Detecting *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 in Soil and Symptomless Banana Tissues

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Abstract

Tropical race 4 (TR4) of *Fusarium oxysporum* f. sp. *cubense* (Foc) is a quarantine pathogen in many banana-producing regions of the world. Preventing further dissemination and precluding incursions into areas where it has not been observed is critical for maintaining local and commercial banana production. Trading of symptomless but infected banana planting material, movement of machinery with adhering infested soil, and the use of traditional banana-based packing material facilitate the spread of Foc. A PCR-based detection tool for TR4 was used to analyze field samples from symptomatic and symptomless banana plants and soils collected in TR4-affected areas. Foc TR4 was detected in all infected samples by conventional or nested PCR analyses. The results will be used to develop and implement TR4 quarantine and management strategies.

INTRODUCTION

The tropical race 4 (TR4) strain of *Fusarium oxysporum* f. sp. *cubense* (Foc) is a quarantine pathogen in many banana production regions of the world. It is present in East and Southeast Asia and has reached epidemic proportions in many large-scale plantations. Most of the known cultivars of banana are susceptible to the TR4 strain, which threatens approximately 80% of world production (Ploetz, 2005). Once Foc enters an area, it is impossible to eradicate and fungicides are ineffective. Therefore, preclusion, containment and host resistance are the most important disease management strategies. Since the development of resistant and commercially well-accepted genotypes is a long-term strategy, controlling further dissemination in Southeast Asia and prevention of new incursions into TR4-free areas is highly desired to maintain local and commercial banana production (Dita et al., 2010; Ploetz et al., 2011; Molina et al., 2011). This requires a sensitive and highly specific diagnostic test that enables early detection of the pathogen (Lin et al., 2009). We have recently published the development of a molecular diagnostic for Foc TR4 (Dita et al., 2010) that specifically identifies the pathogen in fungal cultures and in symptomatic plants. Here, we report the detection of Foc TR4 in symptomless tissues and soils, which are two potential sources of TR4 dissemination.

MATERIALS AND METHODS

Sampling Procedures

Samples of symptomatic and symptomless plants and associated soils from different sources were processed (Table 1). Soil sub-samples were taken from three

equidistant sites around the plant (at 50 cm from the pseudostem base and at 20-30 cm depth) avoiding any banana root tissues. The three samples were then pooled in a composite sample. Plants were sampled by taking pseudostem pieces (10×3 cm) at 50 cm from the pseudostem base. Samples were immediately stored on dry ice and shipped to Plant Research International, The Netherlands for further processing.

DNA Extraction

DNA isolation from plant tissues was performed as described by Dita et al. (2010). DNA from soil was extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. DNA samples were diluted to 10 ng/µl and stored at -20°C until use. Quality of soil DNA was verified by PCR using the universal bacterial rDNA primer set U968 and R1378 (Heuer et al., 1997).

PCR Reactions

Single PCR reactions for Foc TR4 detection were performed according to the conditions described by Dita et al. (2010). The nested PCR included two rounds of amplification using the primer set iNL11/ iCNS1 (O'Donnell et al., 2009) for the first round and a duplex PCR with both ITS1/ITS4 (White et al., 1990) and FocTR4-F/ FocTR4-R primer sets (Dita et al., 2010) for the second round. Nested PCR conditions for the first round were as described by O'Donnell et al. (2009) and for the second round as described by Dita et al. (2010).

RESULTS AND DISCUSSION

Limiting the dissemination of Foc TR4 is a top priority in the absence of resistant cultivars (Dita et al., 2010; Lin et al., 2009). We showed that samples from all of the symptomatic plants reacted with the Foc TR4 diagnostic (Table 1), except two samples from the Philippines (Phi30B and Phi2SV), which were infected with a different Foc strain (data not shown). Even by using single or conventional PCR, Foc TR4 was detected in some soil samples from Taiwan (TS3, TS7 and TS9), demonstrating that in certain situations Foc TR4 can be easily detected in soil without additional methodologies, such as nested PCR. Nevertheless, we developed and applied a nested PCR strategy to screen all initially negative samples, which apparently resulted from low Foc TR4 DNA concentrations in the soil, as the nested PCR approach gave positive results for these samples (Table 1). Overall, these verifications open up important possibilities for Foc TR4 detection and Fusarium wilt disease management in banana. These include the ability to screen for the presence of the pathogen in the soil prior to planting to support the decision-making and risk analysis processes and early detection of the pathogen in tissues of symptomless planting material, as well as in established plantations. Further studies are required to detect Foc TR4 in water used for irrigation, which is another potential and important means of dissemination. These results provide a significant part of the basis for the design and application of national and international quarantine services to detect and contain Foc TR4.

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Tables

Table 1. Samples used for detection of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 and the response to single primer pair or nested PCR diagnostics.

Sample ID	Cultivar[a]	Plant stage[b]	Location	Source	Sample processed	Single primer pair PCR	Nested PCR[c]
AuD1	Grand Naine	Symptomatic	Darwin	Australia	Plant	+	nd
AuH1	Grand Naine	Symptomless	Darwin	Australia	Plant	+	nd
TS1	Grand Naine	Symptomless	Chaojhou	Taiwan	Soil	-	+
					Plant	+	nd
TS3	Grand Naine	Symptomatic	Chaojhou	Taiwan	Soil	+	nd
					Plant	+	nd
TS6	Grand Naine	Symptomless	Wandan	Taiwan	Soil	-	nd
					Plant	+	nd
TS7	Grand Naine	Symptomatic	Jiuru	Taiwan	Soil	+	+
					Plant	+	nd
TS8	Grand Naine	Symptomless	Jiuru	Taiwan	Soil	-	+
					Plant	+	
TS9	Grand Naine	Symptomatic	Luye	Taiwan	Soil	+	+
					Plant	+	nd
Phi126C	Grand Naine	Symptomatic	Kapalong	Philippines	Plant	+	nd
Phi39B	Tall William	Symptomatic	Kapalong	Philippines	Plant	-	nd
Phi2SV	Latundan	Symptomatic	Kapalong	Philippines	Plant	-	nd
ChlamyD2	n.a.	n.a.	n.a.	n.a.	Foc-colonized substrate	+	+
HSoil	n.a.	n.a.	n.a.	n.a.	Not infested soil	-	-
HPlant	n.a.	n.a.	n.a.	n.a.	Healthy plant	-	-

[a] Cultivar: banana cultivar sampled or planted in the area.
[b] Plant stage: corresponds to visual evaluation for Fusarium wilt symptoms.
[c] Nested PCR: nd: corresponds to samples not determined by Nested PCR;
n.a: not applicable as samples were prepared in the laboratory or greenhouse used as positive or negative control.