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Identifying Fusarium and Combinations in the Gibberella Fujikuroi Species Complex

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ABSTRACT

Mango malformation, caused by Fusarium mangiferae, represents the most important floral disease of mango. The first symptoms of this disease were noticed in the beginning of 2005 in plantations at Sohar in the Eastern U.P. of India. The affected inflorescences were abnormally enlarged and branched with heavy and dried-out panicles. Based on morphology and DNA-sequence data fir the genes encoding translation elongation factor 1 α and β-tubulin, the pathogen associated with these symptoms was identified as F. mangiferae.

Keywords— Salt Gibberella Fujikuroi Complex, Translation Clongation Factor 1 α and β-tubulin

I. INTRODUCTION

Mango (Mangifera indica) is an important perennial crop in the Eastern U.P. of India and in 2004 the production of local varieties of this fruit exceeded 8,600 tones (http://www.maf.gov.om/). In many mango growing regions of the world, an infectious disease known as mango malformation has been reported to limit production and cause substantial economical loss (Kumar et al., 1993). The disease was first observed in India in 1891 and has since been reported from a number of countries in Asia, Africa and the Americas (Marasas et al., 2006).

Mango malformation is characterised by the abnormal development of vegetative shoots and inflorescences. The vegetative form of the disease is observed more frequently on young seedlings, where axillary or apical buds produce mishapen shoots, have shortened internodes and brittle leaves that are significantly smaller than those of healthy plants. Malformed shoots tend to remain compact thus giving rise to a bunchy-top appearance. The major symptoms of inflorescence malformation include abnormally branched and thickened panicles that produce up to three times the normal number of flowers. These flowers are unusually enlarged, sterile and do not bear fruit (Kumar et al.,1993; Ploetz, 1994; Marasas et al., 2006).

Many factors such as physiological abnormalities, viral infection, mite (Aceria mangiferae) infection and fungal infections have been suggested as possible causal agents of the disease (Kumar et al., 1993), Various Fusarium species have been associated with the disease (Marasas et al.,2006). Although there are unpublished reports of at leas tdifferent taxa in this genus causing symptoms of malformation on mango (C. Lima, personal communication; G. Rodriguez, personal communication), a fourth taxon, F. mangiferae, is the only one which has conclusively been shown to cause mango malformation (Briz et al., 2002).

To confirm the presence of mango malformation in Eastern U.P. of India we used DNA sequence comparisons and morphology to identify F. mangiferae in symptomatic tissue.

II. MATERIALS AND METHODS

Samples of malformed inflorescences were collected from infected trees and surface-sterilized by submerging pieces of plant tissue in a sodium hypochlorite (1%) solution and then in 70% ethanol for 1 min each. Samples were then rinsed in sterile distilled water and dried on sterile filter paper before plating small flower pieces onto 39g 1^{-1} potato dextrose agar (PDA, Biolab, Merck). Following incubation at 25^0C for 7 days, pure fungal cultures were obtained by single conidial spore transfers onto 20 g 1^{-1} PDA medium. All isolates are stored and maintained in the Fusarium collaction of the Tree Protection Co-operative Programme.

To determine the identity of the fungus recovered from the diseased mango tissue, three representative isolates were characterised based on morphological characteristics and DNA sequence comparisons. In order to observe morphological features, isolates were grown on 39 g 1^{-1} PDA, synthetic low nutrient agar (Nirenberg and O' Donnell 1998) and carnation leaf agar (Fisher et al., 1982).o

After incubation at 25 C for 10 days under near-ultraviolet light, the isolates were examined using a light microscope and the diagnostic characters noted by Britz et al. (2002).

For the DNA comparisons, the first -700 and and -500 bases of the genes encoding translation elongation factor

1 α(EF1 α) and β-tubulin, respectively, were sequenced. For this purpose, genomic DNA was extracted using the CTAB (N-Acetyl-N, N, N-trimethy1-ammonium bromide) method (Steenkamp et al., 1990). After purification with G50 Sephadex columns (Sigma, Steinheim, Germany), PCR products were sequenced in both directions using the original PCR primers, an ABI PRISM Big Dye terminator v 3.0 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) and a 3730 DNA analyzer (Applied Biosystems). The electropherograms were visualised and corrected where necessary with Chromas Lite 2.0 (Technelysium, Australia) and BioEdit version 7.0.5.2 (Hall, 1999). All EF1! "nucleotide sequences were compared using the BLAST search tool (Altschul et al., 1990) to those in the Fusarium identification database (Geiser et al., 2004) and all #-tubulin nucleotide sequences were compared to those in the database of the National Centre for Biotechnology Information (NCBL, www.ncbi.nlm.nih.gov) to obtain preliminary identifications.

III. RESULTS AND DISCUSSION

Nucleotide sequences were aligned using MAFFT version 5.8 with the L-INS-i option effective (http://align,bmr.kyushu-u-ac.jp/mafft/online/server/). These alignments included the sequences generated in this study, as well as those for representatives of the recognized species in the GFC (Geiser et al., 2005) obtained from Gene Bank. Relevant sequences for all of the known unique phylogenetic lineages in the so called Asian Clade of the GFC were also included. The resulting aligned database were analyzed separately as well as combined because they were previously shown to represent homogenous partitions PAUP* 4b10 (Swofford, 2003) were used to perform neighbour-joining distance (NJ) analyses and parsimony analyses using heuristic searches of 1000 random addition replicates and tree bisection reconnection branch-swapping analyses. Maximum likelihood (ML) analyses were performed with PHYML v2.1 (Guindon and Gascuel, 2003) and Bayesian analyses were performed with MrBayes v3.1 (Ronquist and Heuelsenbeck, 2003).

Morphological examination revealed that all three isolates produced macroconidia with 3-5 septa and oval microconidia in false heads from mono- and polyphialides. None of the representative isolates produced chlamydospores under the conditions tested. These morphological characters are typical of F. mangiferae, as well as most fungi previously recognized as F. subglutinans sensu lato (Britz et al., 2002).

Results of DNA sequence comparisons indicated that the fungi isolated from diseased Omani mango flowers, represent F. mangiferae. Similarity searches and sequence comparisons revealed that the EF1 α and β-tubulin sequences of the Oman isolates are identical to that of F. mangiferae NRRL 25226 (Gene Bank Accessions AF160281 and U61561; Steenkamp et al. 2000). Their EF1 α sequences differed at two nucleotide positions from that of the ex-holotype isolate (FCC4581; Briz et al., 2002). However, results of the phylogenetic analyses clearly showed that the Eastern U.P. of India isolates from part of the so-called Asian Clade of the GFC, where they are most closely associated with known F. mangiferae isolates. This results conclusively demonstrate that the India isolates from malformed mango inflorescences represent F. mangiferae.

To the best of our knowledge, F. mangiferae and mango malformation has not previously been reported in the Eastern U.P. of India. Mango malformation has the potential to have a significant negative impact on the mango industry in India and the Middle East as this disease significantly reduces yields. As a result, the spread of the disease must be halted by removing and burning affected trees, as well as by planting non-infected nursery supplies.

IV. CONCLUSIONS

This study further indicated that infections caused by F. mangiferae are not systemic, with infections of apical and lateral buds most likely originating and disseminating from malformed panicles. It is expected that long term treatment of fungicide sprays accompanied with removal of infected panicles (the main source of inoculum) will be cumulative and result in disease reductions annually, eventually achieving negligible levels of malformation in treated orchards over time. Critical periods when infection occurs need to be determined, as these could be the focus for effective application schedules.

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