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First report of *Fusarium solani* causing coffee black berry disease in China

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Introduction

Coffee black fruit is a common symptom in Arabica coffee producing areas, which seriously affects the yield and quality. There are many reasons for black fruit, such as dry fruit caused by physiological factors, black fruit caused by pests and diseases. Here, we report the identification of the pathogens on a suspected coffee black berry disease sample based on morphology and molecular phylogenetic data.

Materials/Methods

Typical diseased black fruit were collected for pathogen isolating, and the obtained single fungal colonies were observed for conidia morphology under the microscopy. The leaves of healthy coffee seedlings and coffee fruit were inoculated with mycelia mass and conidia suspension of the single colony isolates, respectively, and the pathogenicity was observed 7 days later. Total genomic DNA was extracted from fungal mycelia. The ribosomal internal transcribed spacer (ITS) was amplified by using primers ITS1 and ITS4, β-tubulin gene by Bt2a and Bt2b, translation elongation factor (TEF-1a) by EF1-526F and EF1-1567R, 28S rDNA by LROR and LR5, and subcloned as recombinant plasmids for sequencing. The ITS, TEF, tubulin, and 28S rDNA single gene sequence tree and combined ITS- TEF gene sequence tree were constructed using MEGA 6.0.

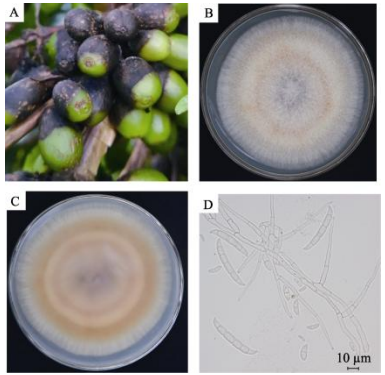

Fig 1: Symptoms and pathogenic characteristics of coffee *Fusarium* sp. black berry disease. A: Field symptoms; B: Colony morphology on PDA medium (front); C: Colony morphology on PDA medium (reverse); D: Conidia morphology (40×).

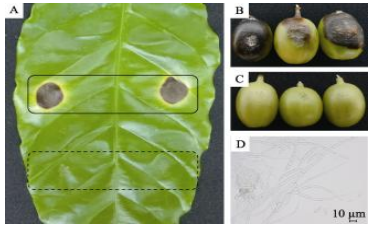

Fig. 2: Pathogenicity determination and conidia morphology of re-isolated of pathogen. A: The solid frame represents the inoculation of mycelium block, and the dotted frame indicates inoculation of sterile PDA (CK); B: Inoculates coffee fruit with conidia suspension; C: Inoculates coffee fruit with water (CK); D: Conidia morphology of re-isolated of pathogen (40×).

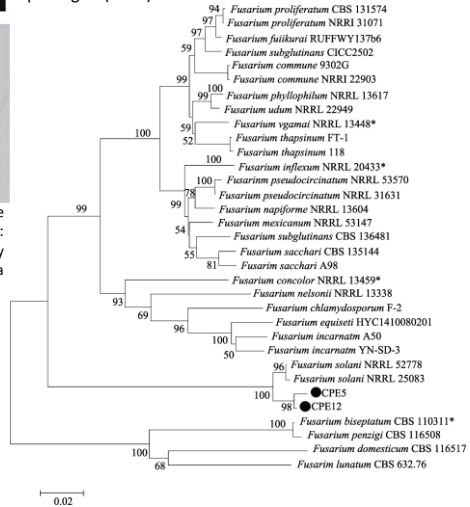

Fig 3: Phylogenetic tree constructed through NJ method based on combined *ITS-TEF* gene sequences.

Results

The fungal isolation had a round, felt-like colony on PDA medium, the mycelium was off-white, the surface was sparse, and light yellow pigment appeared on the back. The conidia had 1~8 septums, its length was 6.08~65.3 μm and width was 2.76~9.03 μm. Small conidia were kidney-shaped and large conidia were sickle-shaped. The pathogenicity test showed that the infected coffee leaves and fruits the symptoms similar typical symptoms as observed from the diseased fruits under natural conditions. Koch's postulates were fulfilled by re-isolating the fungus and verifying its colony and morphological characters. Molecular identification results showed that ITS, β-tubulin, TEF, 28S rDNA, four single gene clustering tree, and ITS-TEF gene sequence clustering results were consistent, indicating all that CPE5 and CPE12 belong to *Fusarium solani*. Therefore, the pathogen was confirmed as *Fusarium solani* by morphological and molecular identification.

Conclusion/Perspectives

To the best of our knowledge, this is the first reported the case of *Fusarium solani* infection on fruit of coffee in China.

Acknowledgments

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