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First Report of Fusarium Wilt in Banana Caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 in India

T. Damodaran, V. K. Mishra, S. K. Jha, R. Gopal, S. Rajan, and I. Ahmed

Affiliations ∨

Authors and Affiliations

T. Damodaran¹ †

V. K. Mishra¹

S. K. Jha¹

R. Gopal¹

S. Rajan²

I. Ahmed²

¹Department of Microbiology, ICAR-Central Soil Salinity Research Institute, Regional Research Station, Lucknow-226 005, India; and

²Department of Crop Improvement and Biotechnology, ICAR-Central Institute of Sub-tropical Horticulture, Lucknow, India

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India is the largest producer of the bananas in the world with an annual production of 29.72 million metric tons (FAO 2014). About 70% of the production is of the Cavendish cultivar Grand Naine (AAA). Recently, the area under the Grand Naine cultivar increased significantly in the state of Uttar Pradesh, India. In June 2017, symptoms of Fusarium wilt of banana were observed in a block of Grand Naine in the Faizabad district in Uttar Pradesh (26.46379°N, 81.59987°E). The affected plants exhibited distinct yellowing symptoms of mature leaves progressing toward the younger leaves. The laminae of the emerging leaves were markedly reduced and shriveled. The leaves collapsed gradually, bending at the petiole. The pseudostem showed longitudinal splitting at the base with distinct vascular discoloration (reddish brown). By September 2017 approximately 42 ha was observed to be affected by the disease. Further survey confirmed symptoms of the disease in the regions of Kushi Nagar (26.73988°N, 83.88697°E) and Ambedkar Nagar (27.10120°N, 81.3112°E). Infected

vascular strands collected from pseudostem tissues with vascular discoloration were subjected to morphological, molecular, and vertical compatibility group (VCG) identification. The infected strands were surface sterilized and plated on 1/4 strength potato dextrose agar (PDA) medium with 0.5% streptomycin sulfate (streptomycin sulfate 1.2 ml per 240 ml of PDA), an antibacterial agent. White hairy cottony colonies with aerial mycelium producing abundant microconidia were developed after 48 h of inoculation that phenotypically resembled *Fusarium oxysporum* (Leslie and Summerell 2006). The fungus was characterized mainly by nonseptate microconidia formed in false heads on short monophialide and chlamydospores with a smooth or rough wall. Molecular confirmation by polymerase chain reaction (PCR) was made using primers FocTR4-F (5'-CACGTTTAAGGTGCCATGAGAG-3') and FocTR4-R (5'-GCCAGGACTGCCTCGTGA-3') (Dita et al. 2010), which are specific for *F. oxysporum* f. sp. *ubense* tropical race 4 (*Foc* TR4). An amplification product of 463 bp specific for VCG 01213 (*Foc* TR4) was obtained, which on sequencing (MG458303) confirmed the identity as *Foc* TR4. VCG testing (Puhalla 1985) established that the fungal isolates obtained from isolated samples were compatible with VCG 01213/16, confirming the presence of *Foc* TR4 in India. In continuation, the isolates (CSR-F-1 and CSR-F-2) used for confirmation were analyzed for pathogenicity to fulfill Koch's postulates on 50-day-old healthy tissue culture plantlets of Grand Naine under polyhouse conditions. Inoculum production, inoculation, and molecular diagnosis were analyzed according to the standardized protocol (Dita et al. 2010) The wounded roots of the plantlets were dipped for 30 min in 10^6 spores/ml of potato dextrose culture broth, and plants were then planted in pots of 3-kg soil capacity with sand media under 28°C (70% humidity). We used three replicates of the two isolates with five plants in each replicate in the study. Five plants treated with water, and five plants treated with *F. oxysporum* f. sp. *lycopersici* were also used in the study as a control. After 45 days of incubation, both isolates caused typical wilting and internal discoloration symptoms of Fusarium wilt. The *Foc* was reisolated from the infected plants on 1/4 strength PDA. All the symptomatic plants inoculated with *Foc* TR4 showed amplification of the diagnostic amplicon 463 bp in PCR analysis, confirming that *Foc* TR4/VCG 01213/16 was the causal agent, whereas the control sets remained asymptomatic. The entire experiment for pathogenicity confirmation from inoculation to the PCR amplification was repeated twice during July to October 2017 with an interval of 45 days per experiment, to validate the results. The isolates were processed and submitted to ICAR-National Bureau for Agriculturally Important Microorganisms, Mau, India (authorized national repository). To our knowledge, this is the first report of *Foc* TR4 in India.

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The American Phytopathological Society

(APS)

📍 3352 Sherman Court, Suite 202, St. Paul, MN

55121 USA

☎ +1.651.454.7250

FAX +1.651.454.0766



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 Details

 Figures

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