STATEGY FOR COMBATING FUSARIUM WILT IN BANANAS THROUGH COVENTIONAL BREEDING,GMO'S AND CULTIVATION TECHNOLOGY

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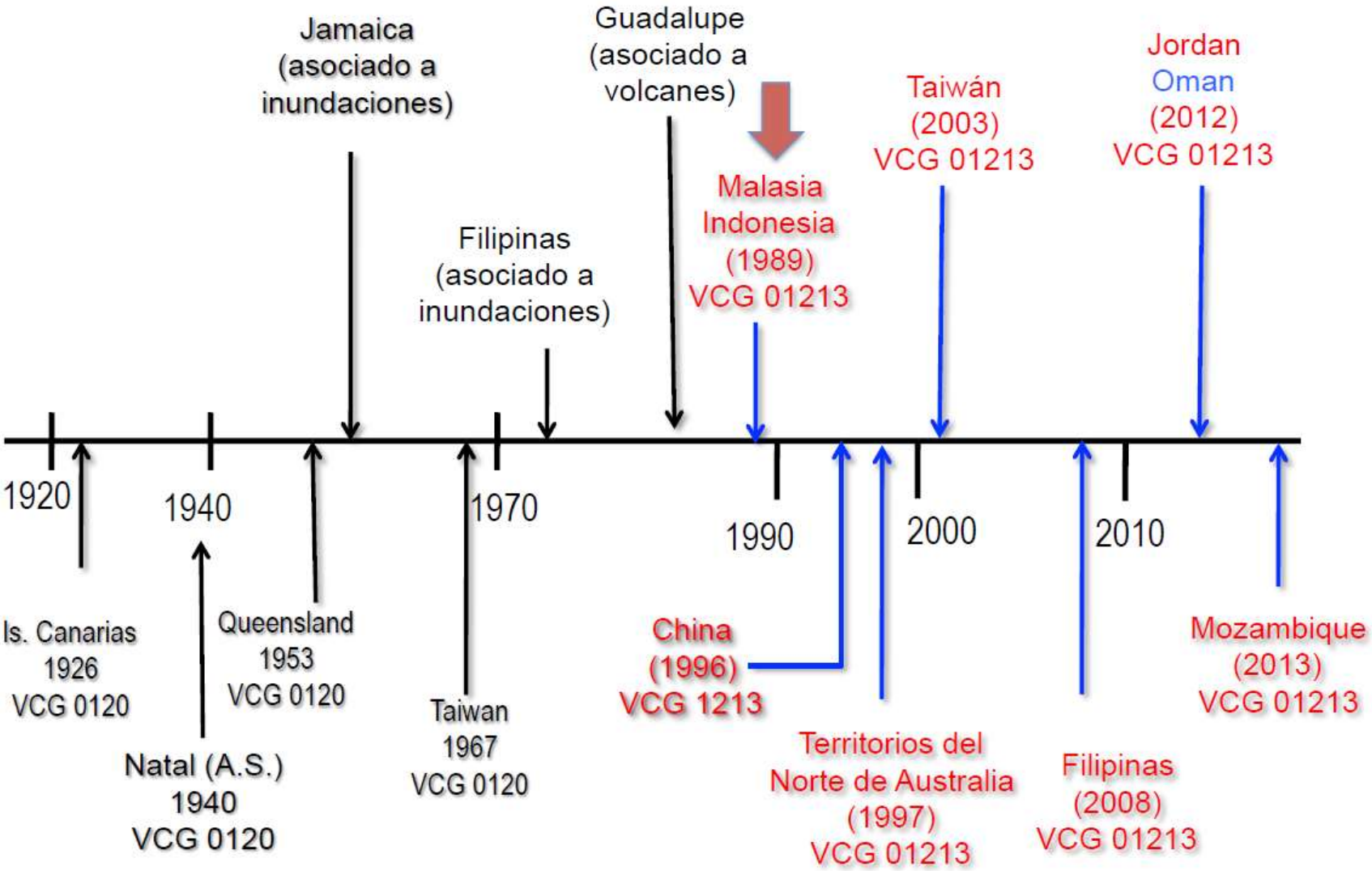
Short History of FOC race 4 in Cavendish



Background

- ✓ ***Banana is one of the most important fruits in China***
- ✓ ***2014Cultivation area 319,500 hectares,Yield (8,660,000 t)***
- ✓ ***90% of cultivars are cavendish***

STATEGY FOR COMBATING FUSARIUM WILT

banana and leek rotation prevents from Fusarium wilt

Chemical Control

biological control

genetic resistance

crop rotation

traditional crossbreeding (difficulty)

cell engineering

Molecular breeding

non-model species

Resistant genes

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Control of Fusarium wilt in banana with Chinese leek

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Abstract The inhibitory effects of Chinese leek (*Allium tuberosum*) on *Fusarium oxysporum* f. sp. *cubense* (*Foc*) and on *Fusarium* wilt incidence were studied in order to identify a potential efficient way to control the disease. Adopting the rotation system of Chinese leek-banana reduced the *Fusarium* wilt incidence and disease severity index by 88–97 % and 91–96 %, respectively, and improved the crop value by 36–86 %, in an area heavily infested by *Foc* between 2007 and 2009. As a result of inoculation in the greenhouse, Chinese leek treatment reduced disease incidence and the disease severity index by 58 % and 62 %, respectively in the variety Baxi (AAA) and by 79 % and 81 %, respectively in the variety Guangcompletely inhibited the growth of *Foc* race 4 on Petri dishes, suppressed the proliferation of the spores by 91 % and caused 87 % spore mortality. The findings of this study suggest that Chinese leek has the potential to inhibit *Foc* growth and *Fusarium* wilt incidence. This potential may be developed into an environmentally friendly treatment to control *Fusarium* wilt of banana.

Keywords *Fusarium oxysporum* · Panama disease · Banana · *Allium tuberosum* · Crop rotation · Biocontrol

Introduction

Decrease of the Fusarium wilt incidence in the field production adopting the CL-Banana rotation system

Guangfen (ABB) Vs CL

Baxi (AAA) Vs Cl

Guangfen (ABB) intercropping with Baxi Vs CL

The fruit of Guangfen (ABB) Vs CL

Conventional Banana Breeding

Resistance evaluation of Banana germplasm

National banana Germplasm

- Fields
- Greenhouse
- In vitro

Resistance evaluation of Banana germplasm

- Get 9 resistance varities from 60 banana germplasm

Pahang (*malaccensis*, AA) No symptom

Pa (Rayong) (AA) ID= 51.79±16.97

The establishment of efficient single-cell original somatic embryo regeneration system

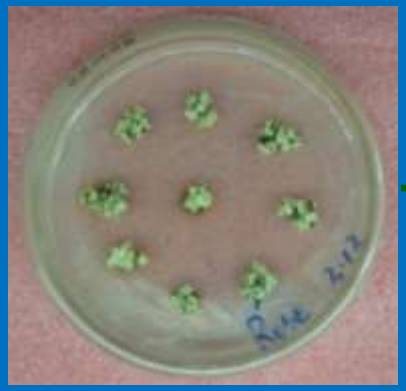








Mutagenesis

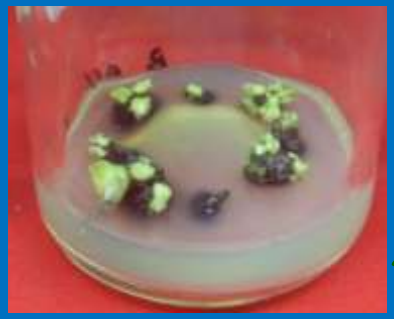


Multiple buds

Embryogenic callus

NaN_3、EMS

Colchicine



4 subcultures

◆ Resistant Varieties Breeding

➢ Screening somaclonal variants of Cavendish

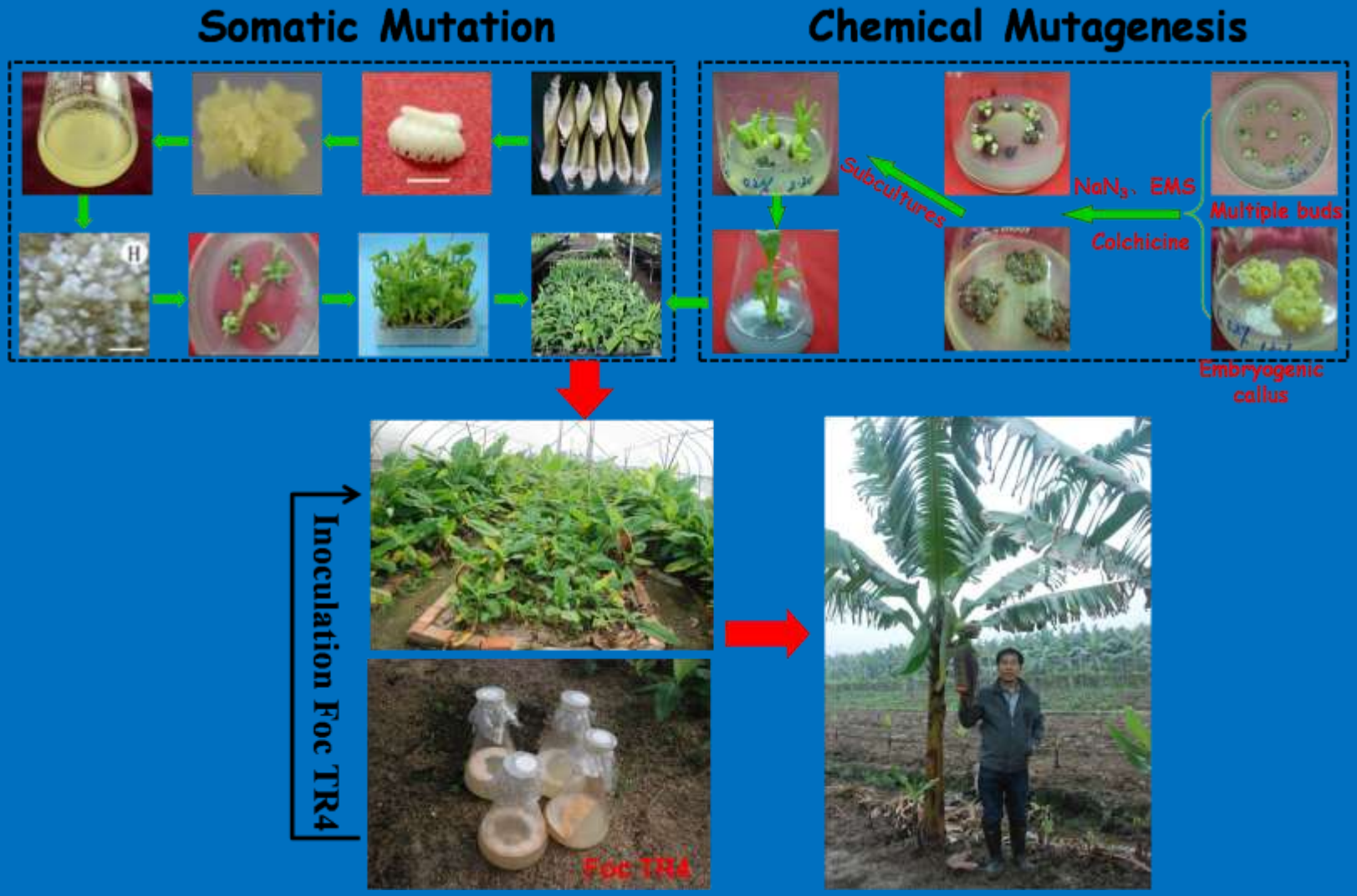
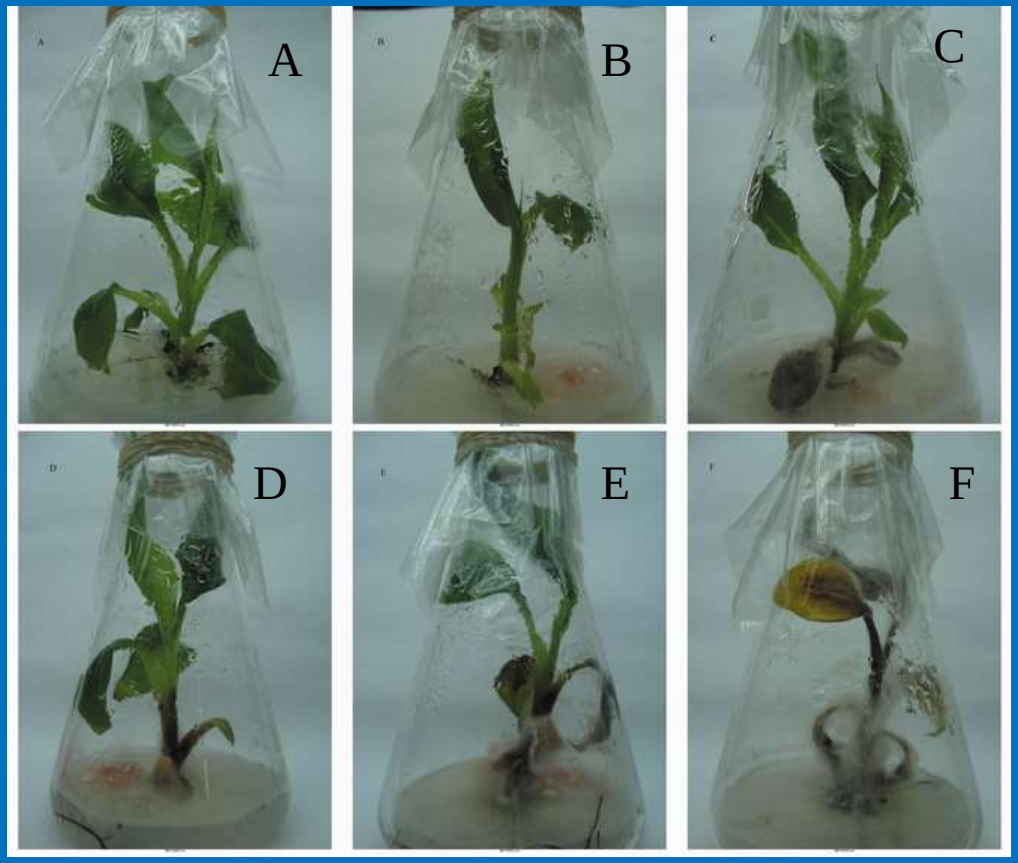


Fig. 1 Cavendish Somaclonal Selections Resistant to Foc TR4

Cavendish somaclonal selections resistant to Foc TR4

Evaluation of resistance to banana Fusarium wilt

- **Established in vitro, pot and field three evaluation systems**



- **Rapid identification of disease resistance in vitro**

- A, Control plant; B-F: Disease incidence of banana seedlings treated with *Foc* TR4 spore suspension

- Rapid screening of Musa species for resistance to Fusarium wilt in an in vitro bioassay. (European Journal of Plant Pathology，2010） （Patent No. 200910192176.0）

➢ Greenhouse Evaluation

A: Zj4
Resistant

B: Zj6
Resistant

CK: Baxi
Susceptible

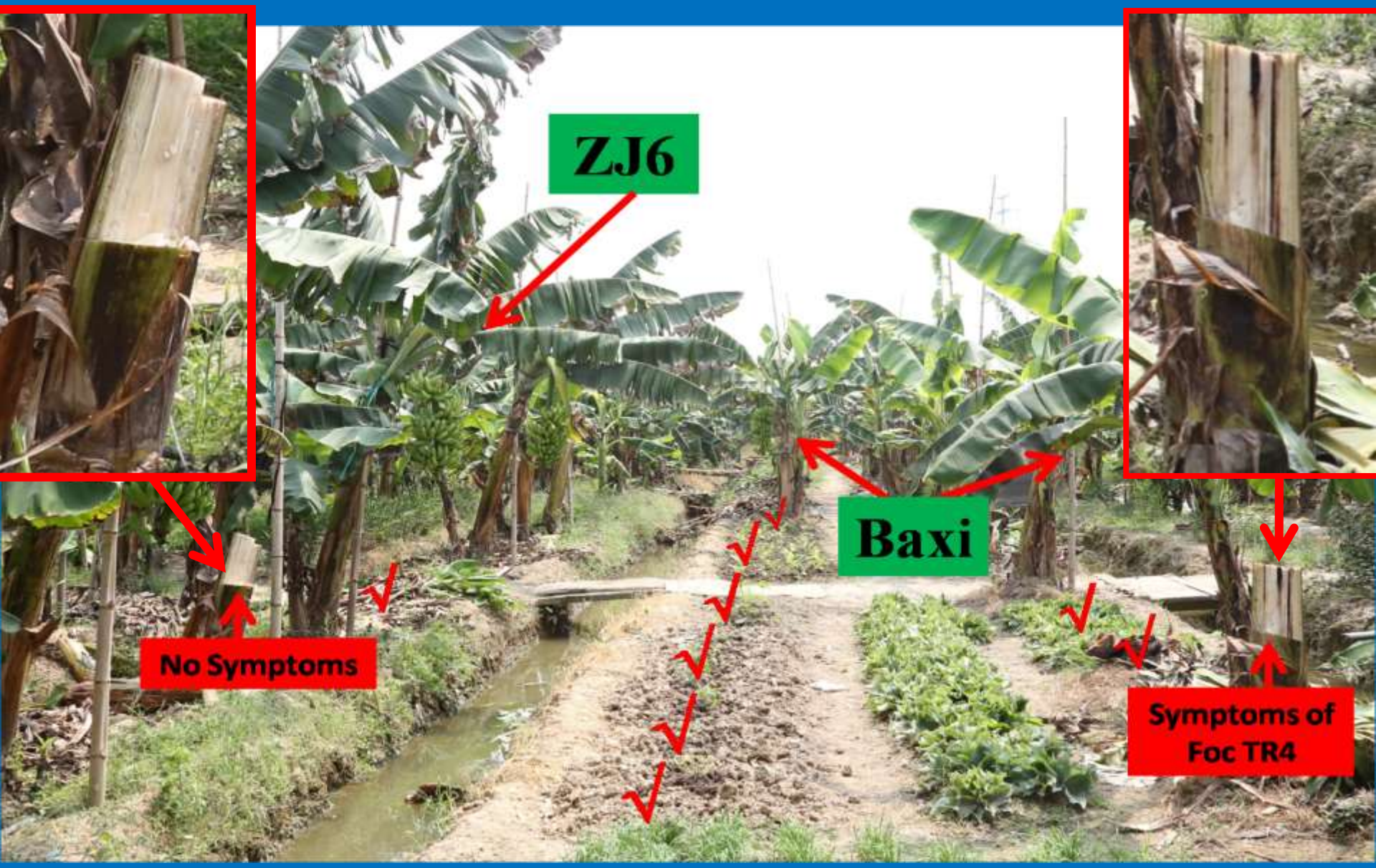
Fig. 2 Greenhouse Evaluation of Cavendish Somaclonal (A: ZJ4, B: ZJ6, CK: Baxi) for Resistance to Foc TR4

New cultivar: ZJ-06

Incidence of disease: Baxi (48.2±2.6%); ZJ-06(3.2±0.82)

Note: Red arrows indicate the died plantlet.

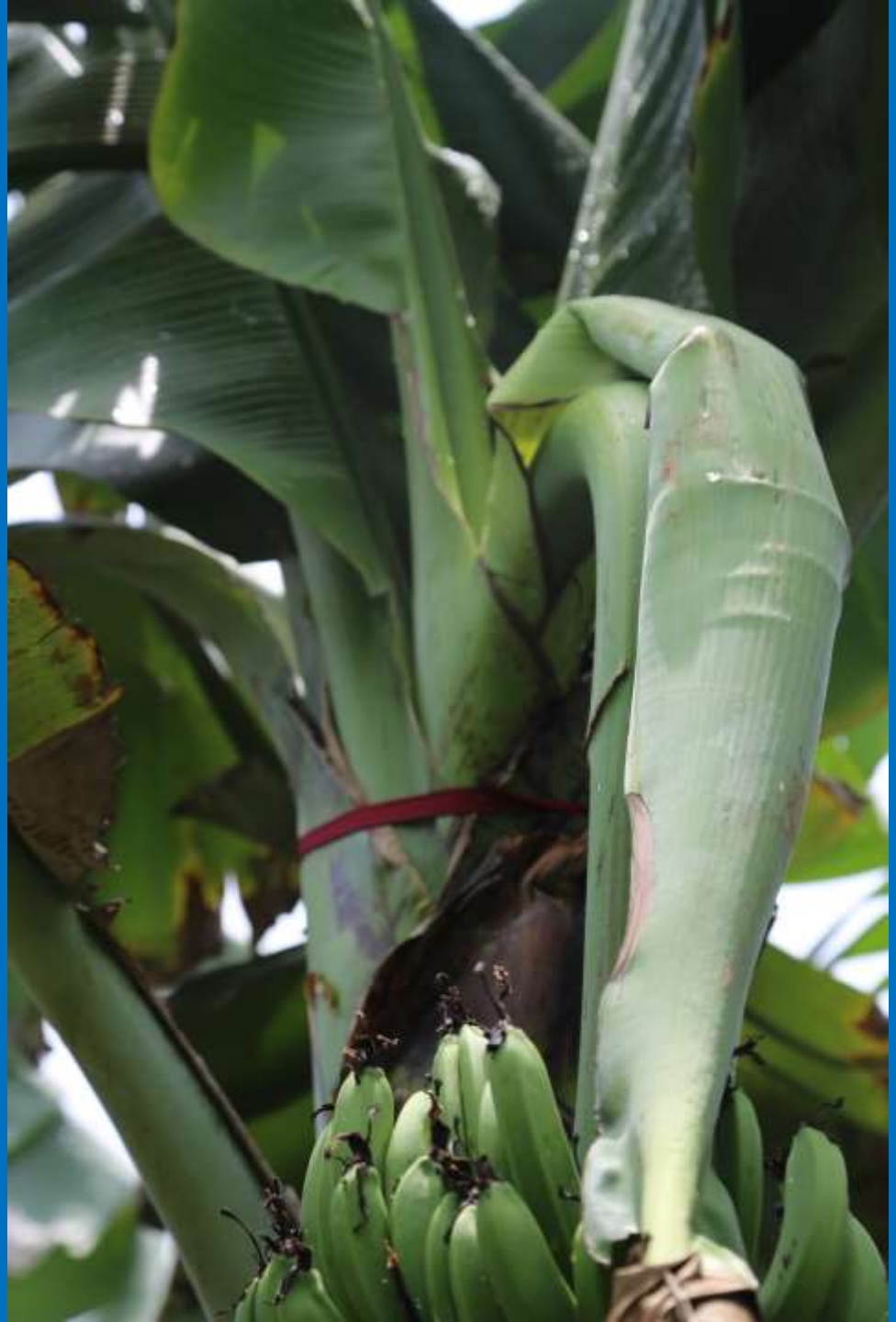
➢ Field Evaluation



Note: '√ ' indicates the banana plant death caused by Foc TR4

➢ Field Evaluation

中蕉4号



➢ Comparison of Main Agronomic and Economic Traits

	Zhongjiao No.6 (ZJ6)	Zhongjiao No.4 (ZJ4)	CK: Baxi
Production cycle	360~380 d	340~350 d	340~360 d
Plant height	2~3 m	2~3 m	2~3 m
Bunch weight	24.7 kg	26.2 kg	~25 kg
Yield per hectare	44.4 T	47.2 T	~45 T
Number of hands per bunch	6~8	6~8	6~8
Number of fingers per bunch	148	139	~145
Fruit (finger) weight	176	177	~175
Fruit length	23.6	25.5 cm	~25
Fruit diameter	12.5	12.74	~12.5
Pulp colour at maturity	Ivory	Ivory	Ivory
Mature fruit peel colour	Bright yellow	Bright yellow	Bright yellow
Predominant taste	Sweet	Sweet	Sweet
Infection rate (Foc TR4)	**5~15%**	**0~5%**	**>50%**

- Results were concluded based on multi-positions of field testing over 5 years in heavily infected fields.

➢ ZJ4 & ZJ6

Fig. 4 Two new Cavendish varieties ('ZJ4' and 'ZJ6') selected from somaclonal variants with high resistant to Foc TR4.

ZJ4

- Selection of somaclonal variation from ‘Baxi’
- High resistant to Foc TR4
- Good bunch, hand, finger shape
- Superior fruit quality and taste, sweet-smelling pulp
- TSS 20.9%, TA 0.277%, SS 15.8%，Vc 7.44mg/100g

ZJ6

- Selection of somaclonal variation from ‘Baxi’
- High resistant to Foc TR4
- Good bunch, hand, finger shape
- Superior fruit quality and taste, sweet-smelling pulp
- TSS 18.8%, TA 0.25%, SS 15.8%，Vc 4.35 mg/100g

Banana Molecular Breeding

Studying on genetic transformation of banana (*Musa* spp. Cavendish) for improving its cold tolerance

Cold treament:

- transgenic banana plants overexpressing *MpICE1*/*MpMYBS3* and control plants were subjected to the prechill chamber at 10℃ for 0, 1, 3, 6, 24 and 48 h
- Leaves were harvested to determine cold-responsive genes regulated by *MpICE1*/*MpMYBS3*

candidate genes:

ICE1,*MYBS3*,*CBF1*,*CBF3*,*COR*,*HOS1 and SIZ1*

Experimental Methods:

Agrobacterium tumefaciens-mediated transformation

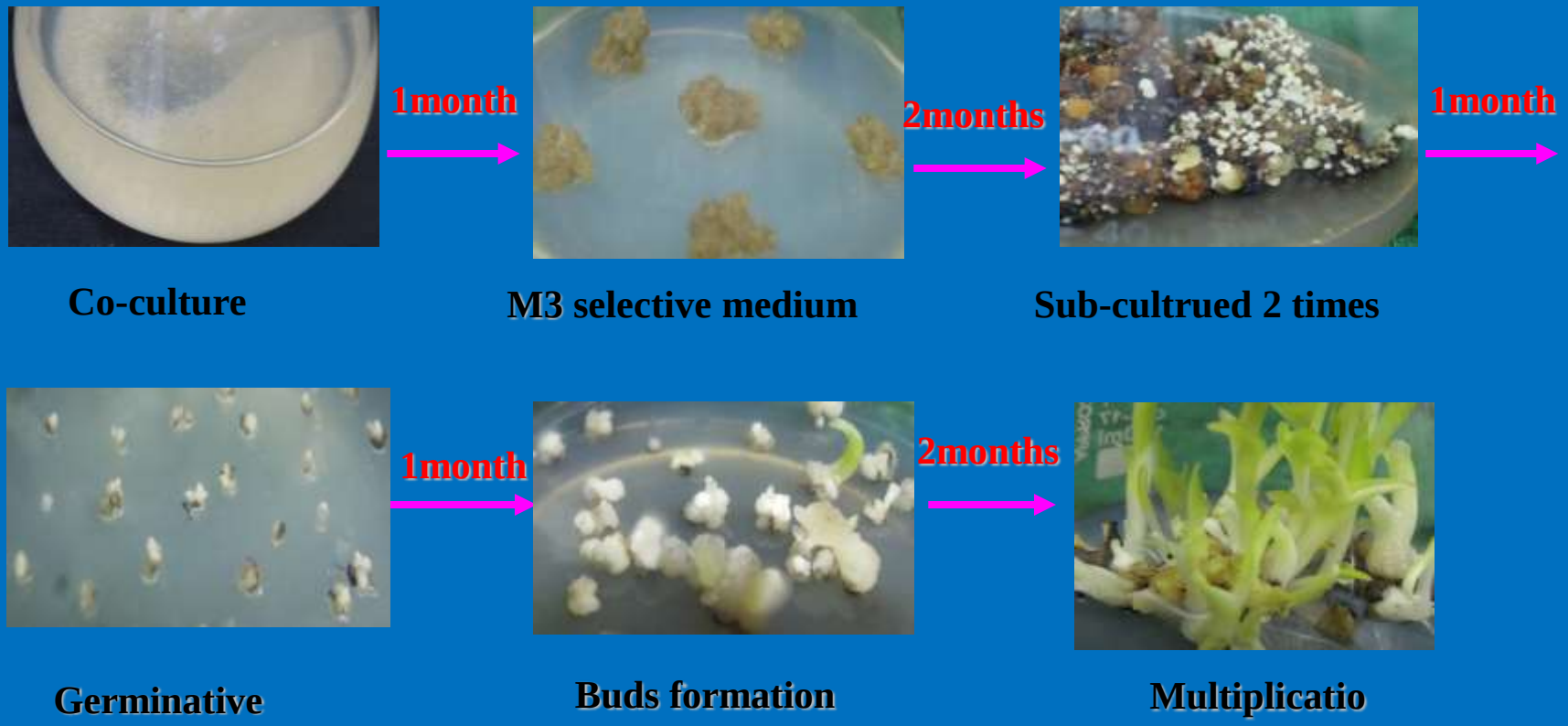


Figure 1. The genetic transformation process of *MpICE1* transferred into embrygenic cell suspension of banana

Cultivar: *Musa spp*. Cavendish, cv Grande Naine (Control)

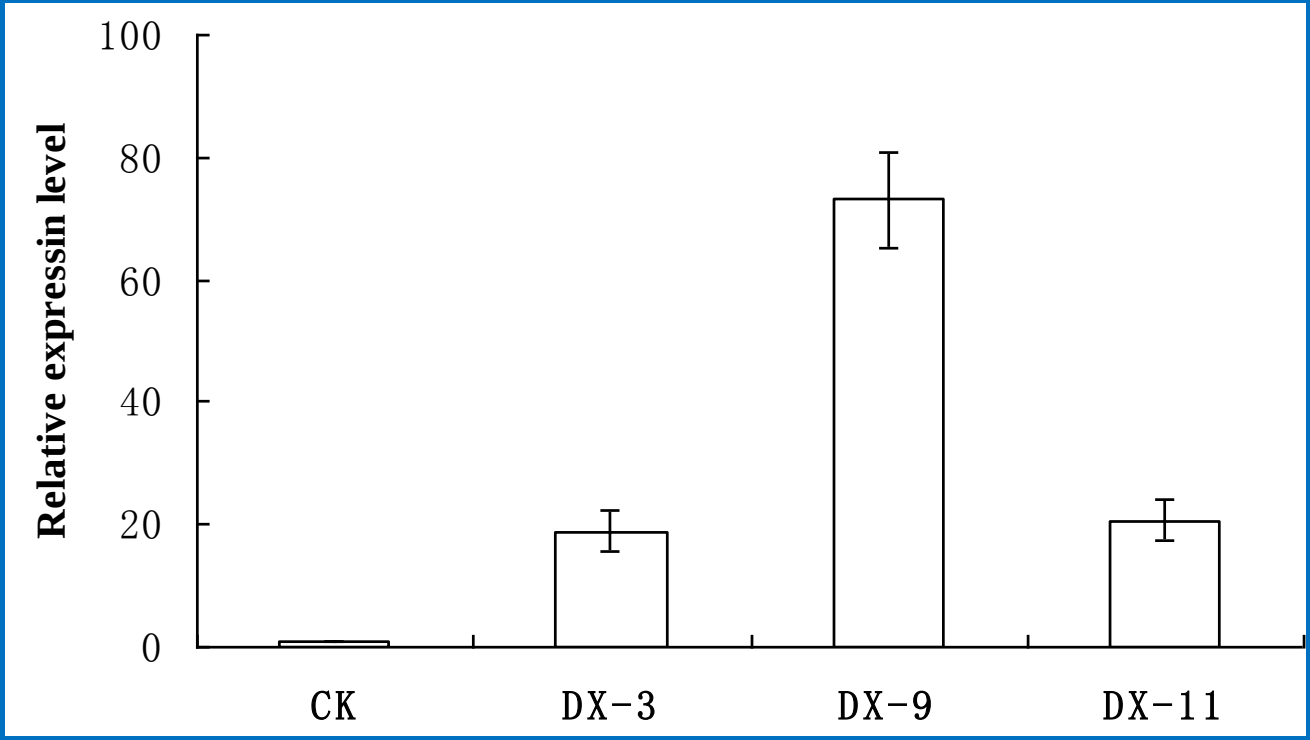
GMO: Overexpression a cold tolerant gene ICE1 from Dajiao in Grande Naine

Condition: grown in a growth chamber at 4°C for 2 days (day/night), a photon flux density of 240 μE throughout a 12-h photoperiod, and a relative humidity of 60-80%.

DX-13 **DX-15** **Control**

Cold tolerance evaluation of P*ubi::MpICE1* transgenic banana plants

Expression Profiles and Physiological data analysis of *MpICE1* Transgenic Banana Plants



Relative expressin level of *MpICE1* in wild-tipe and in the transgenic banana plants

Host-induced gene silencing of ergosterol synthesis genes confers strong resistance to Fusarium oxysporum f.sp. cubense Tropical Race 4

Host-induced gene silencing of cytochrome P450 lanosterol C14α-demethylase–encoding genes confers strong resistance to *Fusarium* species

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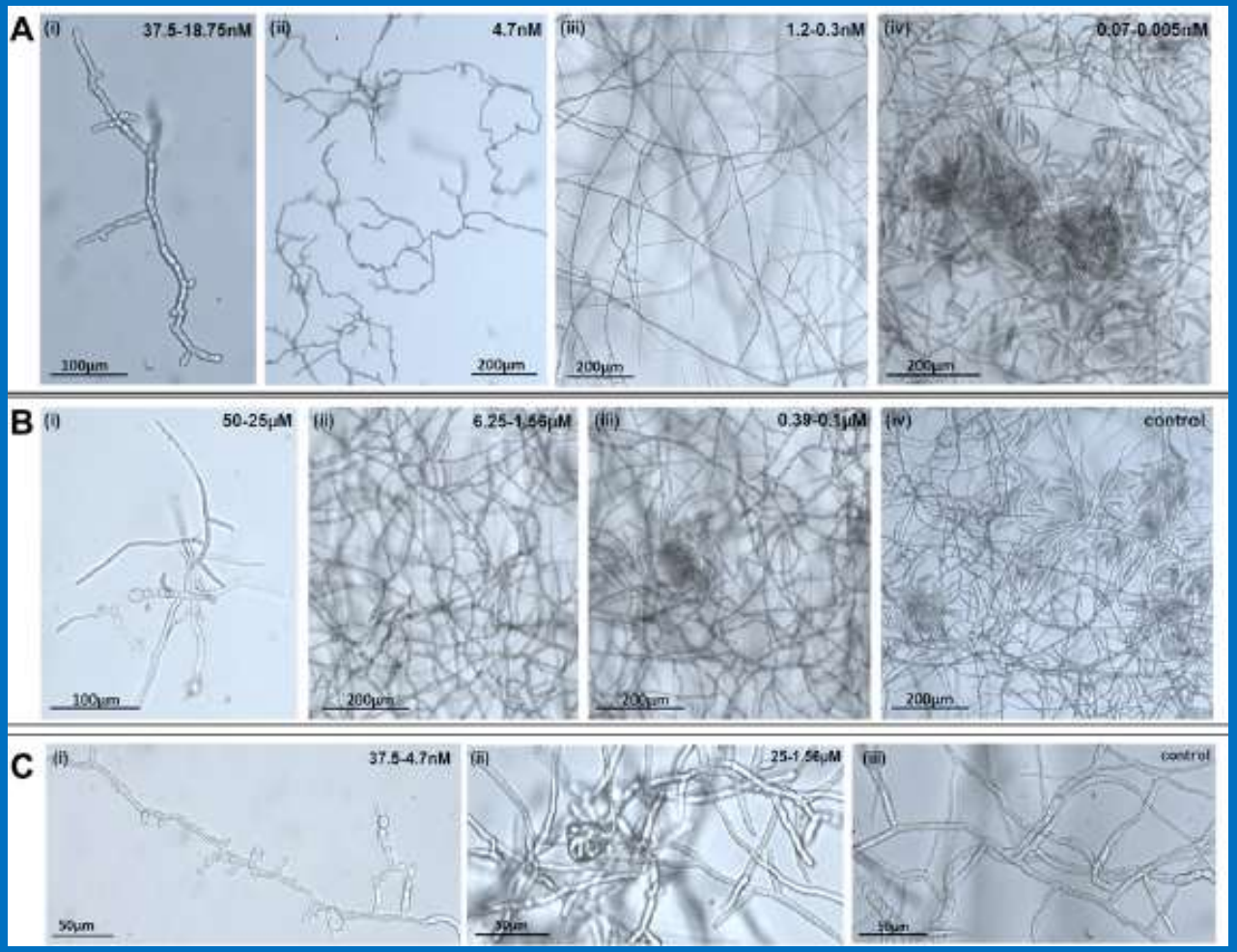


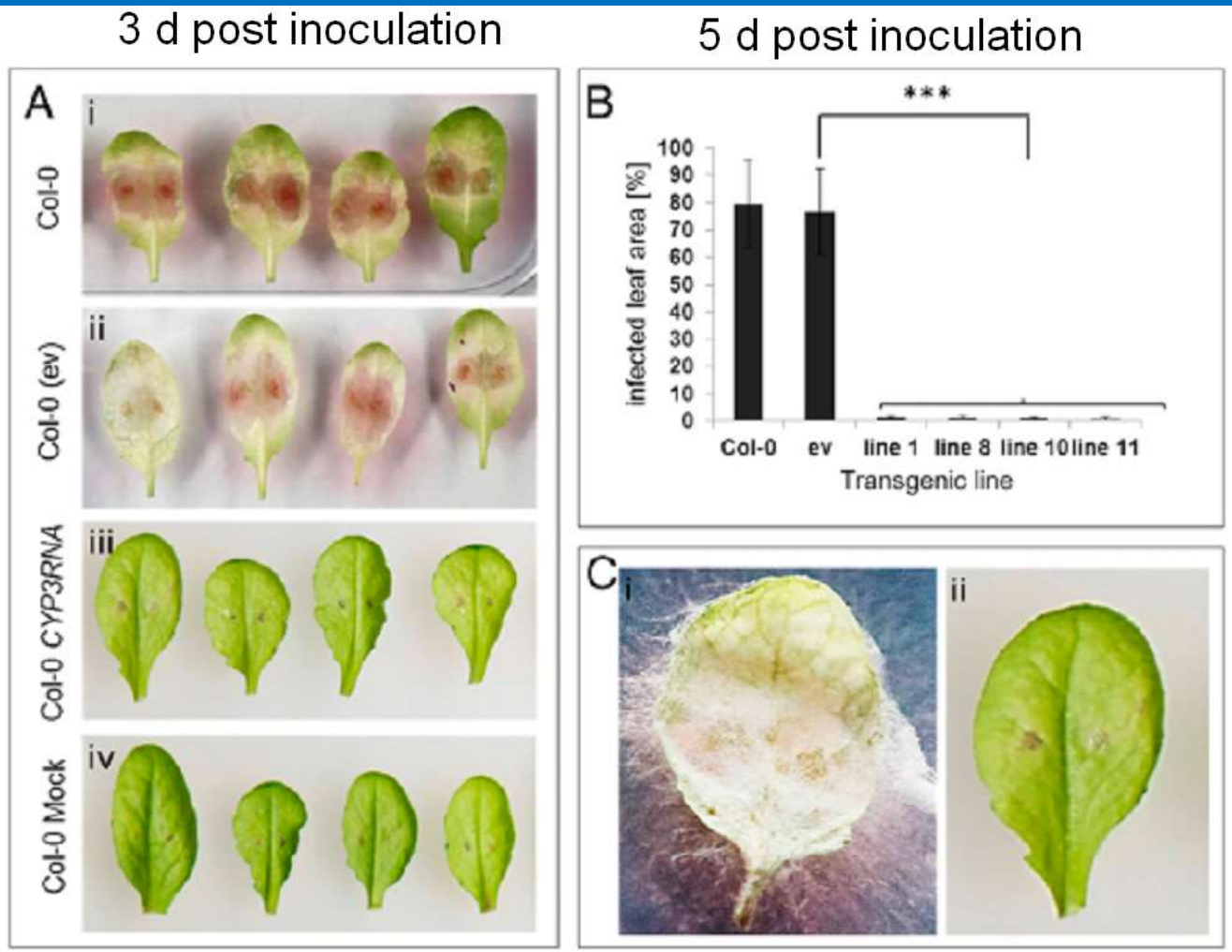
CYP3RNA 48hours

Tebuconazole 48hours

CYP3RNA （i）
Tebuconazole（ii）
72hours

In axenic cultures of Fusarium graminearum, in vitro feeding of CYP3RNA, a 791-nt double-stranded (ds)RNA complementary to CYP51A, CYP51B, and CYP51C, resulted in growth inhibition as well as altered fungal morphology, similar to that observed after treatment with the azole fungicide tebuconazole, for which the CYP51 enzyme is a target.

Koch, A., et al. (2013) PNAS 110, 19324-19329

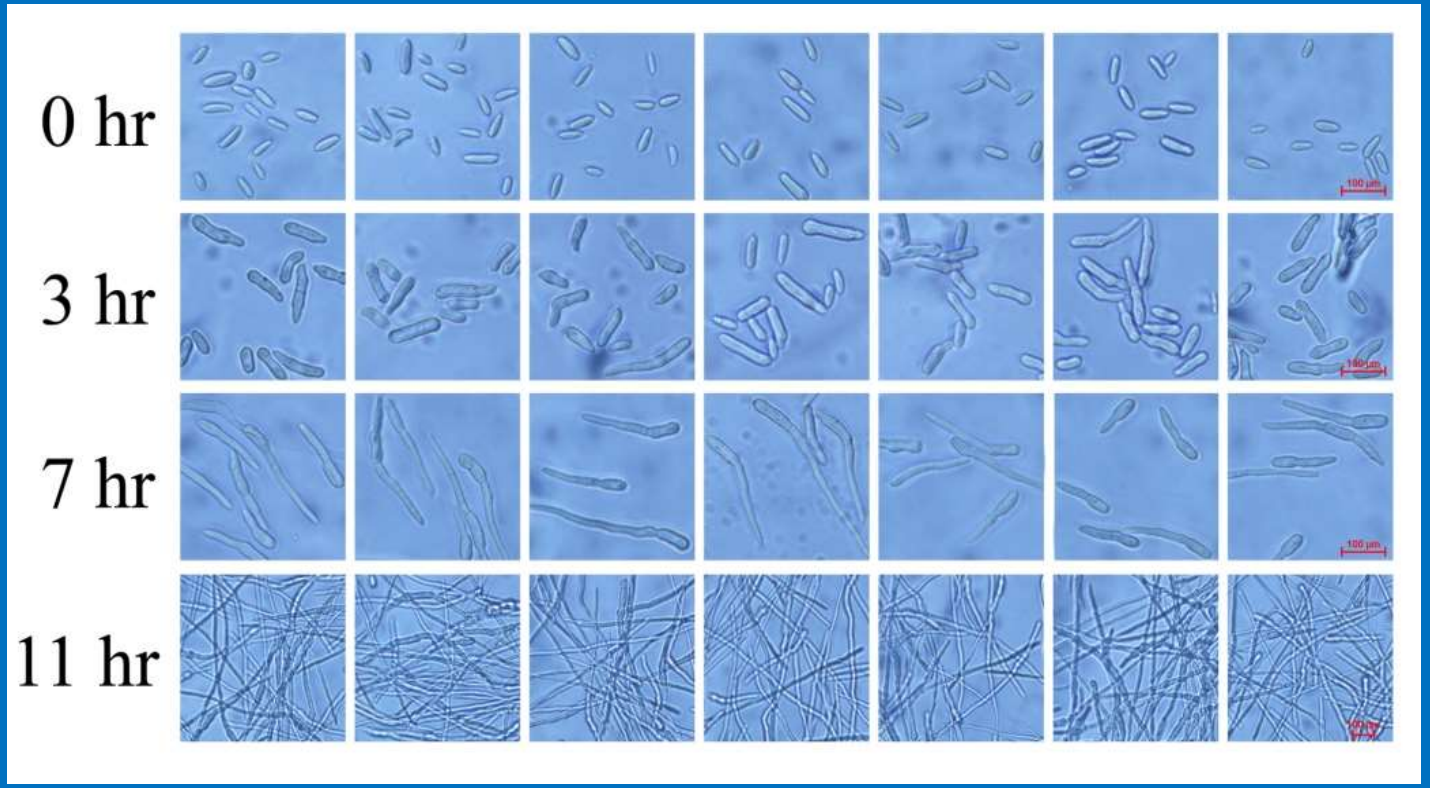


Infection symptoms on Arabidopsis leaves following inoculation with F. graminearum. Detached leaves of 5-wk-old plants were treated with 5×10^4 macroconidia mL^{-1}

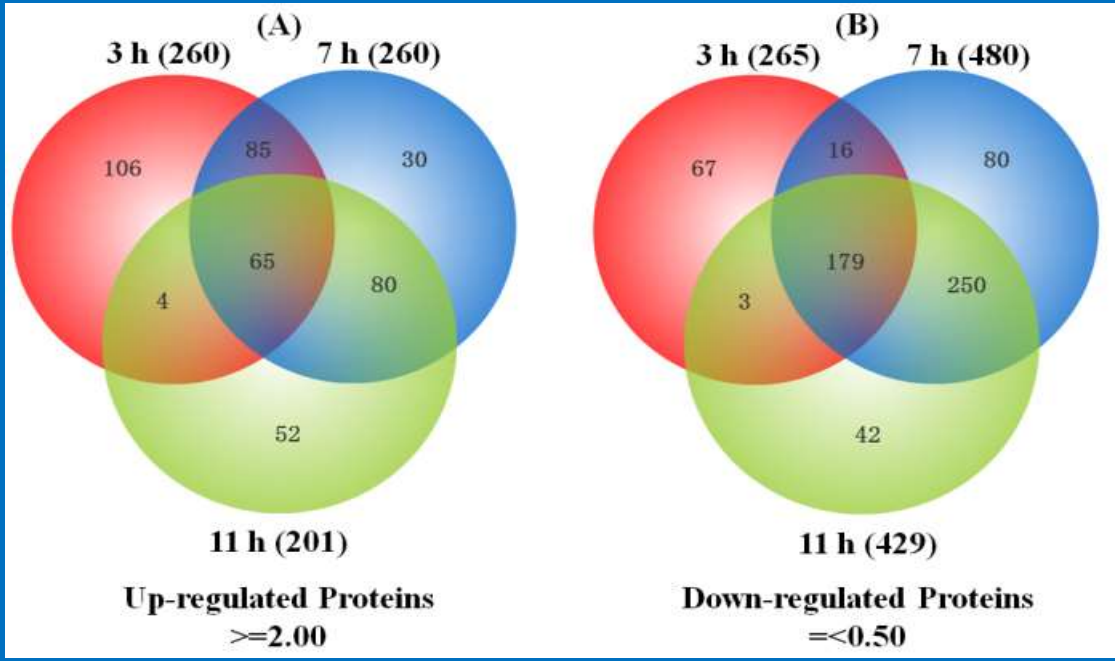
Expression of the same dsRNA in Arabidopsis rendered susceptible plants highly resistant to fungal infection.

Koch, A., et al. (2013) PNAS 110, 19324-19329

Proteomic profiling of Fusarium oxysporum f.sp. cubense Tropical Race 4 during early development



Foc TR4 early growth morphology. The images taken at (A) 0 hour, as control; (B) 3 hours, spore swelling and germination tube emergence; (C) 7 hours, germination tube elongation from conidia and (D) 11 hours, full mature mycelia are visible.



P<0.05	3 hours (115/114)	7 hours (116/114)	11 hours (117/114)	Sum
Up-regulated	260	260	201	422
Down-regulated	265	480	429	637
Total	525	740	630	1059

±2.0倍作为确定差异表达蛋白的阈值

Banana genetic transformation of host-induced gene silencing vector *(ERG-6, ERG-11)*

***ERG-6* 20 lines**

***ERG-11* 50 lines**

GMO: Overexpression two disease tolerant genes ERG6 dsRNA and ERG11 dsRNA from Foc TR4 in Grande Naine respectively

Condition: grown in a green house at 25-35℃, a photon flux density of 500 μE, and a relative humidity of 60-80%

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et al….

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资源评价与育种

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分子育种

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Dr. Dong Tao

栽培技术

Dr. Huang yonghong

栽培生理

Dr. Gao huijun

品质形成机理

Thank You 

敬请批评、 指正！

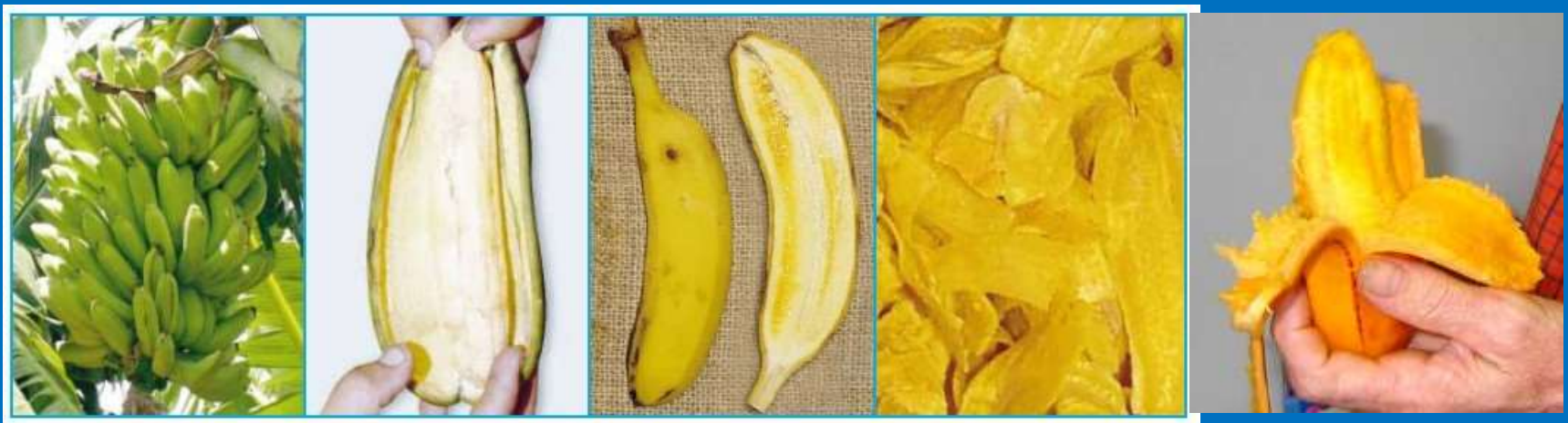

Figura 2. Características del nuevo plátano híbrido SH-4001