

First report of *Fusarium oxysporum* f. sp. *ubense* tropical race 4 (VCG 01213/16) associated with Cavendish bananas in Laos

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The popular Cavendish banana (*Musa* spp., AAA) constitutes about 45% of all bananas grown worldwide (Lescot, 2015). Mainland China is one of the leading producers of Cavendish bananas, and to satisfy local demand for the fruit, commercial production is expanding into neighbouring countries such as Laos, Myanmar and Vietnam. In Laos, local banana varieties such as “kuay nam” (Silk, AAB), “kuay hom” (local Cavendish, AAA) and “kuay klai” (Horn Plantain, AAB) are preferentially grown for food and income generation. The expansion of Cavendish production from China into Laos, therefore, presents significant risks to these varieties, such as the introduction of *Fusarium oxysporum* f. sp. *ubense* tropical race 4 (TR4; VCG 01213/16), a soil-borne fungus that causes severe epidemics and crop losses in Cavendish plantations in southern Chinese provinces. In November 2016, Laos Department of Agriculture staff reported wilting plants from Luang Namtha and Borkeo provinces. In February 2017, yellow leaf symptoms typical of banana Fusarium wilt were observed in commercial Cavendish plantations in Luang Namtha and Vientiane provinces of Laos. When plants were cut open, the pseudostems displayed a dark-red to brown discoloration of the vascular tissue, and the inner rhizome revealed a ring of yellow-brown staining. Infected vascular strands were collected from five plants (three from Luang Namtha province, two from Vientiane province) for morphological, molecular and vegetative compatibility group (VCG) identification. Infected vascular strands were plated out onto potato dextrose agar (PDA) containing 0.04 g/L streptomycin. The fungal cultures were single-spored and identified as *F. oxysporum* based on cultural characteristics and spore morphology (Nelson *et al.*, 1983). Total DNA was extracted from each fungal culture for molecular identification. Amplicons of the expected sizes for Foc TR4-specific primers (Dita *et al.*, 2010) and Foc race 4-specific primers (Lin *et al.*, 2009) were obtained for all five samples. VCG testing (Puhalla, 1985) established that the fungal isolates were all compatible with VGC 01213/16. For two isolates collected from Luang Namtha, pathogenicity testing was performed by adding a 30-mL spore suspension (10^8 conidia/mL) onto the potting soil in which 4-mo-old “kuay hom” plants were grown. Each isolate was inoculated onto three plants, and the control plants were treated with sterile distilled water. The infected plants

were then incubated in a screened shade house under ambient temperature conditions. After 8 weeks, the Foc TR4-inoculated plants produced typical wilting and internal discoloration symptoms of Fusarium wilt. *Fusarium* spp. were re-isolated from the inoculated plants and identified as Foc TR4/VCG 01213/16 by PCR (Dita *et al.*, 2010), thereby completing Koch's postulates. Many large and small-scale Cavendish plantations of less than 5 years old are affected by Fusarium wilt in Luang Namtha and Vientiane provinces, sometimes at a high incidence. The incursion of Foc TR4 in Laos does not only threaten the rapidly expanding Chinese Cavendish banana production, but also susceptible popular varieties grown by small farmers for local markets in Laos.

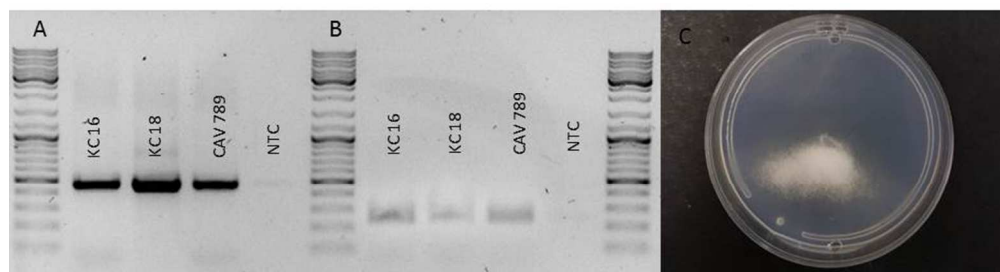
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Typical symptoms from plants in Laos infected with *Fusarium oxysporum* f. sp. *cubense* tropical race 4. **A.** External symptoms of leaf necrosis and chlorosis of a Cavendish banana in Laos. **B.** Dark red internal discoloration inside a banana pseudostem. **C.** Leaf yellowing and internal discoloration (inset) symptoms of Fusarium wilt in a “kuay hom” (local Cavendish, AAA) plant 8 weeks after inoculation with Foc TR4 / VCG 01213/16.

133x102mm (150 x 150 DPI)



Molecular and vegetative compatibility group (VCG) identification of *Fusarium oxysporum* f. sp. *cupense* (Foc) tropical race 4 isolates from Laos. **A.** Foc TR4 isolates produced a 488-bp amplicon following amplification with primers described by Dita *et al.* (2010). KC 16 and KC 18 are Foc TR4 isolates collected in Laos, and CAV 789 (VCG 01213/16) is a positive control previously characterized. NTC refers to no template control. **B.** A 242-bp amplicon amplified with Foc race 4-specific primers described by Lin *et al.* (2009). **C.** Heterokaryon formation between a VCG 01213/16 tester and Foc TR4 isolate KC 16 from Laos.

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