.05
1211-VCG01211	2	13849274	2.8	16506753	747	354257	265323	241206	24117	45.5	42.49	2.6	98.41	94.57
1215-VCG01215	2	13813089	2.79	17987339	814	293180	256678	229885	26793	44.28	41.90	2.38	98.39	94.72
GD19-VCG0120/15	1	15675179	3.17	22709385	821	378875	757096	714805	42291	44.26	40.72	3.55	98.3	94.27
45-VCG0120	2	15088942	3.05	18362627	742	301313	269018	239927	29091	47.97	42.21	5.76	98.22	94
RACE2														
JB2013-VCG0128		15405625	3.11	18608584	826	314715	857592	766754	90838	42.6	38.93	3.67	96.71	90.22

Evolution of *Foc* TR4、*Foc* STR4 and Race 1



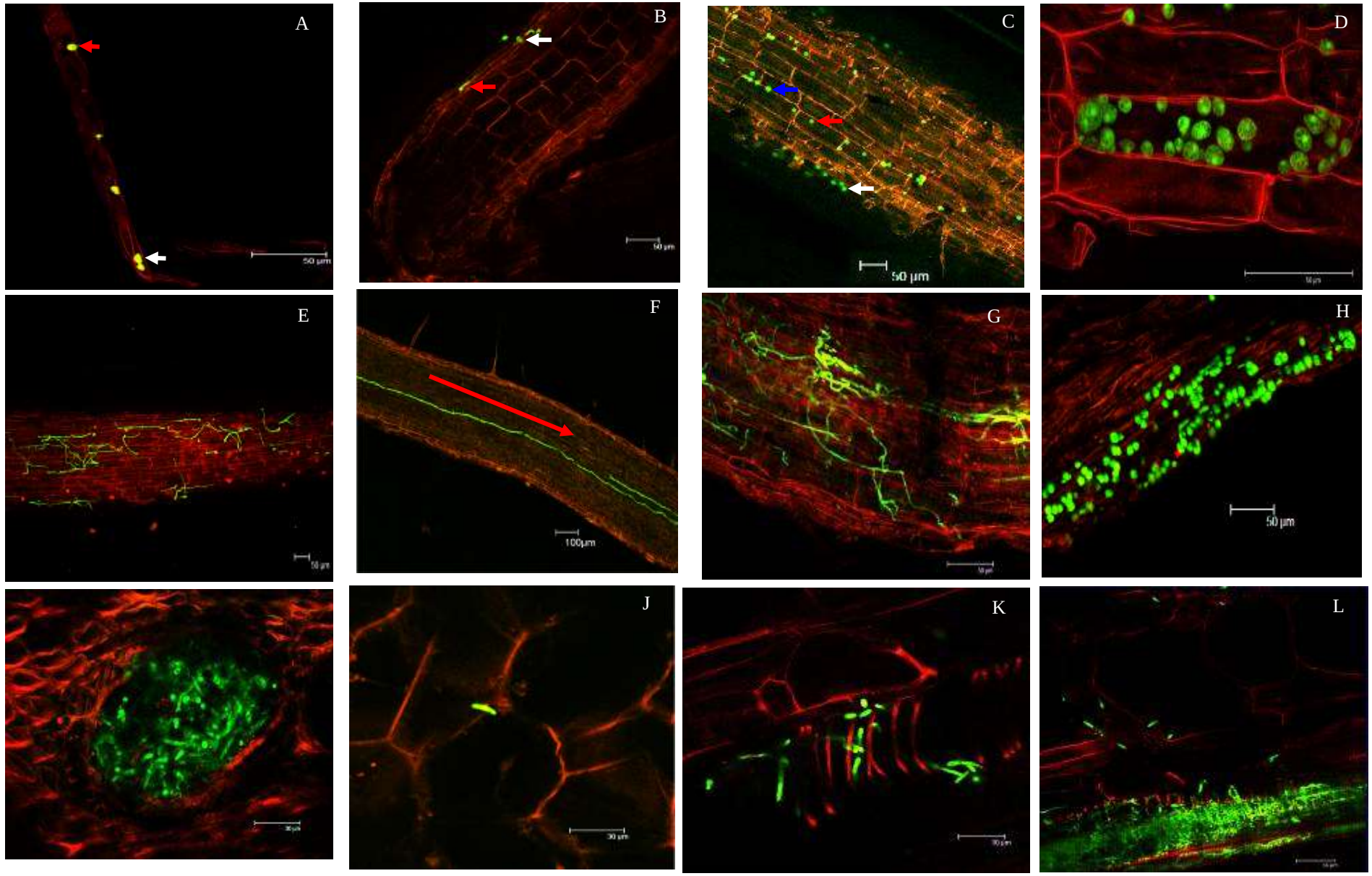
Phylogeny of *Foc* basing on genomic SNP



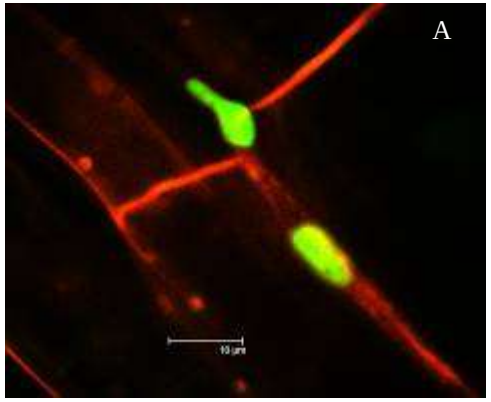
Phylogeny of re-sequencing isolates within 50 conserved genes

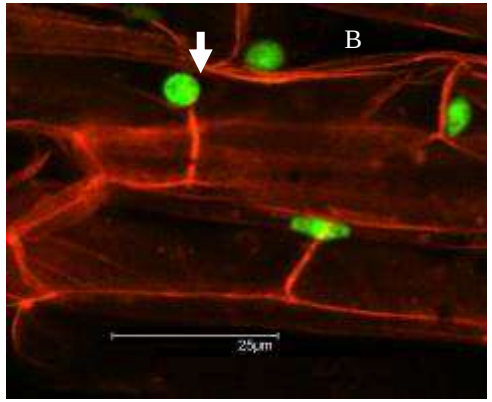
Virulence

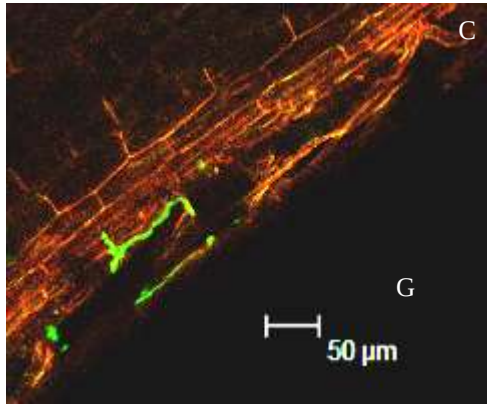
- Infection process
- Mycotoxin
- Effectors

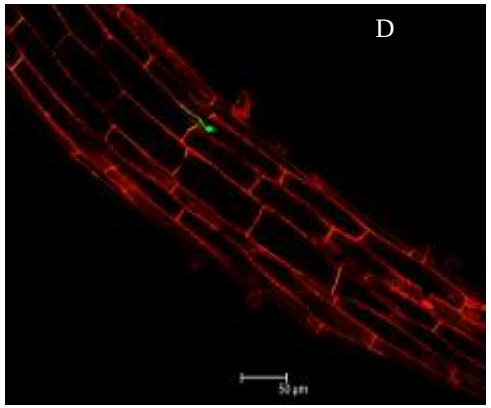


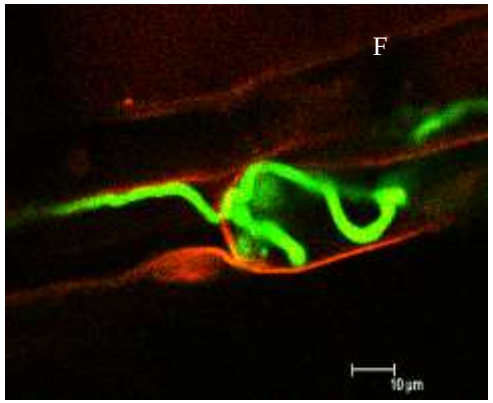
the whole invasion process of *Foc* TR4

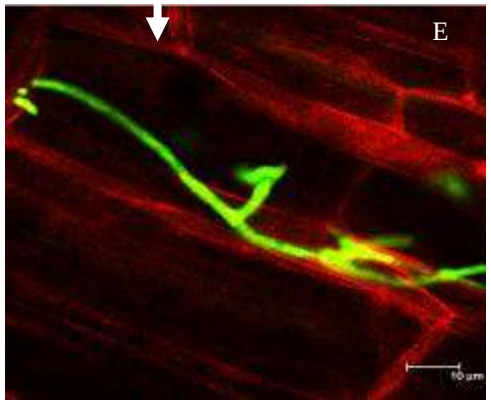


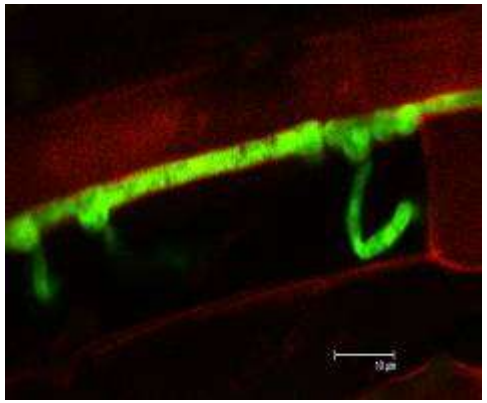




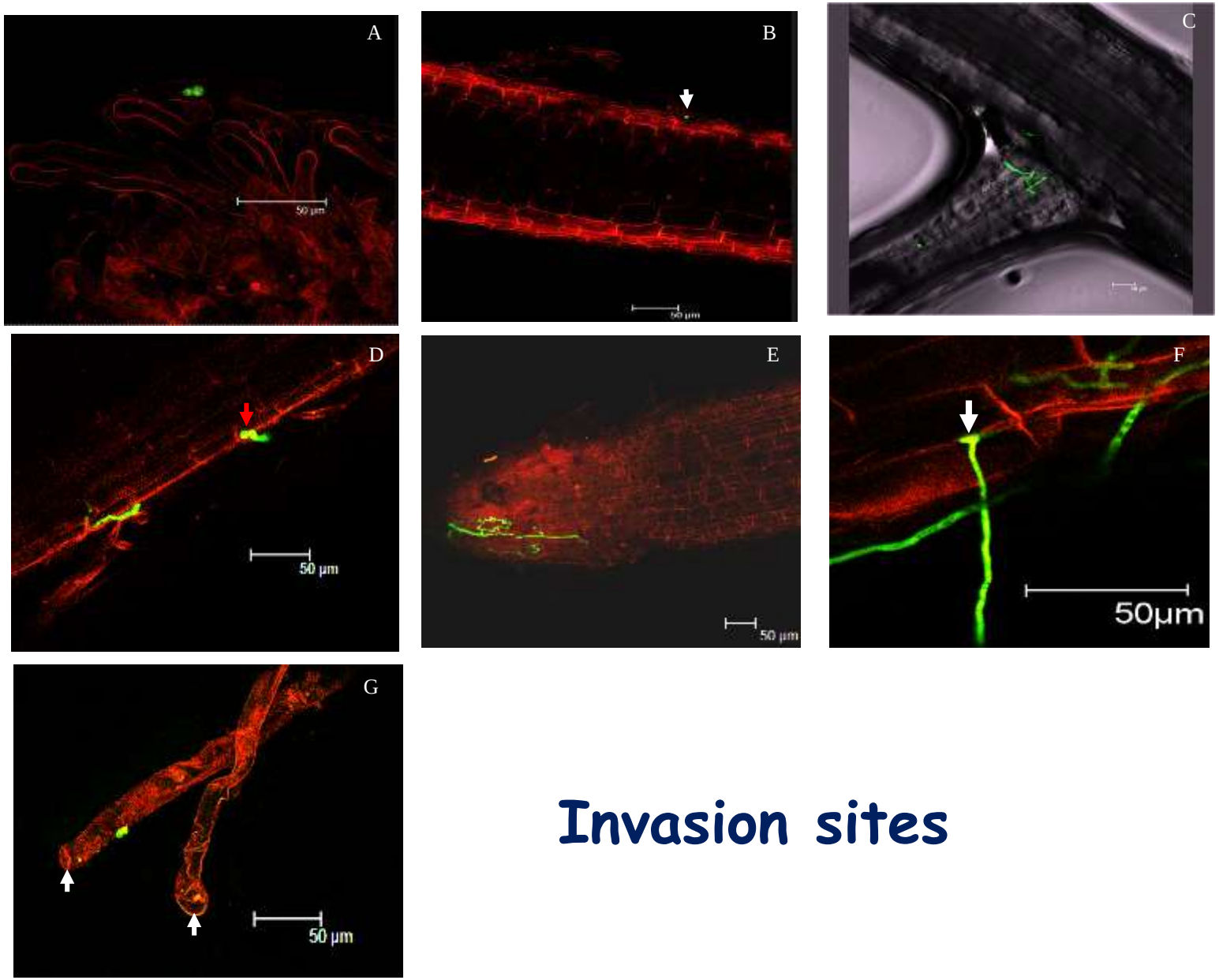






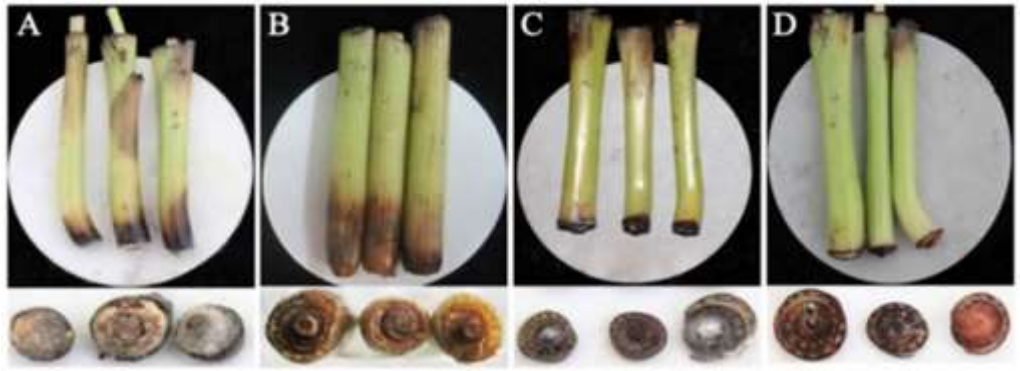


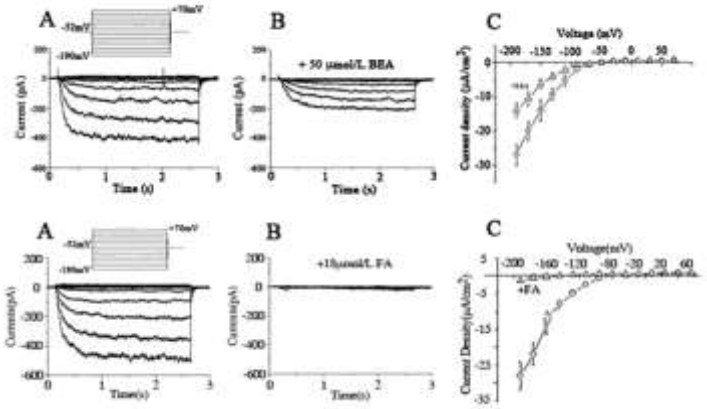
Invasion types



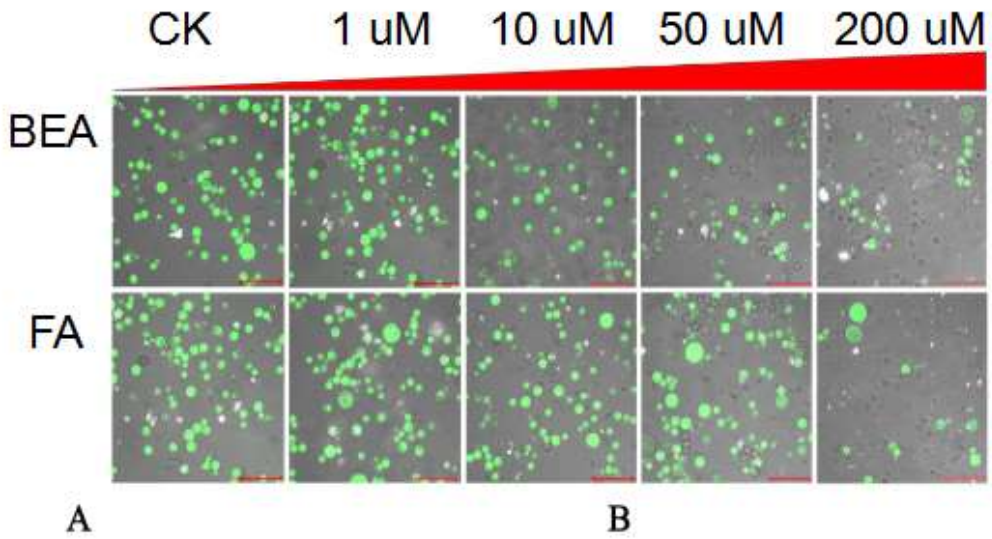
Invasion sites

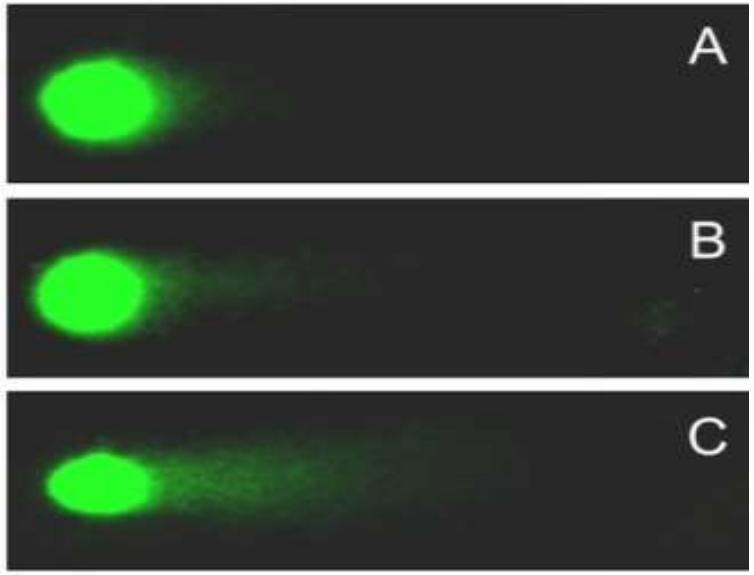
Research on Mycotoxin

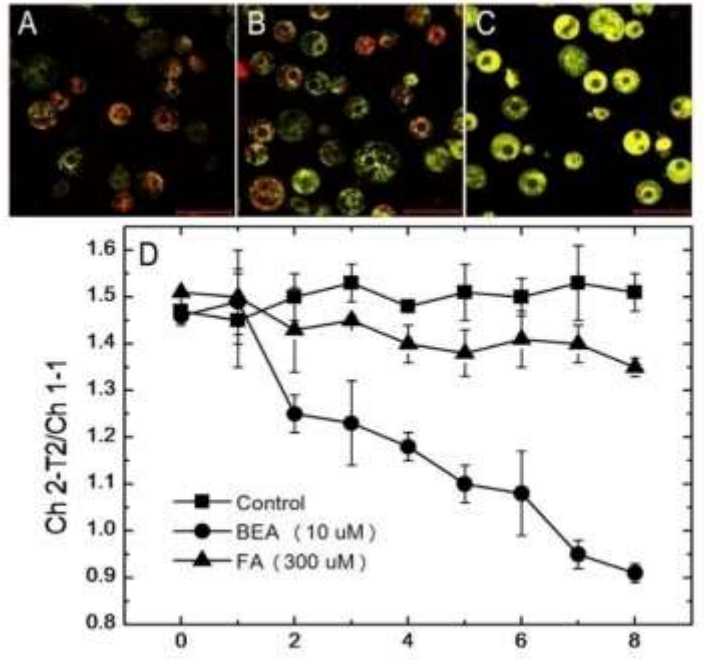




Patch clamp recording technique

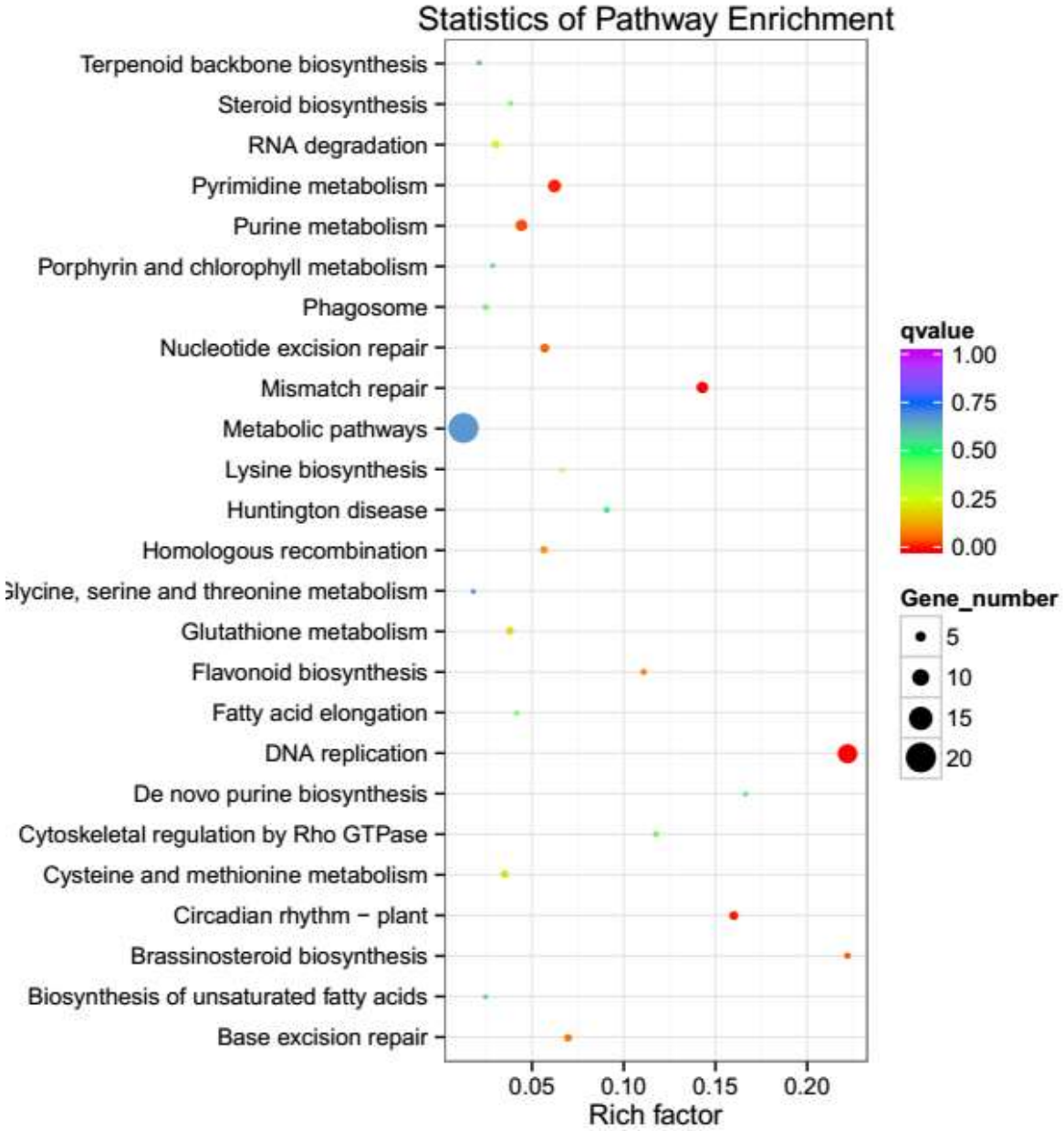




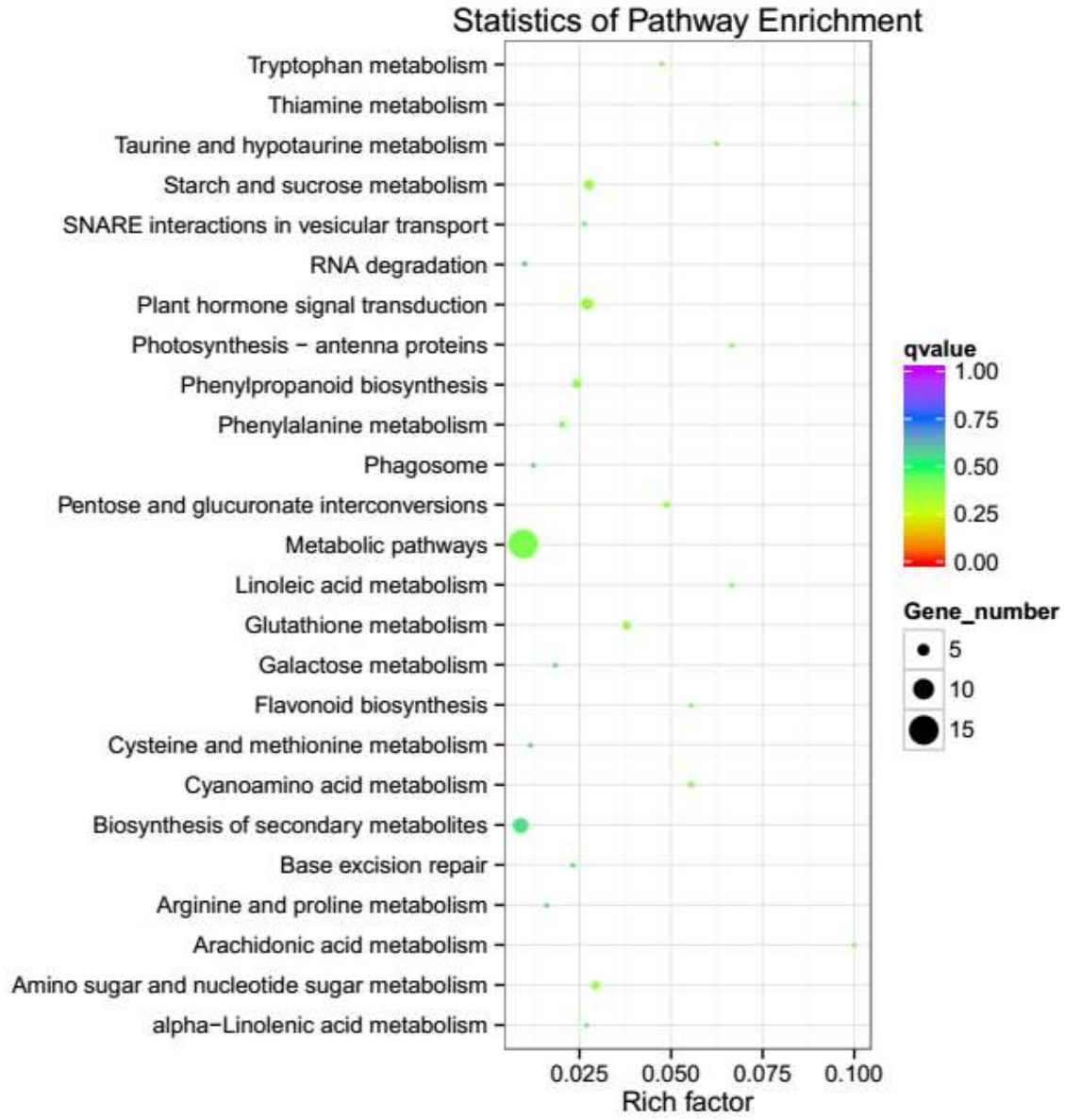


Chunyu Li, Ganjun Yi. 2013. PLOS ONE

Changes in host metabolic pathways caused by BEA

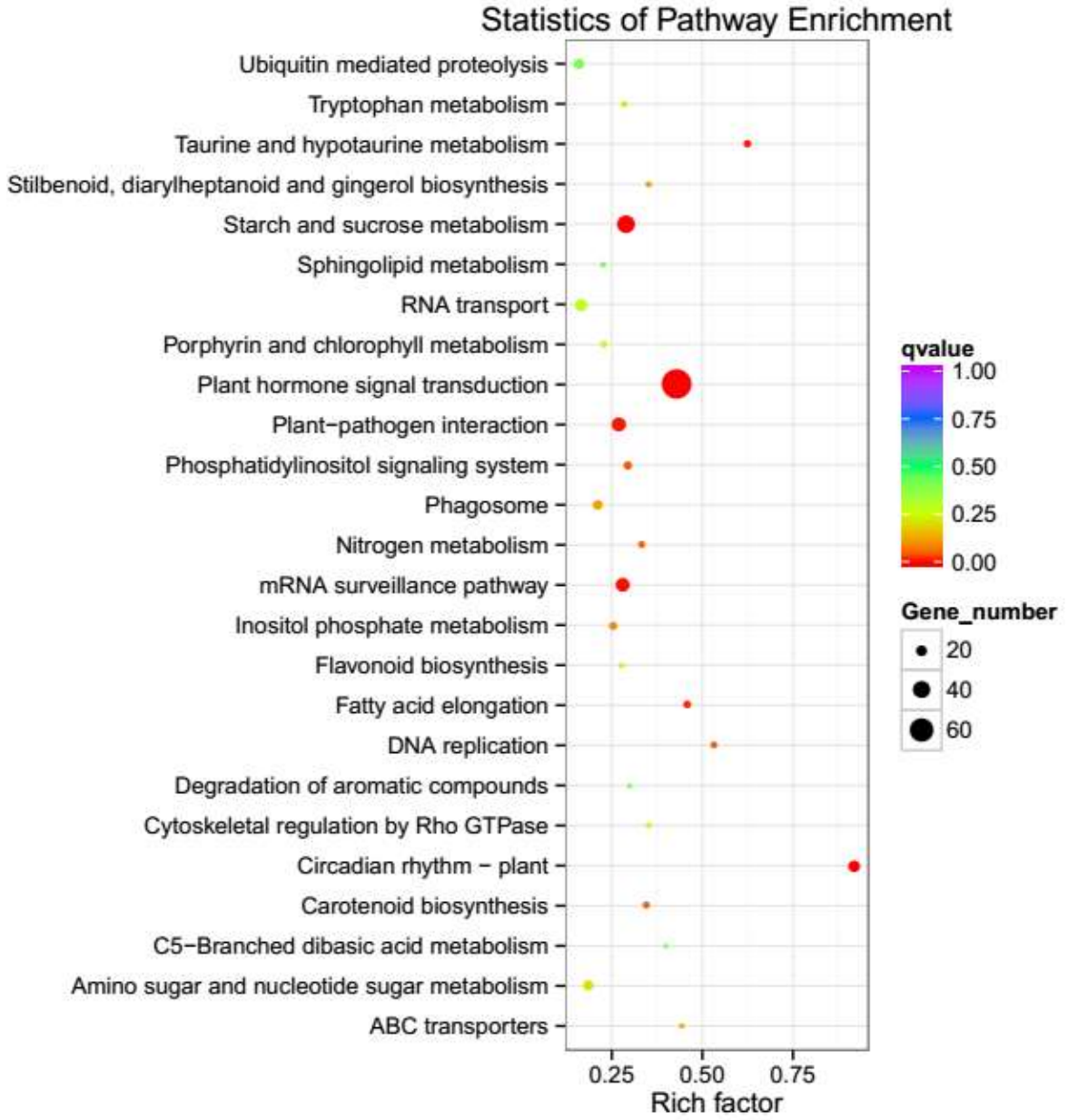


Down-regulated pathways

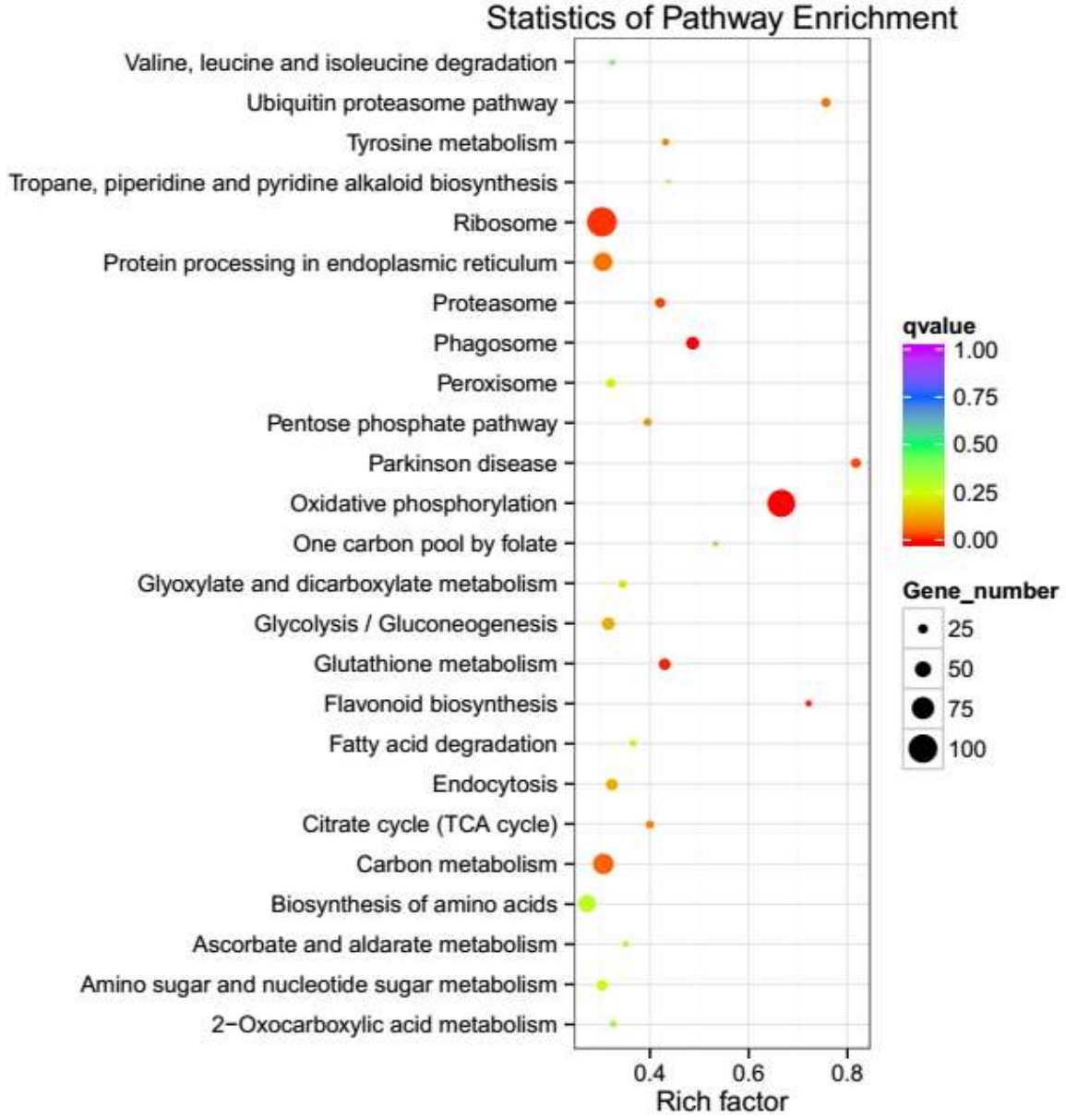


Up-regulated pathways

Changes in host metabolic pathways caused by FA

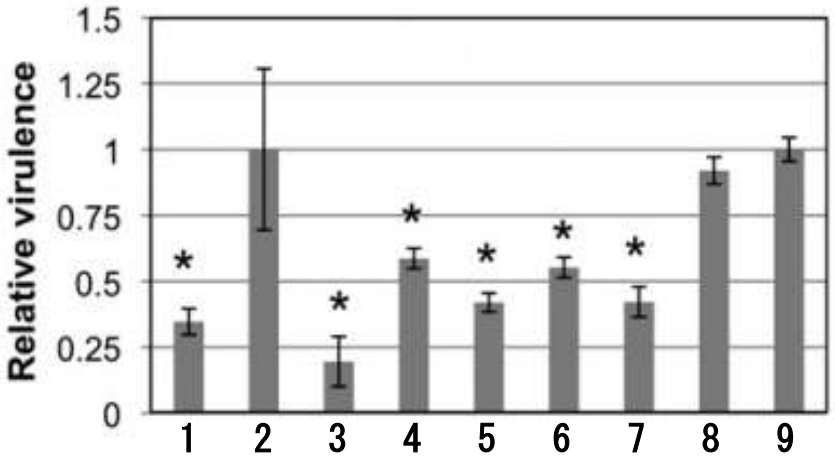


Down-regulated pathways



Up-regulated pathways

Pathogencity of BEA and FA Knockout mutant

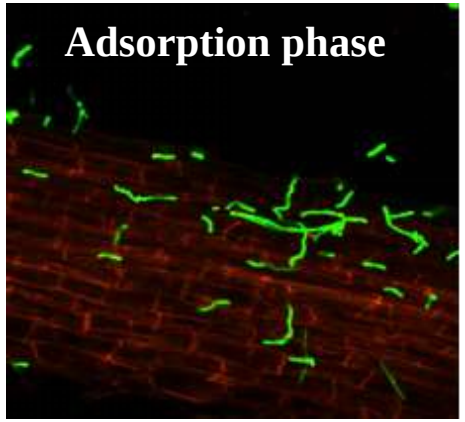


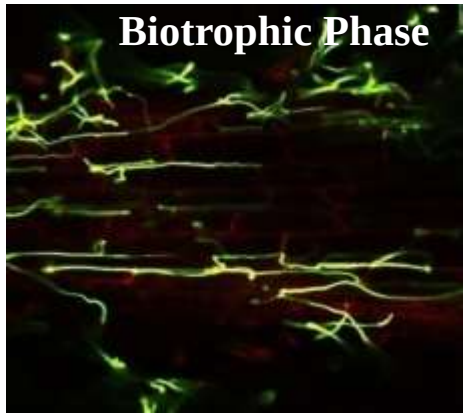
Changes of the BEA and FA biosynthesis in the mutants

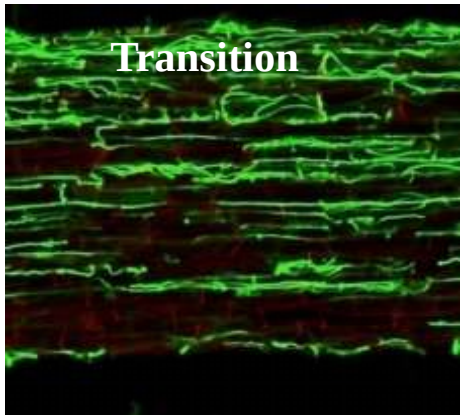
编号	菌株名称	BEA 合成量（μg/g）	FA 合成量（μg/g）	*发病率(%)
1	BEA(-)	420	60.3	35.5[d]
2	*Foc* TR4	2040	57.2	100[a]
3	BEA(-)	380	55.2	19.5[e]
4	FA(-)	1922	<0.05	60.6[b]
5	FA(-)	2036	<0.05	44.2[c]
6	FA(-)	1899	<0.05	57.5[b]
7	FA(-)	1945	<0.05	45.3[c]
8	FA Revertant	2122	44.8	91.5[a]
9	BEA Revertant	2012	58.3	100[a]

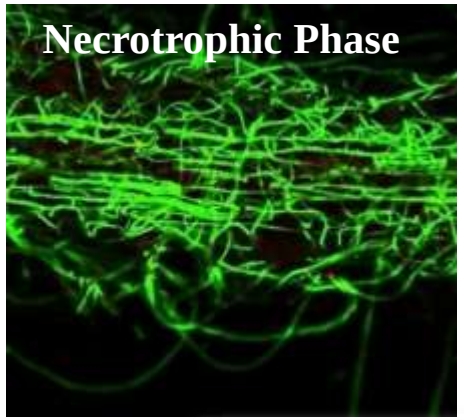
Inoculation test on banana

Research on *Foc* TR4 Effectors

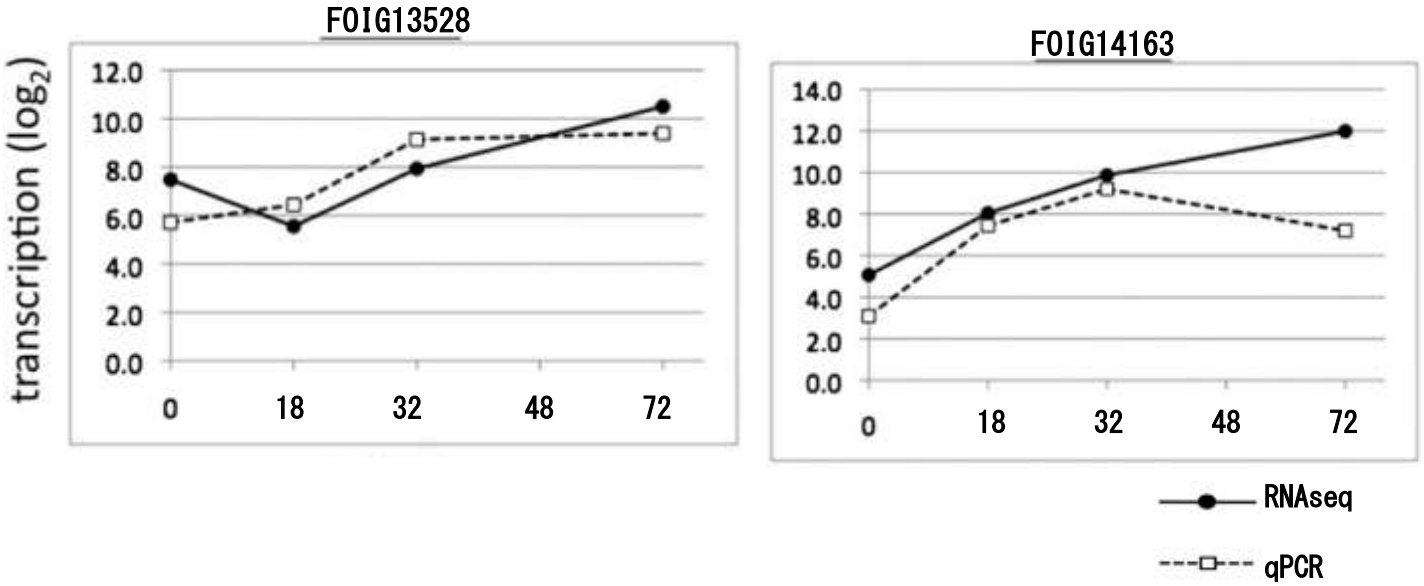


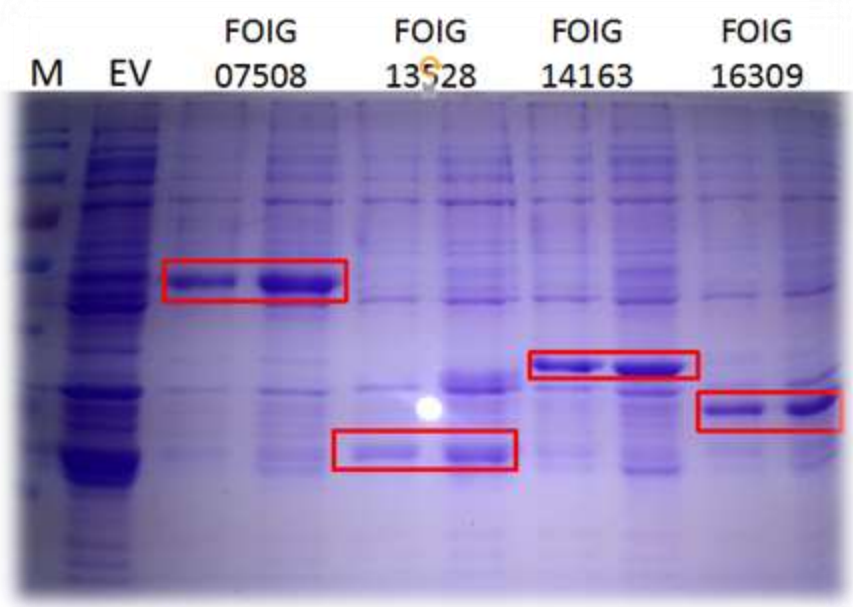






Mining the Effectors expressing during invasion by RNAseq

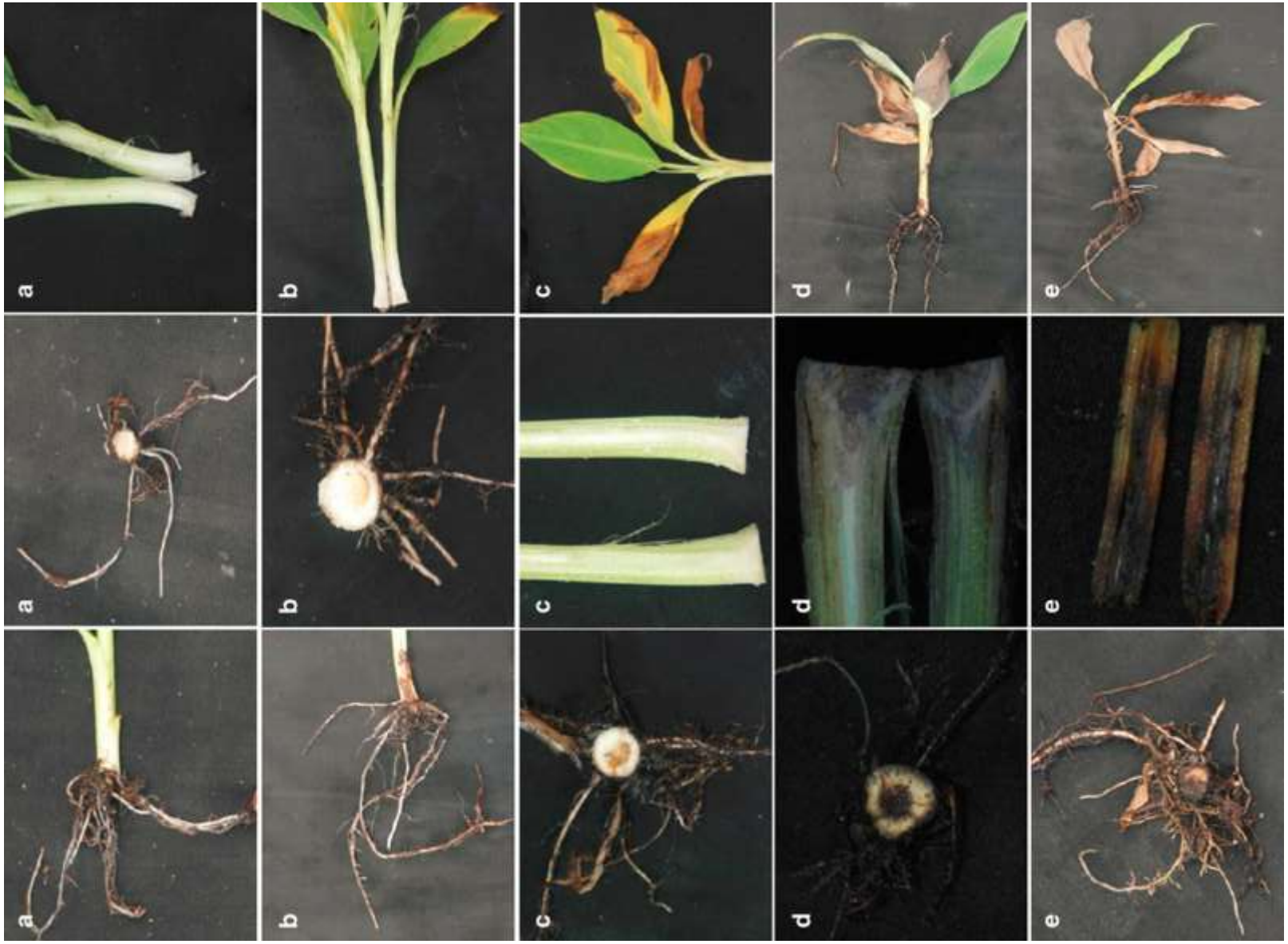




Expressing the Effectors from *Foc* TR4 LS Region and highly expressing during invasion

Remaining moderate virulence

Disease index statistical method



Disease index :

DI= $[(N_{1\text{-}5} \times S_{1\text{-}5})/(N \times S)] \times 100\%$

DI=0 No virulence

DI=1%-25% Weak virulence

DI=26-50% Moderate virulence

DI=51%-75%Strong virulence

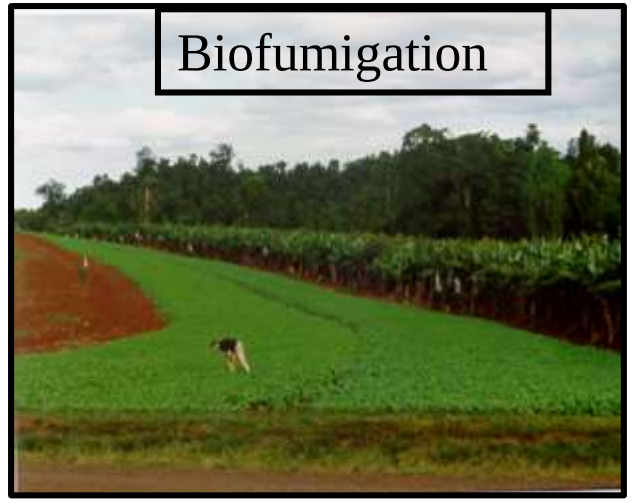
DI=76%-100% Super virulence

Prevention Methods

1. Pre-collecting the local banana germplasm
2. Forbiding the means and source of introduction
   - ◆ Restriction or quarantine of the foreign banana plantlets
   - ◆ Labor's movements
3. Launch of an awareness campaign
4. Introduce on-farm prevention and sanitation practices
   - ◆ Controlling water especially the irrigation
   - ◆ One-gate-banana-plantation
   - ◆ Sanitation of the tools and shoes at the gate

Eradication methods

1. Identification of the disease center;
2. Sanitation of the infected soil and plants with high concentration of fungicides or Quick Lime;
3. Killing the diseased plants with the mixture of glyphosate and carbendazim, then burn it;
4. Or Keeping the diseased plant in its place and don't step into the infected area.



Integrative Control Measures

1. Basing on resistant or tolerant varieties

- Reducing fungicide application
- Facing the threat from the pathogen's evolution

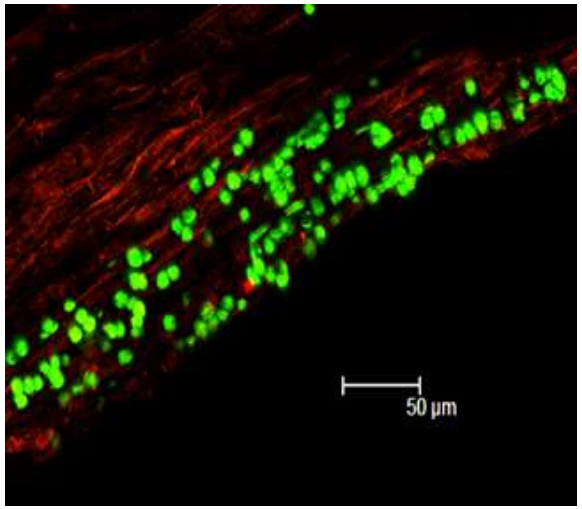
2. Designing a good banana plantation

- Restricting the movement of personnel
- Cutting off the transmission routs, such tools, shoes and etc

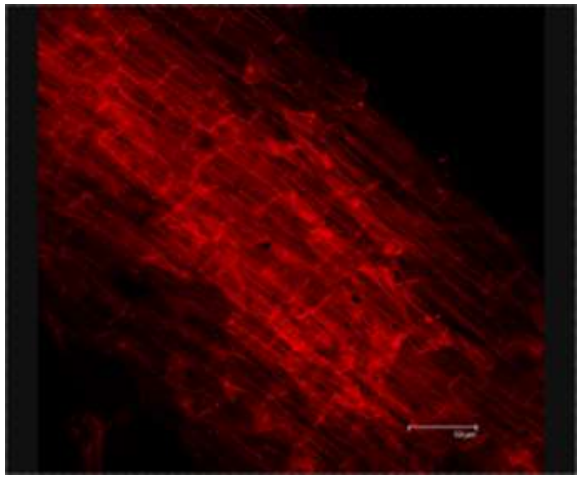
3. **Preparing the Banana Plantlets free of *Foc***
4. **Water treatments:** chlorine dioxide, Lime nitrogen, *Bacillus subtilis*
5. **Soil management.**

PH value, Microbes, organic matters and etc

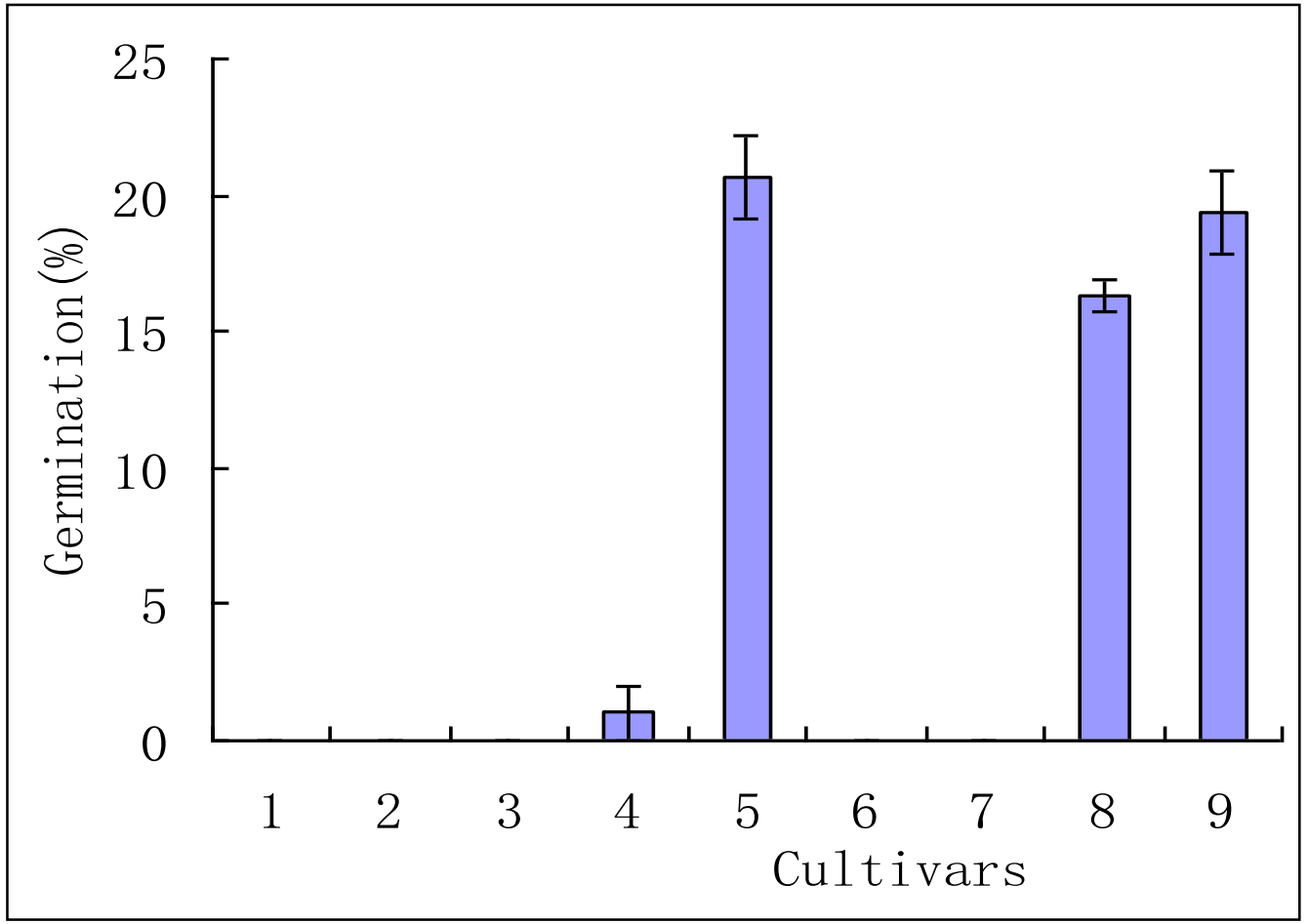
Earlier Screening Method



Susceptible cultivars



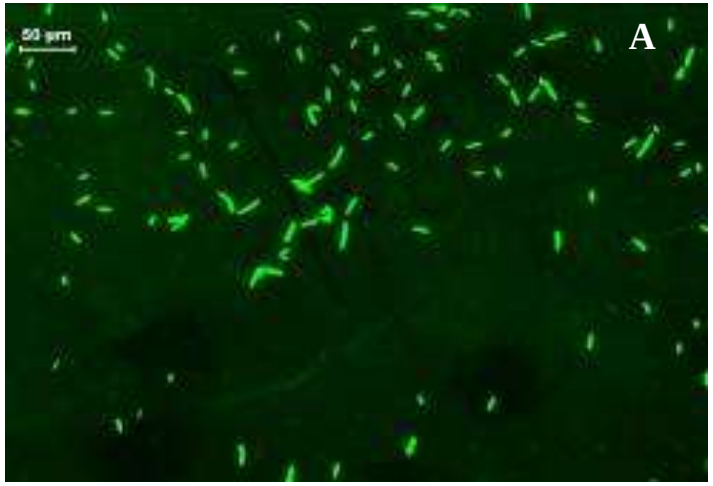
Highly resistant cultivars

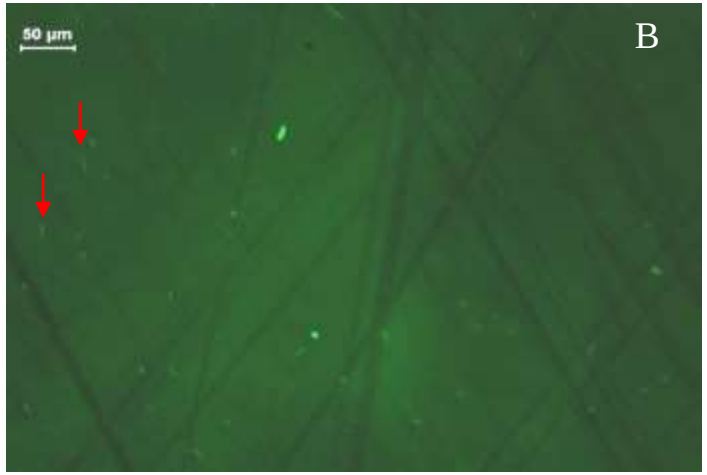


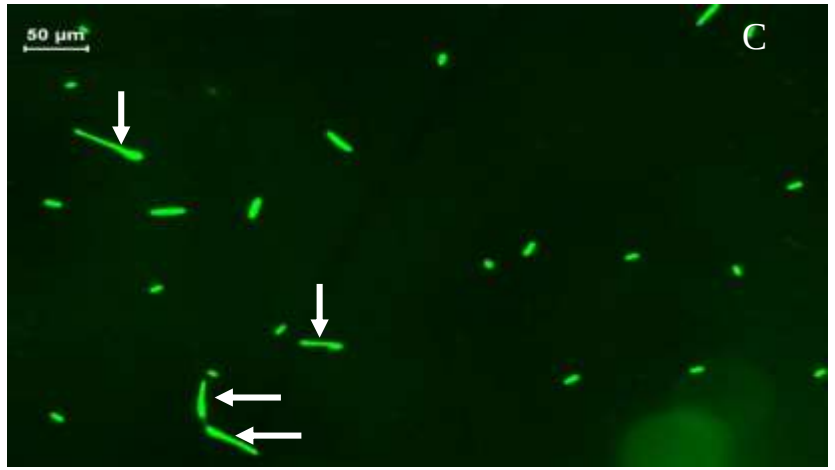
The effect of root exudates on spore germination

1. Musa ABB cv `Dongguan Dajiao`; 2. Musa ABB cv `Zhongshan Dajiao`; 3. Musa AA cv `Haigong`; 4. Musa AAA Cavendish cv `Wilt-resistant #1`; 5. Musa AAA Cavendish cv `Brazilian`; 6. Musa AAA Cavendish cv `Wilt-resistant #5`; 7. Musa ABB Pisang awak cv `Guangfen #2`; 8. Musa AAA Cavendish cv `Dafeng #2`; 9. Musa ABB Pisang awak cv `Guangfen #1`. The germination of differed significantly among the cultivars ($\chi 2$, df=8, $p<0.0001$).

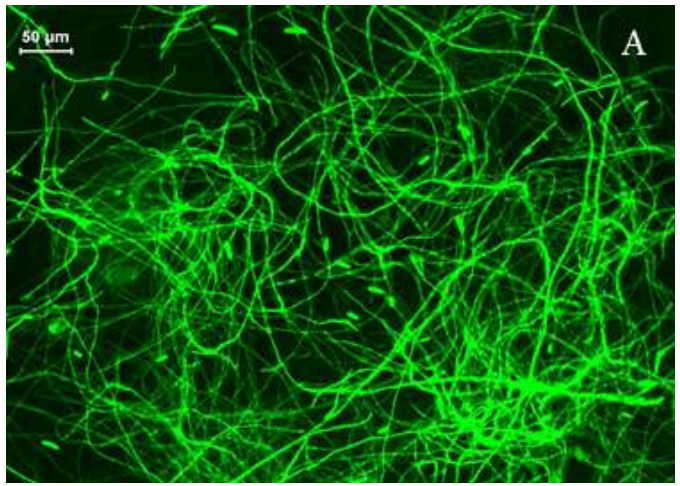
The effect of root exudates on spore germination

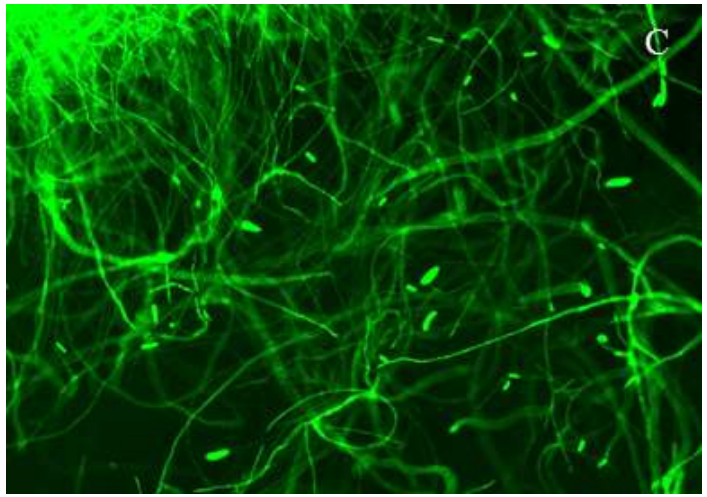


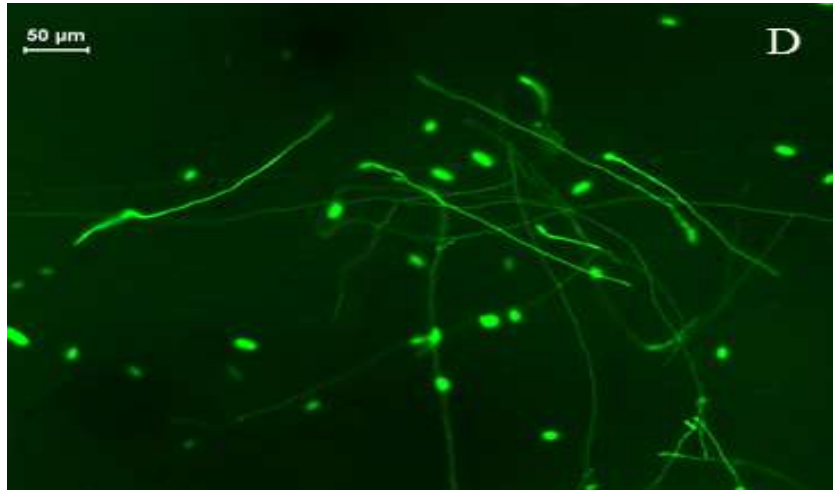




Musa ABB cv `Dongguan Dajiao` Musa AAA Cavendish cv `Wilt-resistant #5` Musa AAA Cavendish cv `Brazilian`

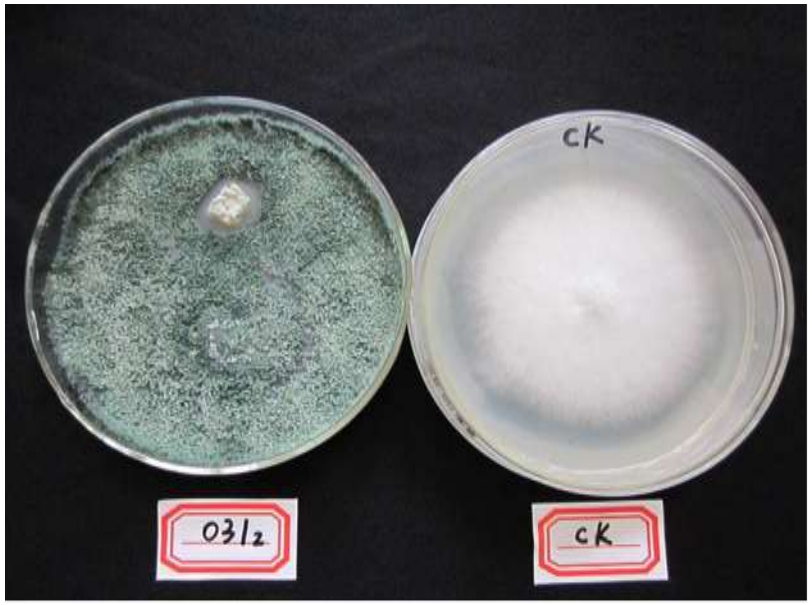


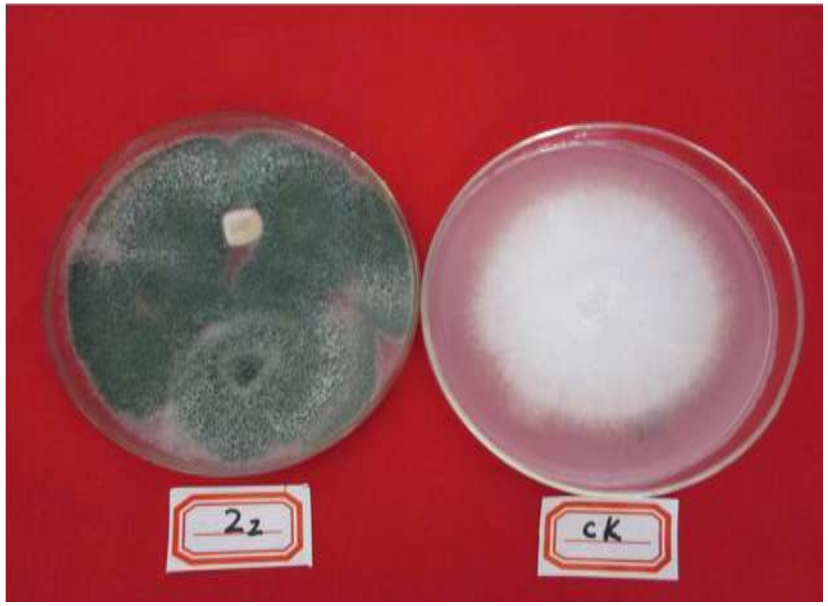




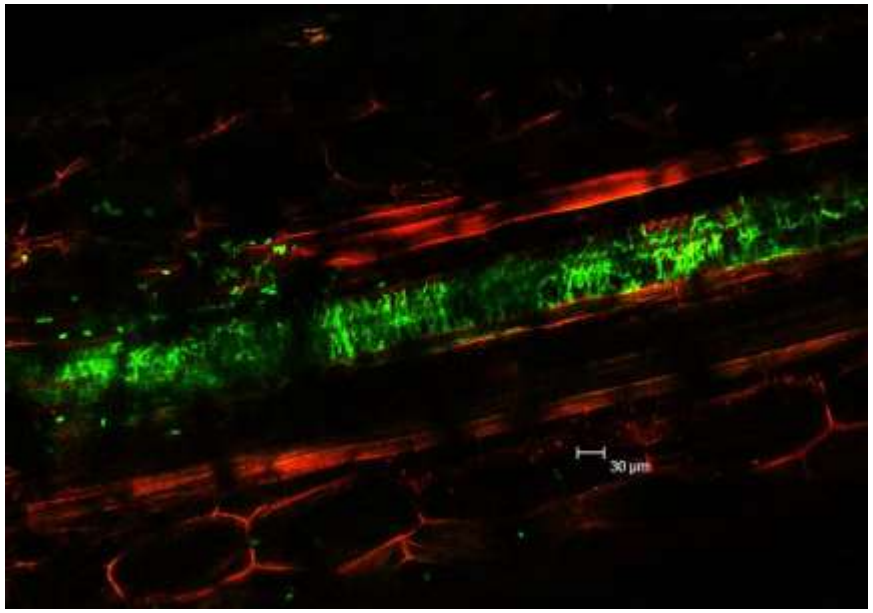
Musa AAA Cavendish cv `Brazilian` Musa ABB Pisang awak cv `Guangfen #1` Musa AAA Cavendish cv `Dafeng #2`

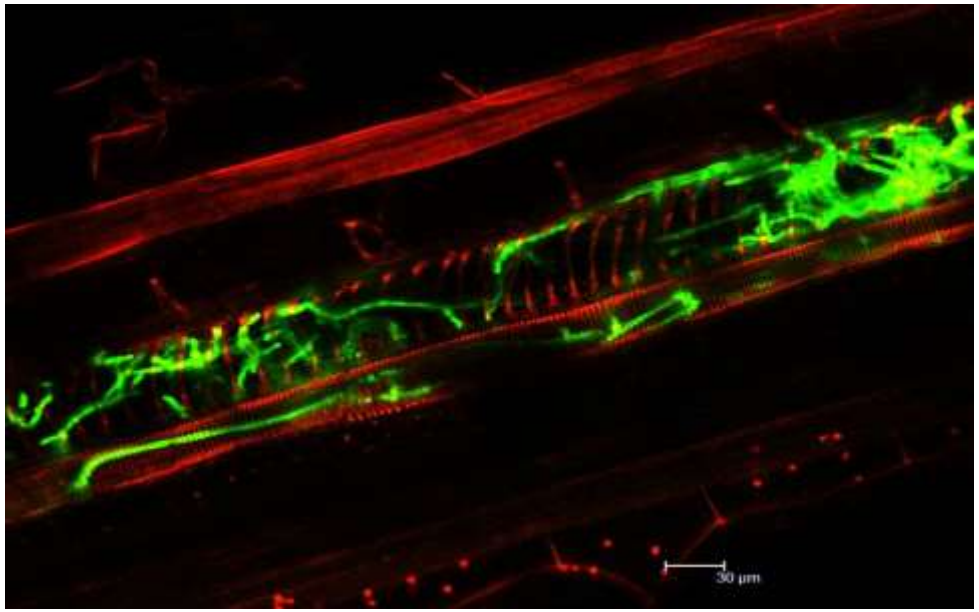
Biology control





Fermentation the banana Pesoudostem with biological strains



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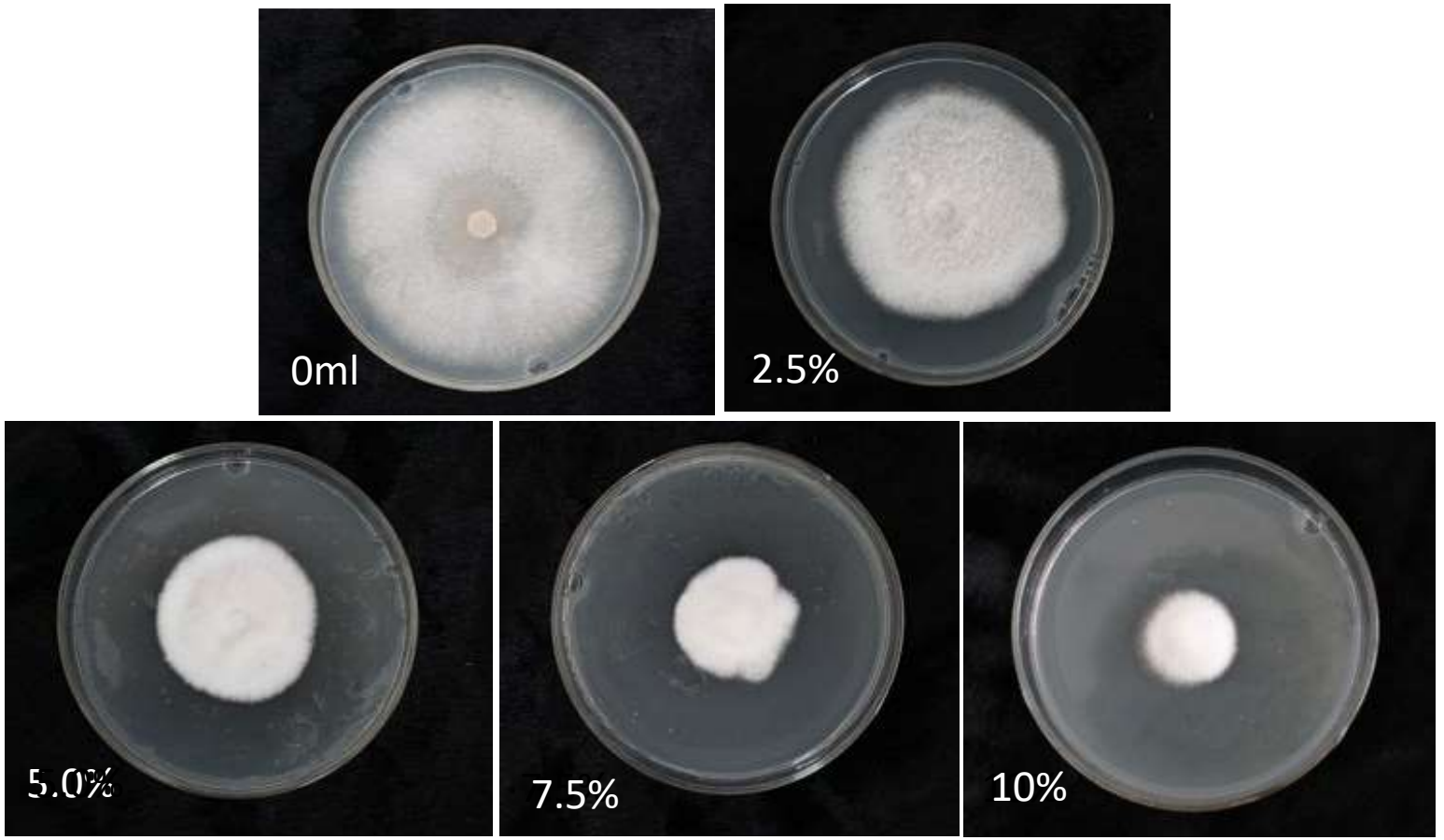
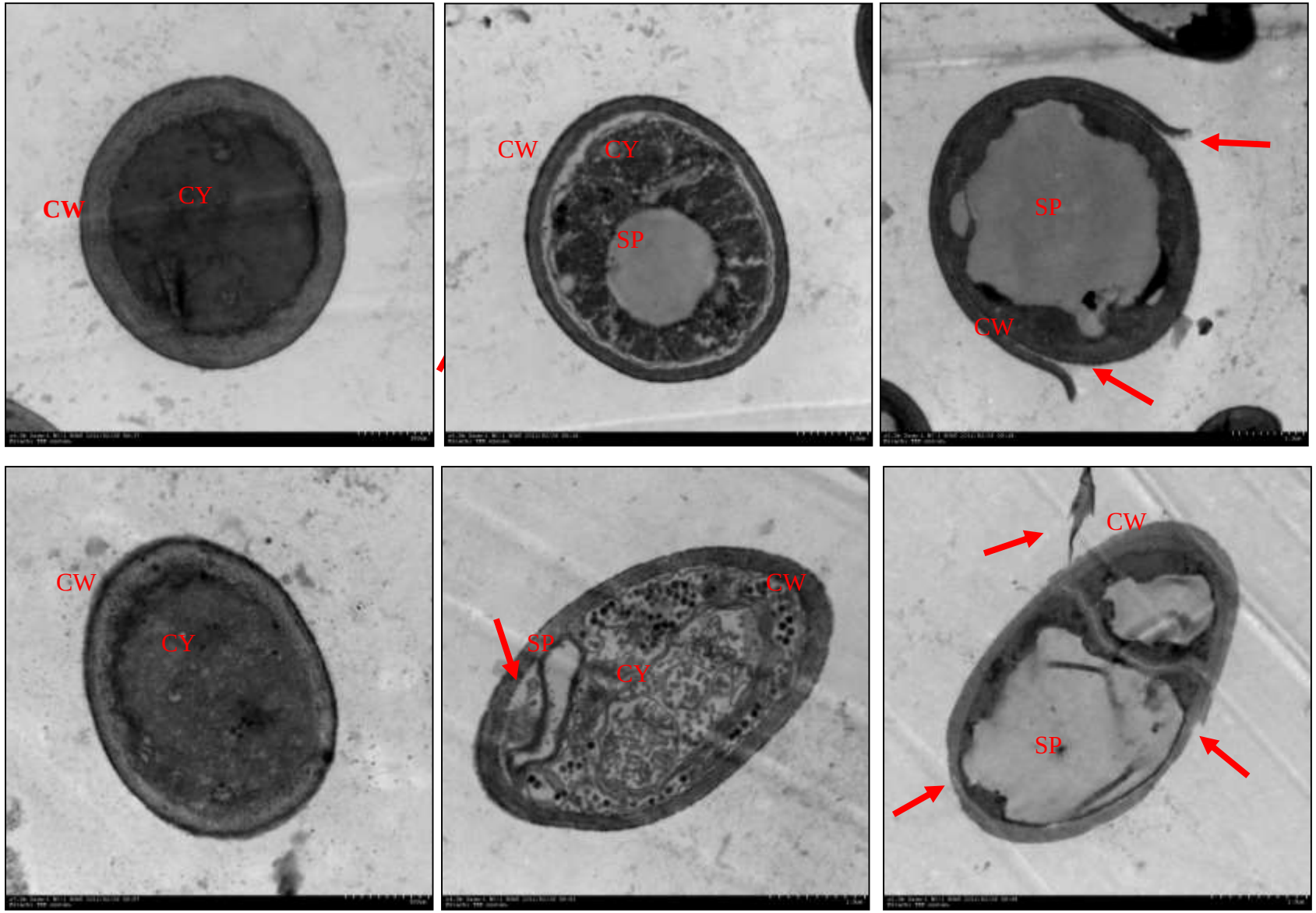
Bioassay in Greenhouse

Chinese Leek control Fusarium Wilt of Banana

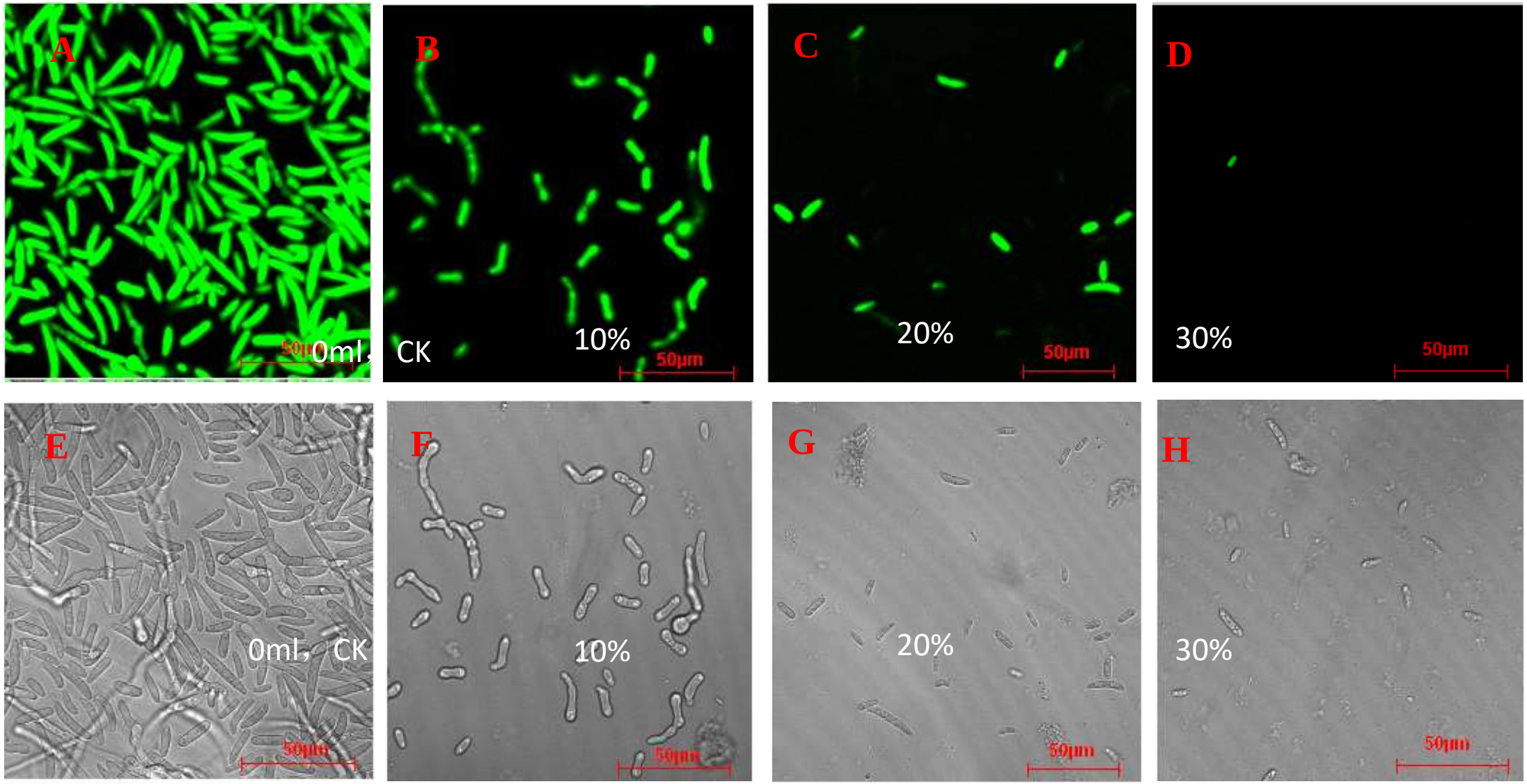
The extract affects *Foc* TR4 mycelial growth



The extract deformed *Foc* TR4 Mycelium



The extract killed *Foc* TR4 spores



Conclusions

1. *Foc* TR4 spread to all of the banana production area in China

2. *Foc* is evolving rapidly, and is a threat for the world banana industry

3. Effectors and mycotoxins are the virulence factors of *Foc* TR4

4. Integrative Control Measures basing on the resistant or tolerant varieties, and other methods including biology control, rotation should be adopted

Acknowledgments

- Group members

1. Dr Ganjun Yi
2. Dr Yuerong Wei
3. Dr Chunhua Hu
4. Dr Cunwu Zuo
5. Dr Guiming Deng
6. Dr Qiaosong Yang
7. Dr Ou Sheng
8. Dr Ruibin Kuang
9. Dr Biswas Monash
10. Dr Huijun Zhao

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   Stellenbosh Univeristy
   South Africa

Dr Altus and Group members

Thank you!