Research

An Additional Threat to 'Cavendish' Banana Growers and Traders: The Infection of Banana Peduncles by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc* TR4)

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Abstract

Fusarium oxysporum f. sp. *cubense* tropical race 4 (*Foc* TR4) is one of the most destructive banana pathogens worldwide. Until now, it has been generally accepted that *Foc* TR4 mainly colonizes the roots, rhizomes, pseudostems, and leaves of banana plants and spreads among plantations with infected planting material, soil residues, and water. Because banana Fusarium wilt is a typical vascular bundle disease, the pathogen is accepted to possess the ability to enter the peduncle. However, no scientific proof has been presented. Recently, more and more signs of peduncle *Foc* TR4 in 'Cavendish' plants in different banana production provinces of China suggest that further investigations ought to be made regarding fungal colonization. We observed discoloration inside some peduncles from the *Foc* TR4-infected plants. The fungus isolated from these symptoms was identified using *Foc* TR4-specific PCR, sequencing, and a host inoculation assay. In a greenhouse bioassay, the *Foc* TR4 isolates from the peduncle showed the capacity for reinfecting Brazilian plants (Cavendish, AAA) up into the leaves. Collectively, we isolated the *Foc* TR4 from the peduncle and directly testified that this pathogen can invade the peduncle via xylem vascular bundle.

Keywords: banana, Cavendish, Fusarium wilt, *Foc* TR4, peduncle

Banana (*Musa* spp.) is one of the most popular and most traded fruits globally and is a major food crop for hundreds of millions of people (Nayar 2010). But global banana production is severely threatened by Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), especially by the virulent strain tropical race 4 (*Foc* TR4) (Maymon et al. 2020; Zheng et al. 2018).

Fusarium wilt of banana is a typical vascular wilt disease. The pathogen normally infects banana plants through roots, and it subsequently invades the rhizome, pseudostem, and leaves via xylem (Dong et al. 2019; Li et al. 2011; Ploetz 2015). The oldest leaves first show yellow symptom progressing from the leaf edges to the midrib, before it becomes necrotic and dies. There is usually a split at the base of the infected leaves (Stover 1962). As more leaves are infected by *Foc*, the whole plant becomes yellow from bottom to top, and dead leaves may form a skirt around the pseudostem. Affected xylem vessels in the pseudostem become visible as continuous reddish-brown streaks when the stem is split longitudinally.

Foc is classified into three races (*Foc* 1, *Foc* 2, and *Foc* 4) based on their infection of different banana cultivars. *Foc* 4 is further divided into *Foc* TR4 and subtropical race 4 (Ploetz 2006). *Foc* 1 was recorded to infect the susceptible 'Gros Michel' cultivar in Panama as early as 1890; thus, Fusarium wilt disease has been commonly known as Panama disease (Stover 1962). It had seriously affected banana production for more than 60 years in tropical America until the 1960s, when the (race 1) resistant 'Cavendish' replaced Gros Michel (Su et al. 1977). Commercial Cavendish cultivars have been planted widely around the world from then on. Unfortunately, relatively recently emerging *Foc* TR4 appears to infect many banana cultivars including Cavendish and is responsible for current banana Fusarium wilt epidemics globally (Ploetz 2006). There is still no completely effective control measure, although deployment of partially resistant/tolerant cultivars might help reduce the impact of *Foc* TR4. Today the pathogen is present in Asian countries, Australia, the Middle East, the Indian subcontinent, and Africa and was also recently detected in Columbia in Central America (García-Bastidas et al. 2014, 2019; Ordonez et al. 2015; Ploetz 2015; Zheng et al. 2018). Therefore, *Foc* TR4 is recognized as one of the most serious diseases in banana production.

China is the largest Cavendish banana-producing country (http://www.fao.org/faostat/en/). The main banana producing areas in mainland China are Guangdong, Guangxi, Hainan, Yunnan, and Fujian Provinces, where banana planting has been a pillar industry

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of agriculture. Researchers in mainland China first discovered *Foc* TR4 infesting Brazilian bananas (Cavendish) in Panyu, Guangdong Province in 1996 (Wang 2006). Then the pathogen spread rapidly in other provinces of mainland China (Lin et al. 2000; Qin et al. 2016; Xie et al. 2005; Zeng et al. 2016). By May 2019, Fusarium wilt of banana had been reported in all banana-producing areas in China, causing serious impact on the banana industry and even leading to the destruction and loss of banana plantations. It has now become the most important factor restricting banana production in China (Li et al. 2019). Although there is no completely effective method to control banana Fusarium wilt, the disease can be managed to some degree through comprehensive measures, such as combining planting of *Foc* TR4-tolerant or partially resistant cultivars with biofertilizer application (Fu et al. 2017; Li et al. 2011). Disease incidence in an experimental field planted with partially resistant Cavendish cultivars in China ranged from 8 to 28.5%, whereas it was 71.5% in the susceptible Cavendish cultivar tested (Zuo et al. 2018).

Due to the planting of resistant cultivars and the improvement of management strategies, a field survey revealed some Cavendish banana plants in Yunnan, Guangdong, Guangxi, Hainan, and Fujian Provinces for which fruit could be harvested from plants with typical external and internal *Foc* TR4 symptoms (Fig. 1A). There is a huge concern that *Foc* TR4 may also move into banana peduncle and even fruit, thereby contaminating fruits with mycotoxins harmful to humans and animals. Previously, there were several *Fusarium* species (including *F. oxysporum* but not *Foc*) that were routinely found in banana fruit associated with crown rot and other disorders, which had the potential for mycotoxin production, and these were probably a much higher risk (Li et al. 2013; Pegg et al. 2019).

Therefore, Cavendish banana peduncle samples of these plants were taken from Yunnan (Ganlanba Town, 21°51′56″ N, 100°55′50″ E) on 11 July 2018, Guangxi (Wuming County, 23°8′45″ N, 108°5′48″ E) on 13 August 2018, and Fujian (Shancheng Town, 24°32′21″ N, 117°21′43″ E) on 23 October 2018, respectively. When the peduncles were cut lengthwise, some xylem vessels appeared reddish brown (Fig. 1B and C). When these peduncles were cut transversely, infected xylem vessels looked like reddish-brown spots (Fig. 1D and E). The symptomatic peduncle tissues were surface sterilized with 75% ethyl alcohol for 30 s and then sterilized with 0.1% mercuric chloride for 30 s. These symptomatic tissues were rinsed in sterile distilled water three times and then placed on potato dextrose ager (PDA) with 40 µg/ml of ampicillin sodium to isolate the pathogen. After incubating for 3 days, white colonies developed (Fig. 2A). Single spores were transferred onto fresh PDA for further identification. A cotton-like white colony with aerial mycelia grew after 7 days of incubation at 28°C in the dark (Fig. 2B). The colony morphology of isolates resembled *Fusarium oxysporum* (Leslie and Summerell 2006). Macroconidia were typically four-septate, and microconidia were aseptate; zero-septate single cells were observed (Fig. 2D).

Total genomic DNA was extracted from mycelium of five isolates incubated for 5 days using the TaKaRa MiniBEST Plant Genomic DNA Extraction Kit (TaKaRa, Kyoto, Japan) according to the manufacturer's instructions. The sequences from the intergenic spacer region were amplified by the polymerase chain reaction (PCR) technique using primer set FocTR4-F/FocTR4-R designed by Dita et al. (2010). These PCR products were observed by agarose gel electrophoresis, and each sample was positive for *Foc* TR4 (Fig. 2C). All the PCR products were then cloned and sequenced in the pMD18-T vector, and the sequences were compared with sequences available on the NCBI GenBank database. NCBI-BLAST analysis showed 100% identity with the sequence of NRRL36114 (*Foc* strain VCG 01213). This result indicated that our isolates were *Foc* TR4, and the amplicon sequences were deposited as accessions MN830360, MN830361, MN830362, MN830363, and MN830364.

One isolate was used in a pathogenicity test by inoculating 60-day-old healthy Cavendish tissue culture plantlets. The wounded roots of 20 plantlets were dipped in a 10^6 CFU/ml spore suspension for 30 min. Five plantlets were treated with distilled water as a negative control. All treated plantlets were planted in 12-cm-diameter pots with pathogen-free coconut bran and soil (volume 1:1). Banana plants and rhizomes treated with the pathogen showed typical wilting symptoms 45 days after inoculation (Fig. 2E and G). No symptoms were observed in the leaves and rhizomes in the negative control treated with distilled water (Fig. 2F and H). Koch's postulates were fulfilled by reisolation and further molecular

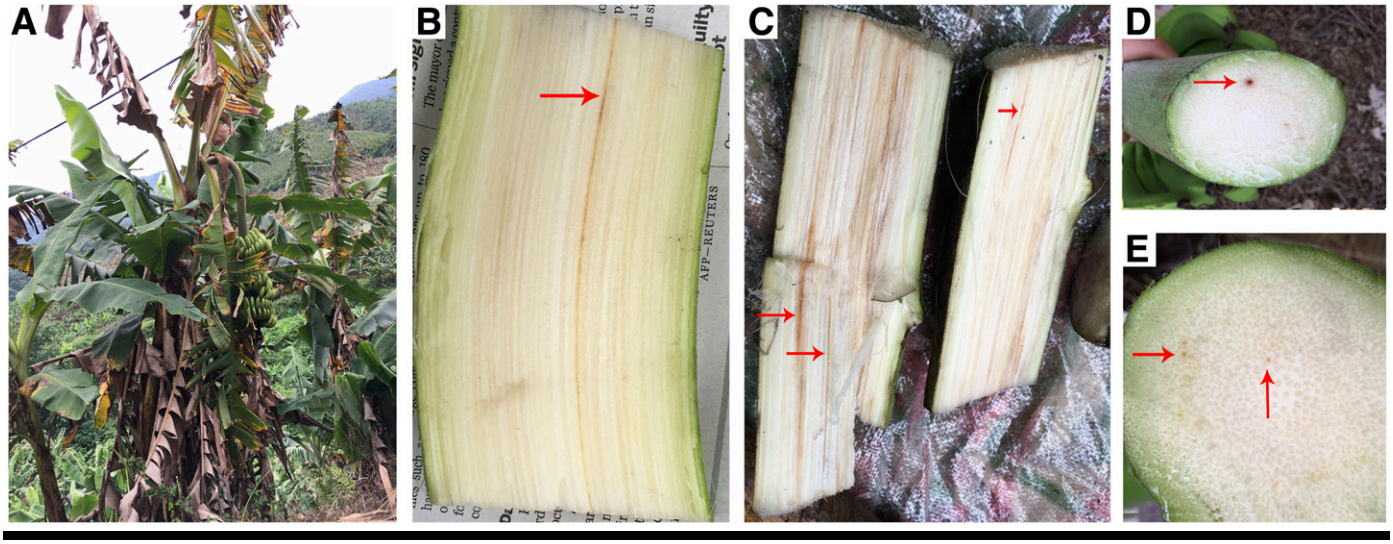

FIGURE 1

Symptoms of Fusarium wilt in the banana peduncle. **A,** Wilt symptoms with fruit setting. **B** and **C,** Wilt symptoms in peduncle cut lengthwise. **D** and **E,** Wilt symptoms in peduncle cut transversely. Red arrows identify reddish-brown vessels.

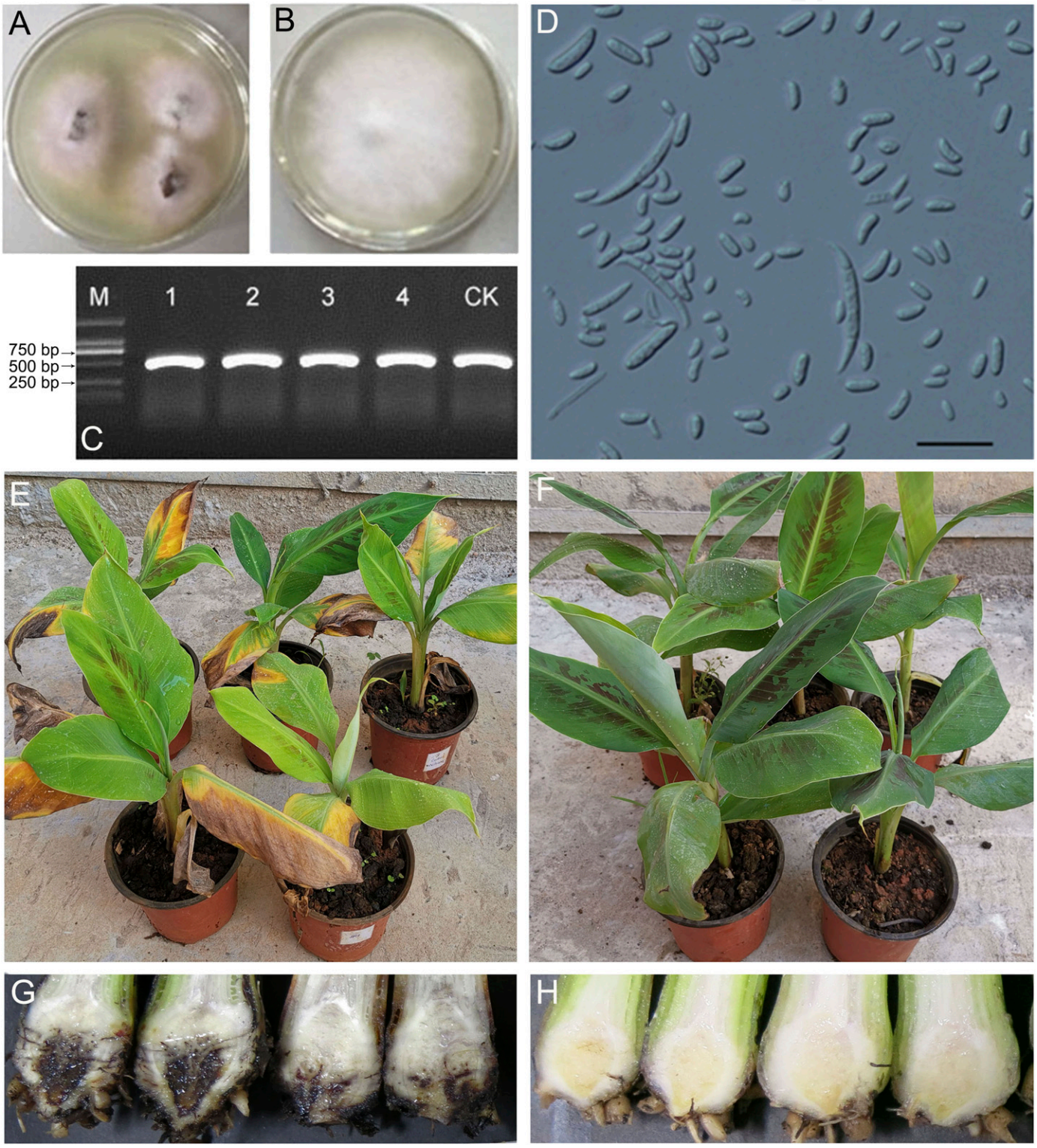

FIGURE 2

Pathogen morphology and molecular identification of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (*Foc* TR4) and Koch's postulates in Cavendish banana. **A,** *Fusarium* colonies developing around symptomatic peduncle tissues. **B,** A *Fusarium* colony developing from a single spore. **C,** PCR identification of *Foc* TR4; lane M = molecular weight maker; lanes 1 to 4 = *Foc* TR4 isolates from symptomatic banana peduncle derived from a single spore; lane CK = positive control; and scale bar = 25 μm. **D,** Macroconidia and microconidia. **E,** Banana plants with *Foc* TR4 symptoms inoculated with the pathogen. **F,** Negative control treated with distilled water. **G,** Banana rhizomes with *Foc* TR4 symptoms inoculated with the pathogen. **H,** Banana stems in negative control treated with distilled water.

identification of the pathogen from the plantlets inoculated with *Foc* TR4. These results suggested that the pathogen was *Foc* TR4.

Our results confirmed that *Foc* TR4 can infect the peduncles. The xylem of such infected banana peduncles exhibited reddish-brown streaks or spots (Fig. 1). Similar *Fusarium* symptom was also reported by Pegg et al. (2019). However, in that review paper there was no detailed description on pathogen isolation and reconfirmation if the isolate could infect a plant again to complete Koch's postulates. The infected banana peduncles are becoming more and more prevalent in Cavendish-cultivated areas in China due to the planting of the resistant cultivars and the improvement of management strategies, which result in an increasing number of infected plants still producing banana bunches.

Therefore, this is the first scientific evidence-based report to document that *Foc* TR4 can reach and infect peduncles, which pathogen could be isolated to infect new plants and may cause potential risk for spreading *Foc* TR4 in other regions (Figs. 1 and 2).

The Cavendish-based banana industry is one of the main income sources for people in the Greater Mekong Subregion (GMS). In order to meet increasing market demand, Cavendish cultivars have been progressively introduced and are now grown abundantly in GMS countries, where banana bunches complete with peduncle are traded in the traditional markets. Vietnam, Laos, and Myanmar have become increasingly under threat from banana Fusarium wilt since *Foc* TR4 recently appeared in these countries (Zheng et al. 2018). Therefore, it is necessary for banana growers and industry stakeholders to avoid moving infected planting material, contaminated nursery soils, and farming equipment, and thus help reduce *Foc* TR4 spreading further to TR4-free banana-growing areas. In this study, the banana peduncle was testified through Koch's postulates as a mediator for spreading banana Fusarium wilt (Fig. 1). Blood disease, Moko disease, and Bugtok disease, all of which are caused by bacterial pathogens, were also reported to occur in the whole banana plant including pseudostem, rhizome, leaf sheath, peduncle, and fruit (Blomme et al. 2017; Liberato and Gasparotto 2006). Thus, pathogen-bearing banana peduncles, as well as infected banana plants, cannot be discarded or carried to other banana-producing areas, especially not to "non-epidemic" areas. We also surveyed whether *Foc* TR4 subsequently infected fruit from the infected peduncles like bacterial pathogens. The crowns and fruits from the same plants with the diseased peduncles were cut, and no typical discoloration could be observed in our study, although *F. oxysporum* could be isolated from crowns by Lassois et al. (2010). However, it was not demonstrated if the *F. oxysporum* strains were indeed *Foc* strains. Some tissues with indistinct discoloration in crowns were used to isolate the pathogen, but no fungus grew from the experimental PDA medium. Because banana Fusarium wilt usually results in host plant death before the banana fruits appear, no fruit has been found to be infected with *Foc* TR4 to date (Pegg et al. 2019). However, *Fusarium* species, such as *F. oxysporum* Schlecht, were found both inside and outside the fruit; hence, the banana fruit behaved as an ideal substrate for development of mycoflora (Jiménez et al. 1993). This suggested that banana fruits are still potentially able to support *Foc* TR4 growth. In summary, here we testified the symptoms caused by *Foc* TR4 in the banana peduncles through Koch's postulates. However, further sampling and research are needed to ascertain whether *Foc* TR4 will reach and contaminate the fruit through the banana peduncle.

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Literature Cited

Blomme, G., Dita, M., Jacobsen, K. S., Vicente, L. P., Molina, A., Ocimati, W., Poussier, S., and Prior, P. 2017. Bacterial diseases of bananas and enset: Current state of knowledge and integrated approaches toward sustainable management. Front. Plant Sci. 8:1290.

Dita, M. A., Waalwijk, C., Buddenhagen, I. W., Souza, M. T., Jr., and Kema, G. H. J. 2010. A molecular diagnostic for tropical race 4 of the banana Fusarium wilt pathogen. Plant Pathol. 59:348-357.

Dong, H., Fan, H., Lei, Z., Wu, C., Zhou, D., and Li, H. 2019. Histological and gene expression analyses in banana reveals the pathogenic differences between races 1 and 4 of banana Fusarium wilt pathogen. Phytopathology 109: 1029-1042.

Fu, L., Penton, C. R., Ruan, Y., Shen, Z., Xue, C., Rong, L., and Shen, Q. 2017. Inducing the rhizosphere microbiome by biofertilizer application to suppress banana Fusarium wilt disease. Soil Biol. Biochem. 104:39-48.

García-Bastidas, F., Ordóñez, N., Konkol, J., Al-Qasim, M., Naser, Z., Abdelwali, M., Waalwijk, C., Ploetz, R. C., and Kema, G. H. J. 2014. First report of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 associated with Panama disease of banana outside Southeast Asia. Plant Dis. 98:694-696.

García-Bastidas, F. A., Quintero-Vargas, J. C., Ayala-Vasquez, M., Schermer, T., Seidl, M. F., Santos-Paiva, M., Noguera, A. M., Aguilera-Galvez, C., Wittenberg, A., Hofstede, R., Sørensen, A., and Kema, G. H. J. 2019. First report of Fusarium wilt tropical race 4 in Cavendish bananas caused by *Fusarium odoratissimum* in Colombia. Plant Dis. 104:994.

Jiménez, M., Logrieco, A., and Bottalico, A. 1993. Occurrence and pathogenicity of *Fusarium* species in banana fruits. J. Phytopathol. 137:214-220.

Lassois, L., Jijakli, M. H., Chillet, M., and Bellaire, L. D. L. D. 2010. Crown rot of banana: Preharvest factors involved in postharvest disease development and integrated control methods. Plant Dis. 94:648-658.

Leslie, J. F., and Summerell, B. A. 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing, Ames, IA.

Li, C., Zuo, C., Deng, G., Kuang, R., Yang, Q., Hu, C., Sheng, O., Zhang, S., Ma, L., Wei, Y., Yang, J., Liu, S., Biswas, M. K., Viljoen, A., and Yi, G. 2013. Contamination of bananas with beauvericin and fusaric acid produced by *Fusarium oxysporum* f. sp. *cubense*. PLoS One 8:e70226.

Li, C. Y., Chen, S., Zuo, C. W., Sun, Q. M., Ye, Q., Yi, G. Y., and Huang, B. Z. 2011. The use of GFP-transformed isolates to study infection of banana with *Fusarium oxysporum* f. sp. *cubense* race 4. Eur. J. Plant Pathol. 131:327-340.

Li, H. P., Li, Y. F., and Nie, Y. F. 2019. Research status of occurrence and control of Fusarium wilt of banana. Hua Nan Nong Ye Da Xue Xue Bao 40: 1-9 (In Chinese).

Liberato, J. R., and Gasparotto, L. 2006. Moko disease of banana (*Ralstonia solanacearum*). Updated on 7/27/2016. https://www.padil.gov.au/.

Lin, C. S., Zhang, S. S., Zhou, Y. F., Huang, Y. Y., and Hu, F. P. 2000. Identification of banana vascular wilt in Fujian. J. Fujian Agric. Univ. 29: 465-469 (In Chinese).

Maymon, M., Sela, N., Shpatz, U., Galpaz, N., and Freeman, S. 2020. The origin and current situation of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in Israel and the Middle East. Sci. Rep. 10:1590.

Nayar, N. M. 2010. The bananas: Botany, origin, dispersal. Hortic. Rev. 36: 118-164

Ordonez, N., Garcia-Bastidas, F. A., Laghari, H., Akkary, M., Harfouche, E. N., Al Awar, B., and Kema, G. H. J. 2015. First report of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 causing Panama disease in Cavendish bananas in Pakistan and Lebanon. Plant Dis. 86:1495-1499.

Pegg, K. G., Coates, L. M., O'Neill, W. T., and Turner, D. W. 2019. The epidemiology of Fusarium wilt of banana. Front. Plant Sci. 10:1395.

Ploetz, R. C. 2006. Fusarium wilt of banana is caused by several pathogens referred to as *Fusarium oxysporum* f. sp. *cubense*. Phytopathology 96: 653-656.

Ploetz, R. C. 2015. Fusarium wilt of banana. Phytopathology 105:1512-1521.

Qin, L. Y., Li, C. S., Wei, S. L., Tian, D. D., Zhou, W., Long, S. F., Huang, S. M., and Li, X. Q. 2016. Investigation on the occurrence characteristics of *Fusarium oxysporum* f. sp. *cubense* race 4 in Guangxi Province. S. China Fruits 45:93-97 (In Chinese).

Stover, R. H. 1962. Fusarial wilt (Panama disease) of bananas and other *Musa* species. Invasive Species Compendium. Commonwealth Mycological Institute, Kew, U.K.

Su, H. J., Chuang, T. Y., and Kong, W. S. 1977. Physiological race of fusarial wilt fungus attacking Cavendish banana of Taiwan. Taiwan Banana Res. Inst. Spec. Publ. 2:1-21.

Wang, Z. Z. 2006. Research progress on banana Fusarium wilt and its control. Plant Quar. 20:198-200 (In Chinese).

Xie, Y. X., Qi, Y. X., Zhang, X., Pu, J. J., and Zhang, H. Q. 2005. Study on culturing characters and pathogenicity of banana Fusarium wilt in Hainan. Plant Prot. 31:72-74 (In Chinese).

Zeng, L., Guo, Z. X., Fan, H. C., Li, H. X., Tang, Z. M., Li, Y., Yang, P. W., Bai, T. T. 2016. Advance in research of banana panama disease and its control in Yunnan Province. Trop. Agric. Technol. 39(4):19-22, 24 (In Chinese).

Zheng, S.-J., García-Bastidas, F. A., Li, X., Bai, T., Xu, S., Yin, K., Li, H., Fu, G., Yu, Y., Yang, L., Nguyen, H. C., Douangboupha, B., Khaing, A. A., Drenth, A., Seidl, M. F., Meijer, H. J. G., and Kema, G. H. J. 2018. New geographical insights of the latest expansion of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 into the great Mekong subregion. Front. Plant Sci. 9: 457.

Zuo, C., Deng, G., Li, B., Huo, H., Li, C., Hu, C., Kuang, R., Yang, Q., Dong, T., Sheng, O., and Yi, G. 2018. Germplasm screening of *Musa* spp. for resistance to *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (*Foc* TR4). Eur. J. Plant Pathol. 3:1-12.

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