STUDIES ON VASCULAR INFECTION OF *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* RACE 4 IN BANANA BY FIELD SURVEY AND GREEN FLUORESCENT PROTEIN REPORTER

Rong F. Xiao*, Yu J. Zhu*, Yan D. Li, Bo Liu**

Agricultural Bio-Resources Institute, Fujian Academy of Agricultural Sciences, Fuzhou, China.

ABSTRACT

Fusarium wilt of banana (*Musa* spp.) caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) is one of the most serious banana fungal diseases in the world. Understanding the infection process of *Foc* is important for development of effective ways in disease control. In order to follow infection and colonization of this pathogen from root to rhizome and pseudostem tissues of banana, a highly pathogenic strain FJAT-3076 of *Foc* race 4 (*Foc*4) was transformed with gene encoding green fluorescent protein (GFP) and the fungus carrying *gfp* (FJAT-3076-GFP) was used to inoculate banana plants (Cavendish cv. B.F.). After inoculation for 3 to 10 d, it was observed that the conidia and their germ-tubes had penetrated into epidermis of young roots. The hyphae were found inside the root xylem 10 d after inoculation in the rhizome and pseudostem xylem after inoculation for 17 d. All plants infected by *Foc* died in 24 d after inoculation. It was also observed that *Foc* had spread all over the xylem and part of hyphae reached the pseudostem surface. Hyphal population was found the highest in the pseudostem, lower in root and least in rhizome. Field survey confirmed that *Foc*4 were mostly present in the base of pseudostem and less in the rhizome. Thus, effective prevention of the *Foc* hyphae movement from the rhizome up to the pseudostem might delay or control banana wilt disease.

Keywords: Banana, infection, *Fusarium oxysporum* f. sp. *cubense*, GFP.

INTRODUCTION

Fusarium wilt of banana, commonly known as Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is one of the most serious fungal diseases in banana, and a major limiting factor of banana production worldwide (Getha & Vikineswary 2002). *Foc* is divided into four races based on host susceptibility. For example, Cavendish cultivars (*Musa* spp. AAA-group), the most commonly planted banana in production area, are highly susceptible to *Foc* race 4 (*Foc*4). The fungus can survive as a saprophyte for numerous years. Once presenting in the soil, *Foc* is not able to be eliminated (Kurtz & Schouten 2009). It initially infects the roots of banana plants, then colonizes the vascular system of the rhizomes and pseudostems, eventually leads to typical wilt symptoms, including foliage chlorosis and necrosis before the plant died. To date, few options are available

in protecting banana from *Fusarium* wilt disease (Forsyth *et al.* 2006). Recently, the application of transformed strains expressing reporter genes and facilitated observation on the development of pathogens within their plant hosts (Paparu *et al.* 2009). In the study of *Fusarium* wilt of banana, Visser *et al.* (2004) had transformed *Foc*4 with *gfp* gene, and observed the fluoresced fungal hyphae within tissues of infected plants. Li *et al.* (2011) developed a GFP-tagged *Foc*4 transformant and studied the pathogenesis using both fluorescence microscopy and confocal laser scanning microscopy (CLSM). They found that fungal hyphae were able to penetrate cell walls directly to grow inside and outside cells in banana roots, and fungal spores were produced in the root systems and rhizomes. However, how *Foc*4 progresses in the vascular tissue of banana roots to the pseudostem, which correlates with *Foc* pathogenicity, is not clearly known. In this study, we report the observa*tion of Foc*4 systemic infection process in a Cavendish banana with GFP reporter, mainly focus on *Foc*4 movement from

---

* Authors contributed equally in this work.

** Corresponding Author:

Bo Liu, Email: liubofaas@163.com

roots to pseudostem. More understanding the infection process of *Foc*4 is required and helpful for development of banana *Fusarium* wilt control.

MATERIALS AND METHODS

***Isolation, identification and distribution of Foc4 in field banana plants*:** Three banana plantations of Cavendish cultivar were chosen for experimental sites at Zhangzhou Country, one of the main banana-producing regions in South of China (Longitude 117.37, Latitude 24.52). A symptomatic plant with yellowing and wilting older leaves and an asymptomatic plant were gathered from each experimental site. Twenty grams of tissue samples were obtained from the different positions of banana plant as follow: root, rhizome, base pseudostem, middle pseudostem and top pseudostem; and they were marked as sample I, II, III, IV and V in turn. The samples were surface disinfected with 10% sodium hypochlorite and homogenized with sterile distilled water. Dilution series were made of each suspension and plated onto Komada medium for isolation of *F. oxysporum* (Komada 1975). For the pathogen distribution inside banana plant, the single-spore isolate from each sample was numbered and the average fungal contents at the same position of three symptomatic or asymptomatic plants were calculated. For phytopathogen identification, 30 single-spore colonies from each sample at different positions of banana were chosen. The mycelia of single-spore isolates were transferred to fresh potato dextrose agar (PDA) medium for 7 d, and then scraped directly from agar plates and used for DNA isolation. Total genomic DNA was extracted according to fungal DNA kit (Omega Bio-tek, Norcross, Georgia). The specific PCR for *F. oxysporum* and *Foc*4 were detected according to Nel *et al*. (2006) and Lin *et al*. (2010), respectively.

***Foc race 4 strain and gfp gene transformation*:** The *Foc* race 4 isolate, FJAT-3076, isolated from pseudostem of a diseased banana in this study was selected for the GFP transformation. The fungus was tested for its aggressiveness on Cavendish banana cv. B.F., resulting in the wilting of 100%. Strain FJAT-3076 was modified with the *gfp* gene using the sGFP expression vector pCT74 according to Lorang *et al*. (2001). Fungal protoplasts of FJAT-3076 were transformed using a polyethylene glycol/CaCl$_2$-mediated transformation method as described by Liat *et al*. (2003). The colony with the highest GFP expression was chosen under a fluorescence stereomicroscope. The mycelia with bright green

fluorescence were submitted to six successive subcultures on PDA without antibiotic, in order to obtain stable transformants (Sarrocco *et al*. 2007). Growth characteristics and pathogenicity of GFP-expressing isolates of *Foc* were verified using the inoculation protocol described by Xiao *et al*. (2009).

***Banana plant inoculation by Foc transformants*:** A *Foc* transformant with the highest GFP expression, FJAT-3076-T2, and the wild-type *Foc* strain FJAT-3076 were chosen for inoculation. The fungi were cultured respectively into 500 mL potato-dextrose broth (PDB) in 2 L erlenmeyer flasks on a shaker at 170 rpm min^{-1}. After 5 d, the conidial suspension was passed through sterile cheesecloth to separate the mycelia from the conidia and adjusted to a final concentration of 10^6 spore mL^{-1}. Due to the high susceptibility to Foc, Cavendish banana cv. B.F. was chosen for plant inoculation. Thirty plants were used for the inoculation trial per strain. Banana cuttings (50-d-old tissue-cultured plant) were inoculated by root dipping in the spore suspensions for 30 min. Two plants per pot (17.0 cm × 17.0 cm × 12.0 cm) were ultimately retained in a greenhouse at 28 °C, 80% relativity humidity and 12 h light/dark. Control plants were treated with sterile water and maintained in the similar situation.

***Vascular colonization of banana plant by Foc examined or viewed under CLSM*:** Following symptom appearance at different phases, microscope observations of *Foc* colonization in banana plant were performed at 3, 10, 17 and 24 d after inoculation. Inoculated and control plants were carefully taken out of the pot and gently rinsed in tap water to wash away soil particles and unattached fungi. Longitudinal sections of root, rhizome, pseudostem and leaf were hand-sectioned and each placed directly on a glass slide in a water drop with a cover slip. Above 50 sections from different positions were viewed by microscope. The microscopic examinations were performed by using a Leica SP5 confocal laser scanning microscope (Wetzlar, Germany) with emitted light at 500–525 nm.

RESULTS

***Identification and distribution of Foc4 in field banana*:** The Komada medium was used to isolate *F. oxysporum* from banana plants at root, rhizome, base pseudostem, middle pseudostem and top pseudostem. The *Fusarium* colonies were obtained only from symptomatic plants; none was found in the asymptomatic plants. All the isolated *Fusarium* spp. were identified as *F. oxysporum* by FOF1/FOR1 primer

set with the amplification of a single 340-bp DNA fragments. Among which, all the isolates were also confirmed as *Foc*4 identified by the *Foc*1/*Foc*2 primer set, except 40% of the isolates from root were not *Foc*4 (Fig. 1 and Table 1). Moreover, there were significant differences among contents of *Foc*4 at different positions of diseased bananas. The fungi existed mostly at base pseudostem with an average concentration of 10.4×10^2 colony forming unit (cfu) g^{-1}, following with at middle pseudostem (4.76×10^2 cfu g^{-1}), root (1.02×10^2 cfu g^{-1}), top pseudostem (0.98×10^2 cfu g^{-1}) and rhizome (0.24×10^2 cfu g^{-1}). Therefore, *Foc*4 existed least in the rhizome, only 23.5 and 2.3% of those in the root and the base pseudostem.
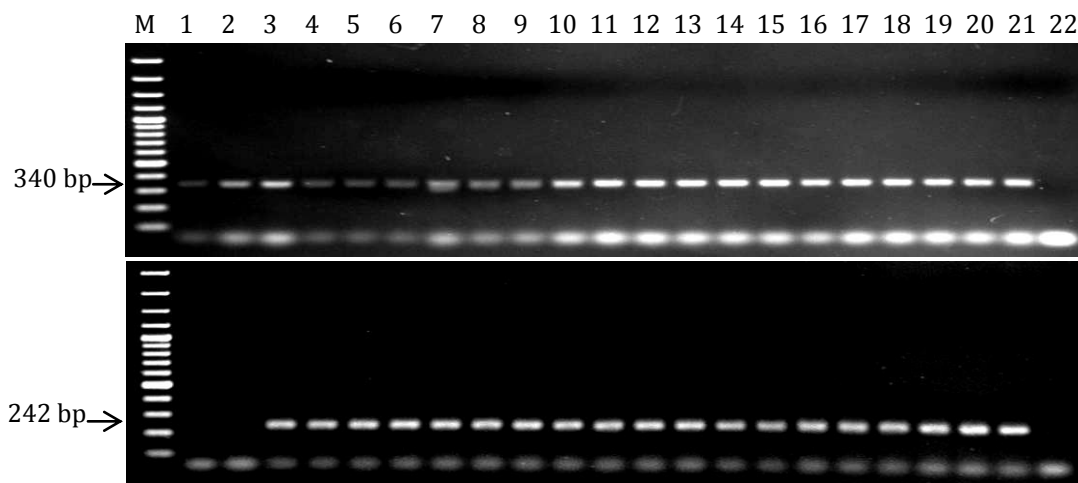

Fig. 1. Amplification of PCR products of 30 random samples which were from 150 samples of wilted banana plant using primer set FOF1/FOR1 (upper panel) and Foc1/Foc2 (lower panel). Lane 1-4, partial samples from diseased roots (S-I); 5-8, partial samples from diseased rhizome (S-II); 9-12, partial samples from diseased base pseudostem (S-III); 13-16, partial samples from diseased middle pseudostem (S-IV); 17-20, partial samples from diseased top pseudostem (S-V); 21: positive control; 22: negative control using sterile dH_2O as the template; M = molecular markers of Gen-100bp DNA ladder.

Table 1. Distribution of *Fusarium* spp. inside symptomatic and asymptomatic banana plants.

Sample	Type of plant	Sampled position	*Fusarium* colony ($\times 10^2$ cfu g^{-1})[a]	Percentage of *F. oxysporum* (%)	Percentage of *Foc*4 (%)	*Foc*4 colony ($\times 10^2$ cfu g^{-1})[b]
S-I		Root	1.70±0.10 c	100	60	1.02±0.10 c
S-II		Rhizome	0.24±0.08 c	100	100	0.24±0.08 c
S-III	Symptomatic plant	Base pseudostem	10.4±1.73 a	100	100	10.4±1.73 a
S-IV		Middle pseudostem	4.76±0.46 b	100	100	4.76±0.46 b
S-V		Top pseudostem	0.98±0.22 c	100	100	0.98±0.22 c
AS-I		Root	0	0	0	0
AS-II		Rhizome	0	0	0	0
AS-III	Asymptomatic plant	Base pseudostem	0	0	0	0
AS-IV		Middle pseudostem	0	0	0	0
AS-V		Top pseudostem	0	0	0	0

[a]The different letters in the same column were significantly different at P < 0.05 by Duncan's multiple range test.
[b]The different letters in the same column were significantly different at P < 0.05 by Duncan's multiple range test.

Green fluorescent colonies, arising from the regenerated protoplasts, became visible at 5 d after transformation. Those putative transformants with highest GFP expression were chosen under a fluorescence stereomicroscope (Fig. 2a) and transferred to six successive subcultures on PDA without antibiotic. Strong constitutive expression of

sGFP occurred and could be visualized in fungal hyphae, microconidia, macroconidia and conidiogenous cells (Fig. 2b-2d). Neither virulence nor morphological characteristics (growth rate, spores or hyphae) of the pathogen was altered by transformation with the plasmid pCT74. A *Foc* transformant with the highest GFP expression, FJAT-3076-T2, was chosen for further study.
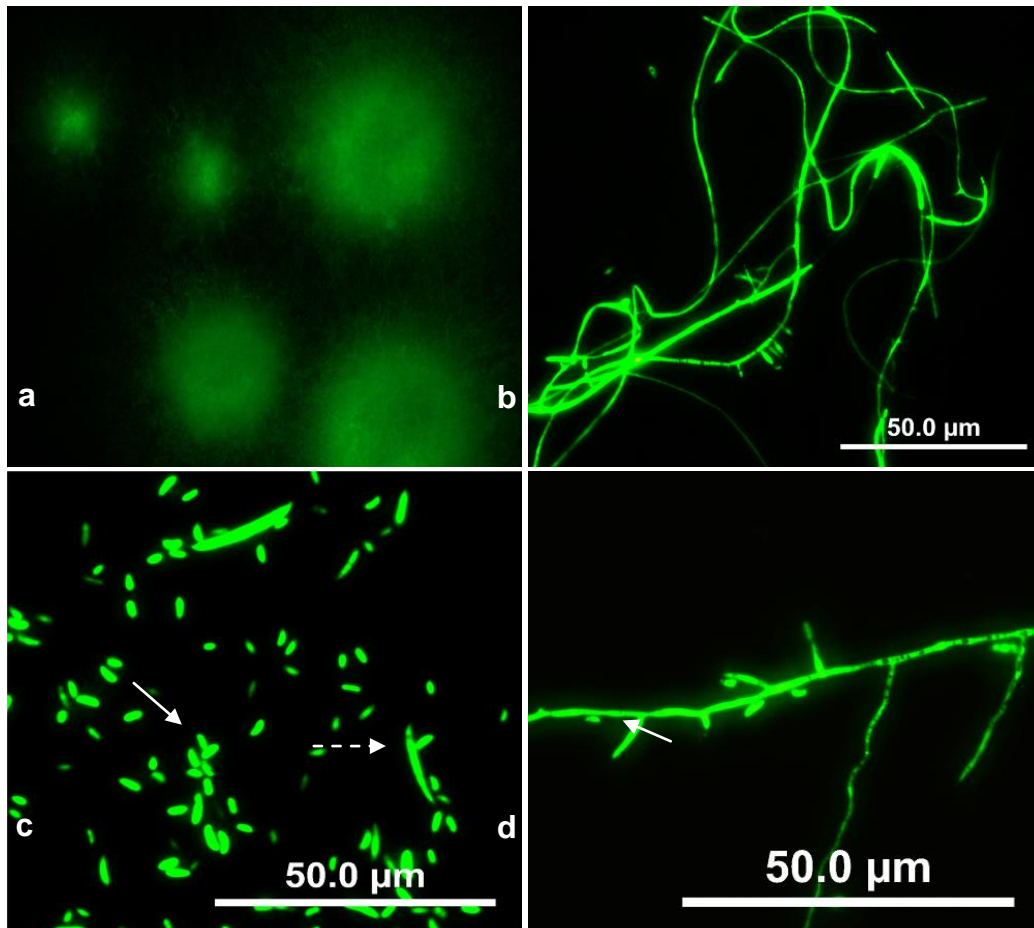

Fig. 2. Structures of transformed *Fusarium oxysporum* f. sp. *cubense* (*Foc*) isolates fluorescing bring green. Fluorescing colonies (**a**) under fluorescence stereomicroscope. Hyphae (**b**), microconidia (**c**, solid arrow) and macroconidia (**c**, dotted arrow) and conidiogenous cells (**d**, solid arrow) of transformed *Foc* under fluorescence microscope.

***Vascular colonization of banana plant by Foc examined or viewed under CLSM*:** In the plant inoculation tests, *Foc*4 transformant FJAT-3076-T2 was as pathogenic as the wild-type strain FJAT-3076, causing 100% mortality rate of banana plants tested at 24 d after inoculation. In order to study the colonization of banana plant by *Foc*4, transformed strain FJAT-3076-T2 was microscopically followed in whole plant and in longitudinal sections from 3 to 24 d after inoculation by CLSM. Inoculated for 3-10 d, the pseudostems and leaves of plant were symptom-free, but most roots performed severe yellowing and

subsequent discolored. The conidia or their germ-tubes of *Foc*4 were observed to penetrate epidermal cells of young roots (Fig. 3a-b) and the hyphae developed in the root xylem (Fig. 3c). However, the green hypha couldn't be visible except root tissue. By 17 d, the wilt symptoms became visible on banana plants inoculated with both transformants and wild-type isolate. The leaves at the bottom appeared yellowing and one half of the rhizome was discolored (Fig. 4). Hyphae were visible in the root, rhizome and pseudostem. On the 24th day after inoculation, the plant became dying and three quarters of the rhizome were discolored. The green-fluorescing

hyphae were visible in the root, rhizome and pseudostem, but not in the leave. Some hyphae reached the pseudostem tissue and were very obvious intercellular growth in these tissues (Fig. 3d). Parts of hyphae were confined in xylem vessel and grew upwardly (Fig. 3e). A few of conidia could be also visible within the root or pseudostem vessels tissues of banana during the course of infection (Fig. 3f).
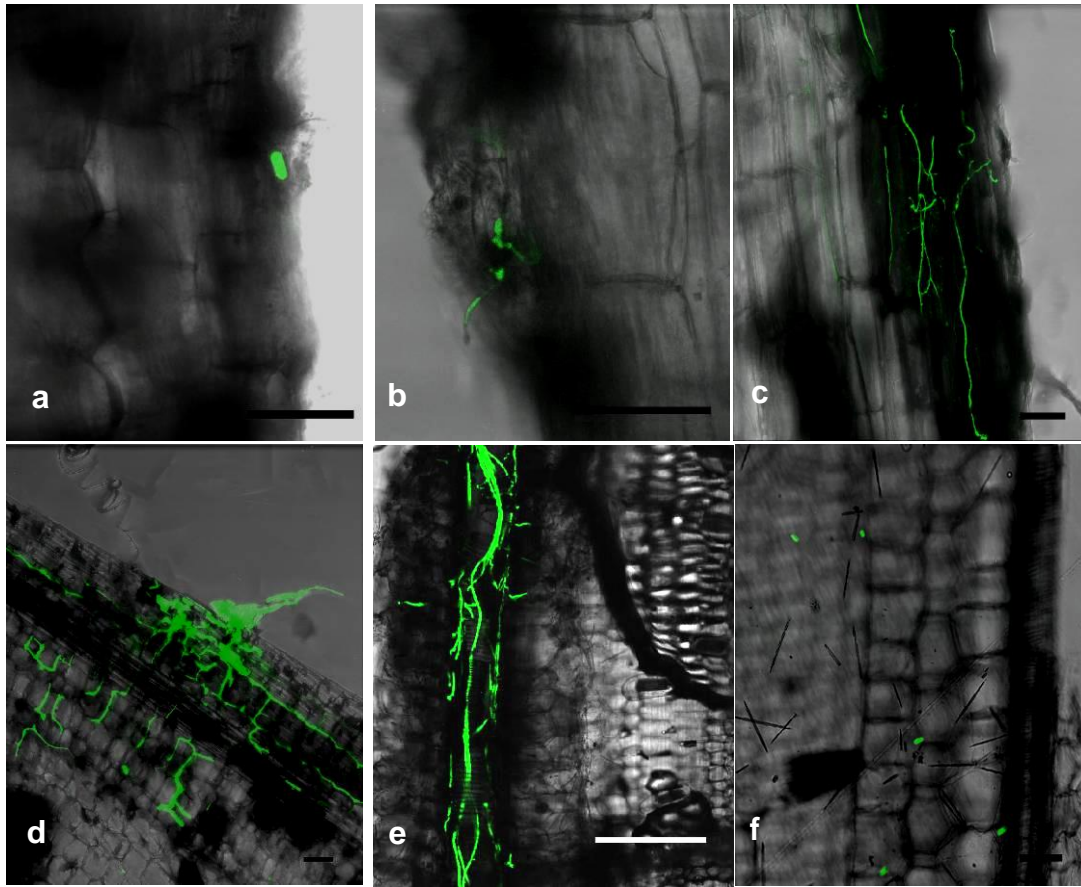

Fig. 3. Fluorescent microscopic images of transformed *Fusarium oxysporum* f. sp. *cubense* strain in banana. (**a**) Conidia were observed to penetration the young roots epidermis 3 days after inoculation. (**b**) Hyphae were observed to penetration the young roots epidermis by day 10. (**c**) Hyphae penetrated and progressed in the root xylem 10 d after inoculation. (**d**) Hyphae were up to the pseudostem tissues and some hyphae reached the pseudostem surface at 24 d after inoculation. (**e**) Hyphae grew upwards and were confined in a xylem vessel at 24 d after inoculation. (**f**) A few of conidia could be also visible at different positions of banana tissues during the course of infection, such as in the pseudostem. Scale bars represent 50 μm.
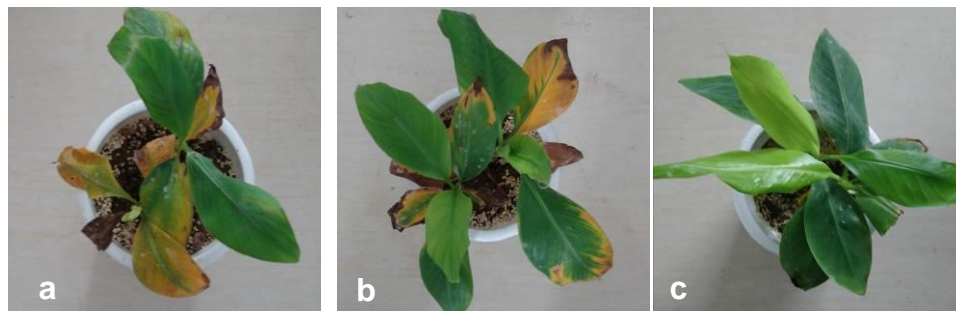

Fig. 4. The symptom of banana plant after 17 d of inoculation. Plants inoculated with wild-type isolate FJAT-3076 (**a**), transformed isolate FJAT-3076-T2 (**b**) showing symptom of *Fusarium* wilt; Control plant (**c**) inoculated with water showing no disease symptom.

Furthermore, obvious differences were also figured out among the *Foc*4 densities at different plant positions (root, rhizome and pseudostem) after inoculation for 24 d. The colonization on the root developed continuously; small hyphal networks were observed within the root tissues by CLSM (Fig. 5a). Only a few hyphae were observed in the rhizome tissues (Fig. 5b), whereas denser hyphal network presented on the surface and inside the pseudostem tissues (Fig. 5c). Therefore, the highest level of hyphal density was in the pseudostem, whereas the level was much less in root and only a few hyphae were found in rhizome.
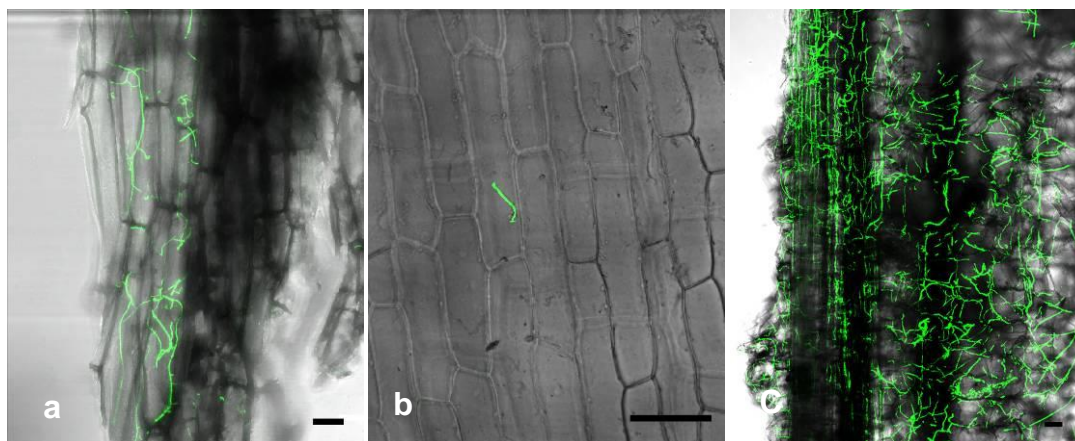

Fig. 7. The hyphal colonization of GFP-transformed *Fusarium oxysporum* f. sp. *cubense* with different densities at different banana positions on 24th day after inoculation. **a**) root, **b**) rhizome and **c**) pseudostem. Scale bars represent 50 μm.

DISCUSSION

Using GFP-expressing strains to monitor invasion and infection, the plant infection by *F. oxysporum* was found to be a complex process, which comprised several stages of host–pathogen interaction: recognition of the host roots and adsorption, penetration of hyphae through the different root tissues, penetration and progression in the xylem, and adaptation to the internal plant environment. To be successful, the fungus should overcome different plant defence responses at each stage (Di Pietro *et al.* 2003; Michielse & Rep 2009; Zvirin *et al.* 2010).

For example, Lagopodi *et al.* (2002) visualized the *F. oxysporum* f. sp. *radicis-lycopersici* (*Forl*) invasion of a susceptible tomato cultivar at 1-3 day after inoculation. *Forl* attached itself to root hairs by means of fungal mycelia and penetrated portions of the root at random, followed by penetration along epidermal cell borders after 4 d. Zvirin *et al.* (2010) mentioned the attachment of *F. oxysporum* f. sp. *melonis* (*Fom*) to a susceptible melon cultivar had occurred along the root epidermis at 3th day after inoculation. *Fom* was discovered in the root xylem by day 4, and then the xylem of the root and hypocotyl were heavily populated with mycelia by day 11 and all seedlings were dead by day 14. The conidia were produced and germinated within the vessels. For infection of banana with *Foc*, Li *et al.* (2011) reported the development of *gfp*-marked *Foc*4 in banana root and rhizome. They observed that chlamydospores of *Foc*4 were attached to banana roots and root hairs as early as 72 h after the plantlets were planted in *Foc*4-infected soil, and that plant penetration occurred through the tips and elongation zone of lateral roots, as well as through natural wounds in secondary root bases after approximately 5 days. A network of fungal hyphae forming on root caps and elongation zone of banana roots at 11 and 15 days after inoculation of cv 'Brazilian', respectively. Fungal hyphae and spores have filled vascular spaces in the rhizome and the surrounding tissue became disorganized at 29th day after inoculation, while 70% of the plants were dead or dying.

Similarly, in current study, it was found that *Foc*4 were observed to penetrate the young roots epidermis of Cavendish banana cv. B.F after inoculation for 3-10 d. However, we observed that *Foc*4 seemed able to penetrate portions of banana root by its conidia or their germ-tubes. The difference of the penetrate type might be due to the individual difference of *Foc*4 isolates or the method of pathogenicity test that resulted in the suitable environment condition for producing chlamydospores or conidia. Furthermore, systemic infection of *Foc*4 in banana was visualized by using green fluorescent protein reporter. A few of hyphae of

Foc was visible within the root vascular cylinde tissues of banana by day 10. The hyphae were up to the rhizome and pseudostem xylem by day 17. All plants were dead at 24 d after inoculation; whilst *Foc* had spread all over the xylem and part of hyphae reached the pseudostem surface. The conidia of *Foc* were also visible at different positions of banana tissues during the course of infection. It could be explained that sporulation and germination of secondary mycelium was crucial for rapid upward colonization (Beckman & Roberts 1995).

An interesting finding in this study was the obvious differences among *Foc* populations at different banana positions during the infection phase, both in field plants by pure culture method and in laboratory plantlets under CLSM examination. In symptomatic banana plants collected from field, *Foc*4 was found to exist least in the rhizome, only 23.5% of those in the root and 2.3% of those in the base pseudostem. Microscopic examination of banana plantlets inoculated with virulent *Foc*4 showed that the highest level of hyphal density was observed in the pseudostem, while root had middle level and rhizome had the least. Sampled equally for about 2 × 2 cm size, few of hyphal pieces could be found in the rhizome tissues, whereas a mass of hyphae could be discovered within the root and pseudostem xylem. It was speculated that the hyphae were blocked or delayed to progress within the rhizome xylem, which could be related with the banana's histophysiological characteristic. The banana plant was a giant perennial herb that consisted of a rhizome and a pseudostem (Robinson 1996). The rhizome consisted of starchy parenchyma cell, and was a nutrients store house that could transfer all the nutrients stored thereto the growing suckers (Wu 2006; Thakorlal *et al.* 2010). Differently, the pseudostem was composed of several tightly packed leaf bases that consisted of aerenchymas in the parenchyma cell (Wu 2006). The plant, moreover, was often able to prevent infection from successfully travelling to and entering the rhizome by the production of gels and tyloses (a resistance mechanism) to seal off the infection (Daly & Walduck 2006). But, if few of hyphae progressed into the pseudostem through the rhizome, the hyphae would spread rapidly in the aerenchymas and travel all the way up to the top of the pseudostem. Eventually, the pseudostem of the plant was easily damaged and died.

Foc was difficult to be controlled using fungicides and be eradicated from the soil using fumigants (Daly & Walduck 2006). However, *Foc* might be delayed or controlled, if it was effectively preventing the hyphae from the rhizome up to the pseudostem, such as injecting fungicide at the points above the rhizome.

ACKNOWLEDGEMENTS

REFERENCES

Beckman, C.H. and E.M. Roberts. 1995. On the nature and genetic basis for resistance and tolerance to fungal wilt diseases of plants. *Advances in Botanical Research Incorporating Advances in Plant Pathology.* 21**:** 35–77.

Daly, A. and G. Walduck. 2006. *Fusarium* Wilt of bananas (Panama Disease). *Agnote.* I51: 1–5.

Di-Pietro, A., M.P. Madrid, Z. Caracuel, J. Delgado–Jarana and M.I.G. Roncero. 2003. *Fusarium oxysporum*: exploring the molecular arsenal of a vascular wilt fungus. *Molecular Plant Pathology.* 4: 315–325.

Forsyth, L.M., L.J. Smith and E.A.B. Aitken. 2006. Identification and characterization of non–pathogenic *Fusarium oxysporum* capable of increasing and decreasing *Fusarium* wilt severity. *Mycological Research.* 110: 929–935.

Getha, K. and S. Vikineswary. 2002. Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f. sp. *cubense* race 4: indirect evidence for the role of antibiosis in the antagonistic process. *Journal of Industrial Microbiology & Biotechnology.* 28**:** 303–310.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Review of Plant Protection Research.* 8**:** 114–125.

Kurtz, A. and A. Schouten. 2009. Shifts in banana root exudate profiles after colonization with the non–pathogenic *Fusarium oxysporum* strain Fo162. *Communications in Agricultural and Applied Biological Sciences.* 74**:** 547–558.

Lagopodi, A.L., A.F. Ram, G.E. Lamers, P.J. Punt, C.A. Van den Hondel and B.J. Lugtenberg. 2002. Novel aspects of tomato root colonization and infection

by *Fusarium oxysporum* f. sp. *radicis–lycopersici* revealed by confocal laser scanning microscopic analysis using the green fluorescent protein as a marker. *Molecular Plant-Microbe Interactions.* 15**:** 172–179.

Li CY, S. Chen, C.W. Zuo, Q.M. Sun, Q. Ye, G.J. Yi and B.Z. Huang. 2011. The use of GFP–transformed isolates to study infectionof banana with *Fusarium oxysporum* f. sp. *cubense* race 4. *European Journal of Plant Pathology.* 131: 327–340.

Liat, O., E. Smadar, C. David and S. Amir. 2001. Early events in the *Fusarium verticillioides*-maize interaction characterized by using a green fluorescent protein-expressing transgenic isolate. *Applied and Environment Microbiology*. 69: 1695–1701.

Lin, Y.H., K.S. Chen, J.Y. Chang, Y.L. Wan, C.C. Hsu and J.W. Huang. 2010. Development of the molecular methods for rapid detection and differentiation of *Fusarium* oxysporum and *F. oxysporum* f. sp. *niveum* in Taiwan. *Journal of Biotechnology.* 27**:** 409–418.

Lorang, J.M., R.P. Tuori, J.P. Martinez, T.L. Sawyer, R.S. Redman and J.A. Rollins. 2001. Green fluorescent protein is lighting up fungal biology. *Applied & Environmental Microbiology.* 67**:** 1987–1994.

Michielse, C.B. and M. Rep. 2009. Pathogen profile update: *Fusarium oxysporum*. *Molecular Plant Pathology.* 10**:** 311–24.

Nel, B., C. Steinberg, N. Labuschagne and A. Viljoen. 2006. The potential of non–pathogenic *Fusarium oxysporum* and other biological control organisms for suppressing *Fusarium* wilt of banana. *Plant Pathology.* 55 217–223.

Paparu, P., A. Macleod, T. Dubois, D. Coyne and A.

Viljoen. 2009. Efficacy of chemical and fluorescent protein markers in studying plant colonization by endophytic non–pathogenic *Fusarium oxysporum* isolates. *BioControl.* 54**:** 709–722.

Robinson, J.C. 1996. *Banana and plantain*. CAB International.

Sarrocco, S., N. Falaschi, M. Vergara, F. Nicoletti and G. Vannacci. 2007. Use of *Fusarium oxysporum* f. sp. *dianthi* transformed with marker genes to follow colonization of carnation roots. *Journal of Plant Pathology.* 89**:** 47–52.

Thakorlal, J., C.O. Perera, B. Smith, L. Englberger and A. Lorens. 2010. Resistant starch in Micronesian banana cultivars offers health benefits. *Pacific Health Dialog.* 16**:** 49–59.

Visser, M., T.R. Gordon, B.D. Wingfield, M.J. Wingfield and A. Viljoen. 2004. Transformation of *Fusarium oxysporum* f. sp. *cubense*, causal agent of *Fusarium* wilt of banana, with the green fluorescent protein (GFP) gene. *Australasian Plant Pathology.* 33**:** 69–75.

Wu, Q.L. 2006. Biological characteristics and tissue culture technique of banana. *Bulletin of Biology.* 41**:** 5–7.

Xiao, R.F., Y.J. Zhu, Y.D. Li and B. Liu. 2009. Green fluorescent protein gene (GFP) transformation of *Fusarium oxysporum* f. sp. *niveum* strain, FOV-135. *Fujian Journal of Agricultural Sciences.* 24**:** 519–522.

Zvirin, T., R. Herman, Y. Brotman, Y. Denisov, E. Belausov, S. Freeman and R. Perl–Treves. 2010. Differential colonization and defence responses of resistant and susceptible melon lines infected by *Fusarium oxysporum* race 1·2. *Plant Pathology.* 59**:** 576–585.