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SHORT COMMUNICATION

Fusarium mangiferae associated with mango malformation in the Sultanate of Oman

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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 Sultanate of Oman. The affected inflorescences were abnormally enlarged and branched with heavy and dried-out panicles. Based on morphology and DNA-sequence data for the genes encoding translation elongation factor 1α and β-tubulin, the pathogen associated with these symptoms was identified as *F. mangiferae.*

Keywords *Gibberella fujikuroi* complex · Translation elongation factor 1α · β-tubulin

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Mango (*Mangifera indica*) is an important perennial crop in the Sultanate of Oman and in 2004 the production of local varieties of this fruit exceeded 8,600 t (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 misshapen shoots, have shortened internodes and brittle leaves that are significantly smaller than those of healthy plants (Kumar et al. 1993). Malformed shoots tend to remain compact thus giving rise to a bunchy-top appearance (Kumar et al. 1993; Ploetz 1994; Marasas et al. 2006). 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).

Historically, the aetiology of the disease has been poorly understood. Many factors such as physiological abnormalities, viral infection, mite (*Aceria mangiferae*) infestation 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 least three different 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 (Freeman et al. 1999; Britz et al. 2002; Marasas et al. 2006). To date, the presence of *F. mangiferae* has been confirmed in Egypt, Florida, Israel, Malaysia and South Africa (Britz et al. 2002; Marasas et al. 2006). Based on results with species-specific PCR primers, the pathogen may also exist in Spain (S. Freeman, personal communication). A recent report from Pakistan cannot be confirmed based on the diagnostic data it contained (Iqbal et al. 2006).

When *F. mangiferae* was first isolated from malformed tissue, Summanwar et al. (1966) recognized it as *F. moniliforme*. Later, Varma et al. (1974) used the name *F. moniliforme* var. *subglutinans* and demonstrated its involvement in both the vegetative and floral forms of the disease. Nelson et al. (1983) recognized the fungus as *F. subglutinans* in the section *Liseola*, which broadly corresponds with the so-called *Gibberella fujikuroi* complex (GFC) (O'Donnell et al. 1998a). To accommodate morphologically and phylogenetically-related isolates of *F. subglutinans* (Steenkamp et al. 2000) that had been shown previously to cause mango malformation (Freeman et al. 1999), Britz et al. (2002) established the taxon, *F. mangiferae*.

Early in 2005, typical symptoms of mango malformation were noticed on mango trees at Sohar, 250 km north–west of Oman's capital Muscat. These symptoms included abnormally enlarged inflorescences with thick, branched and heavy panicles (Fig. 1). To confirm the presence of mango malformation in Oman, we used DNA sequence comparisons and morphology to identify *F. mangiferae* in symptomatic tissue.

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 39 g l^{-1} potato dextrose agar (PDA, Biolab, Merck). Following incubation at 25°C for 7 days, pure fungal cultures were obtained by single conidial spore transfers onto 20 g l^{-1} PDA medium. All isolates are stored and maintained in the *Fusarium* collection of the Tree Protection Co-operative Programme, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

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 l^{-1} PDA, synthetic low nutrient agar (Nirenberg and O'Donnell 1998) and carnation leaf agar (Fisher et al. 1982). 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) and Nelson et al. (1983).

Fig. 1 Malformed inflorescence of mango collected at Sohar in the Sultanate of Oman

For the DNA comparisons, the first ~700 and ~500 bases of the genes encoding translation elongation factor 1α (EF1α) and β-tubulin, respectively, were sequenced. For this purpose, genomic DNA was extracted using the CTAB (*N*-cetyl-*N*, *N*, *N*-trimethyl-ammonium bromide) method (Steenkamp et al. 1999). The two gene regions were amplified with primer set EF1+EF2 [5'-atgggtaagga(a/g)gacaagac-3' and 5'-gga (g/a)gtaccagt(g/c)atcatgtt-3'; O'Donnell et al. 1998b] and T1+T2 [5'-aacatgcgtgagattgtaagt-3' and 5'-tagt gacccttggcccagttg-3'; O'Donnell and Cigelnik 1997], respectively, using previously described PCR reaction and cycling conditions (Geiser et al. 2005). After purification with G50 Sephadex columns (Sigma, Steinheim, Germany), PCR products were sequenced in both directions using the original PCR primers, an ABI PRISM BigDye Terminator v3.0 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 EF1α nucleotide sequences were compared using the BLAST search tool (Altschul et al. 1990) to those in the *Fusarium* identification database (Geiser et al. 2004; http://fusarium.cbio.psu.edu/) and all β-tubulin nucleotide sequences were compared to those in the database of the National Centre for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov) to obtain preliminary identifications.

Nucleotide sequences were aligned using MAFFT version 5.8 with the L-INS-i option effective (Katoh et al. 2002, 2005; 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 (O'Donnell et al. 1998a, 2000; Geiser et al. 2005) obtained from GenBank. Relevant sequences for all of

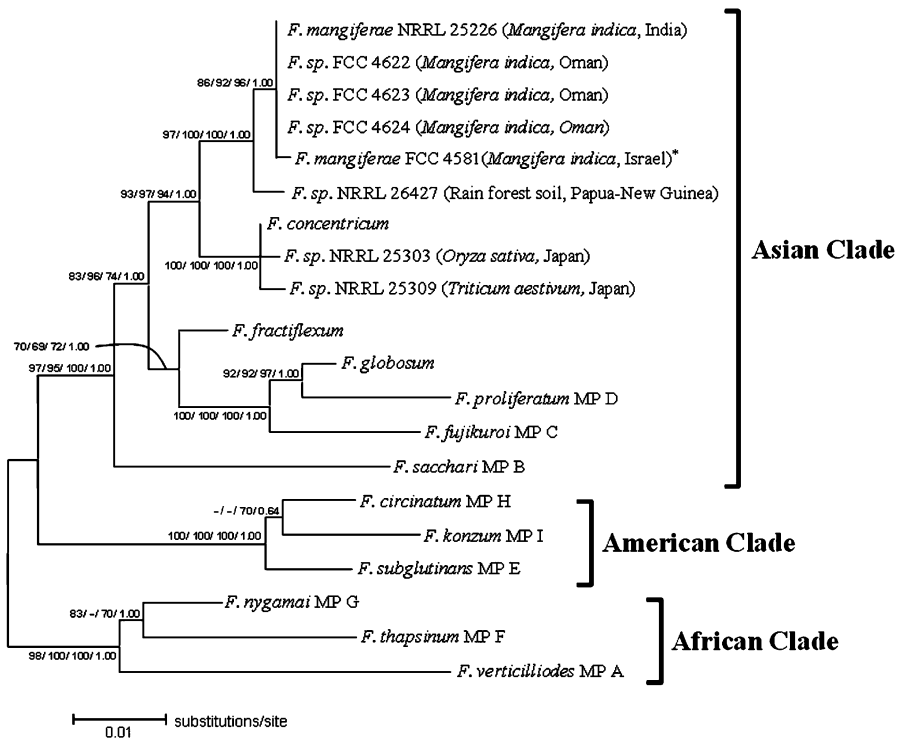

Fig. 2 A maximum likelihood phylogeny of the *Gibberella fujikuroi* species complex based on combined translation elongation factor 1α and β-tubulin sequence data. The mating populations (MP) of this complex, as well as all of the known members of the so–called Asian Clade (O'Donnell et al. 1998a, 2000) are included. The ex-holotype of *F. mangiferae* is indicated with an asterisk and the tree is rooted with the *Fusarium* species in the so-called African Clade. NJ, parsimony and Bayesian analyses of the combined dataset generated trees with topologies similar to that of the ML tree. The topologies of trees generated from analyses of the individual gene datasets were also congruent with these trees. Bootstrap support values >60% based on 1,000 replications and Bayesian posterior probabilities are indicated at the branches in the order parsimony, NJ, ML and Bayesian. Branches with bootstrap support values <60% are indicated with "–"

the known unique phylogenetic lineages in the so-called Asian Clade of the GFC (O'Donnell et al. 1998a, 2000) were also included. The resulting aligned datasets were analysed separately as well as combined, because they were previously shown to represent homogenous partitions (O'Donnell et al. 1998a, 2000). PAUP* 4b10 (Swofford 2003) was used to perform neighbour-joining distance (NJ) analyses and parsimony analyses using heuristic searches of 1,000 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). The latter was based on the Metropolis-coupled Markov chain Monte Carlo search algorithm with 1,000,000 generations, and calculation of Bayesian posterior probabilities after discarding a burnin of 500 generations. Modeltest 3.7 (Posada and Crandall 1998) and MrModelTest 2.2 (Nylander 2004; Posada and Crandall 1998) were used to determine appropriate evolutionary models for the NJ, ML and Bayesian analyses. The General Time Reversible (GTR) model (Rodríguez et al. 1990) with gamma correction for rate variation was used for EF1α, while the Hasegawa-Kishino-Yano (HKY) model (Hasegawa et al. 1985) with gamma correction was used for the β-tubulin dataset. Analyses of the combined dataset utilized the GTR model with proportion invariable sites and gamma correction. ML, NJ and parsimony branch supports were estimated using 1,000 bootstrap replicates.

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 the DNA sequence comparisons indicated that the fungi isolated from diseased Omani mango flowers, represent *F. mangiferae*. Similarity searches and sequence comparisons revealed that the EF1α and β-tubulin sequences of the Oman isolates are identical to that of *F. mangiferae* NRRL 25226 (GenBank Accessions AF160281 and U61561; O'Donnell et al. 2000; Steenkamp et al. 2000; Britz et al. 2002). Their EF1α sequences differed at two nucleotide positions from that of the ex-holotype isolate (FCC4581; Britz et al. 2002). However, results of the phylogenetic analyses clearly showed that the Oman isolates form part of the so-called Asian Clade (O'Donnell et al. 1998a, 2000) of the GFC, where they are most closely associated with known *F. mangiferae* isolates (Fig. 2). These results conclusively demonstrate that the Oman 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 Sultanate of Oman. Mango malformation has the potential to have a significant negative impact on the mango industry in Oman 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 (Marasas et al. 2006). Research is underway to ascertain the distribution of the disease in Oman and the route by which the disease entered the country.

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