Australasian Plant Dis. Notes (2016) 11: 21
DOI 10.1007/s13314-016-0206-5

Fusarium fujikuroi causing fusariosis of pineapple in peninsular Malaysia

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Received: 7 July 2015 / Accepted: 27 May 2016 / Published online: 8 June 2016

Abstract Fusariosis symptoms were detected on pineapple fruits and leaves in several states in Peninsular Malaysia. Eight *Fusarium fujikuroi* isolates were isolated and identified using a partial Translation Elongation Factor -1α (*TEF*) and β-tubulin sequences. The isolates showed 98–100 % similarity with *F. fujikuroi* NRRL43610. Phylogenetic analysis of the combined *TEF* and β-tubulin sequences showed that the eight isolates clustered with *F. fujikuroi* from rice. Koch's postulates were fulfilled on pineapple fruits and leaves confirming pathogenicity of the *F. fujikuroi* isolates.

Keywords *Fusarium fujikuroi* · Pineapple · Fusariosis

Pineapple (*Ananas comosus*) is one of the important commercial fruit crops cultivated in Malaysia (Malaysian Pineapple Industry Board 2015). During a disease survey from December 2010 to November 2011 in pineapple farms in the states of Kedah, Penang, Selangor and Johor, typical fusariosis symptoms were observed on Moris, Josapine and Gandul varieties. Common symptoms were lesion and brown discoloration of the fruitlet, rotten or sunken fruit skin and stem, gum exudation on some fruits, dry rot on leaf, stem bending, chlorosis, increasing number of leaves per spiral and natural cracks on the fruit (Fig. 1a-d), which is similar to pineapple fusariosis described by Rohrbach and Schmitt (1998) and Ploetz (2006). Wilt symptoms were generally observed on the leaves with yellowing or brown discoloration.

Fungi were isolated from the infected fruits and leaves. The tissues between the margin of infected and apparently healthy fruit tissues were sampled. Isolates from infected leaves were obtained from the rotted leaf base and necrotic leaf tissues. The infected areas of the fruits and leaves (cut into 5 × 5 mm pieces) were surface sterilized by soaking in 1 % sodium hypochlorite for 3 min, rinsed with sterile distilled water, and then plated onto peptone pentachloronitrobenzene agar. All of the plates were incubated at 27 ± 1 °C under 12 h alternate light and dark. Mycelial growths from the tissues were then subcultured onto potato dextrose agar (PDA) and single-spored.

Morphological identification was based on procedures in the *Fusarium* Laboratory Manual (Leslie and Summerell 2006). Eight isolates of *Fusarium* produced white to dark purple colonies and white to dark violet pigmentation. Aerial mycelia were floccose and sometimes cottony in appearance (Fig. 2a, b). Macroconidia (3.3–3.6 μm x 37.8–43.7 μm) were slender to relatively straight, 2–3-septa (Fig. 2c). Microconidia (3.4–3.7 μm x 12.2 to 12.9 μm) were obovoid with a truncate base (Fig. 2d). Conidiophores produced mono- and polyphialides, and microconidia formed in short (4 conidia) to medium chains (20 conidia) (Fig. 2e, f). The characteristics observed best matched those of *F. fujikuroi.* The eight isolates that were recovered from the three pineapple varieties from were deposited in culture collection of Plant Pathology Lab, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia.

Genomic DNA of the isolates was extracted using DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany)

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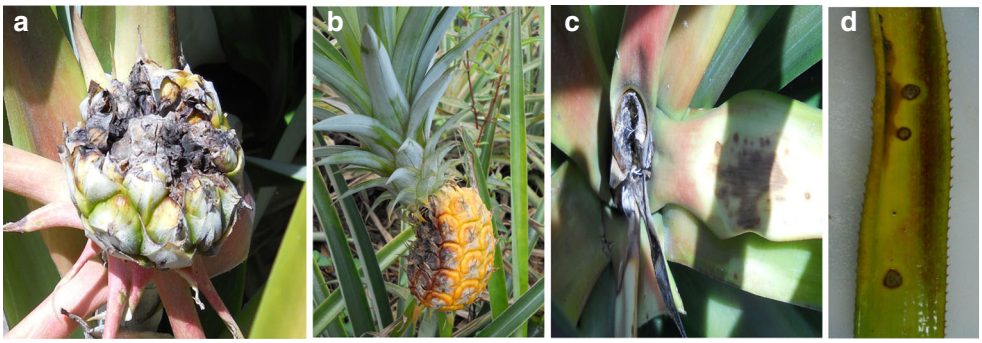

Fig. 1 Fusariosis symptoms in the field **a** Dry rot on top of the fruit, **b** brown lesion on one side of the fruit, **c** rot at leaf base, **d** necrotic leaf spot

according to the manufacturer's instructions. PCR was performed in a MyCycler® Thermal Cycler (Bio-Rad, Hercules, CA, USA). Molecular identification was done using a partial *TEF* (O'Donnell et al. 1998) and β-tubulin (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997) sequences. PCR cycles for amplification of *TEF* was set at initial denaturation at 94 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 35 s, annealing at 59 °C for 55 s, extension at 72 °C for 1.5 min and final extension at 72 °C for 1 min. For β-tubulin, the PCR cycles were initial denaturation at 94 °C for 1 min followed by 39 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min and final extension at 72 °C for 5 min. Based on BLAST results against GenBank and Fusarium-ID databases, the isolates showed 98–100 % similarity to *F. fujikuroi* NRRL43610. All of the sequences were deposited in GenBank (Accession No. from KC584844 to KC584851 for *TEF* and from KC584866 to KC584873 for β-tubulin).

Phylogenetic analysis was done using *TEF* and β-tubulin sequences and analyzed using Molecular Evolutionary Genetic Analysis (MEGA) version 5.1 (Tamura et al. 2011). Several *F. fujikuroi* isolates from rice from Malaysia, Taiwan, Thailand, Korea and the Philippines were included in the analysis (Table 1). Twenty-one members of *Fusarium fujikuroi* species complex representing Asian, American and African clades (O'Donnell et al. 1998), *F. guttiforme*, *F. ananatum* and *F. proliferatum* from pineapple were also included in the phylogenetic analysis (Table 1). The tree was rooted with *F. oxysporum* (O'Donnell et al. 1998).

The Maximum likelihood tree generated (Fig. 3) showed that all of the pineapple fusariosis isolates clustered with *F. fujikuroi* from rice. Isolates of *F. guttiforme* and *F. ananatum* which have been reported to be associated with fusariosis or fruit rot of pineapple, as well as isolates of *F. proliferatum* formed separate clade. The results of the

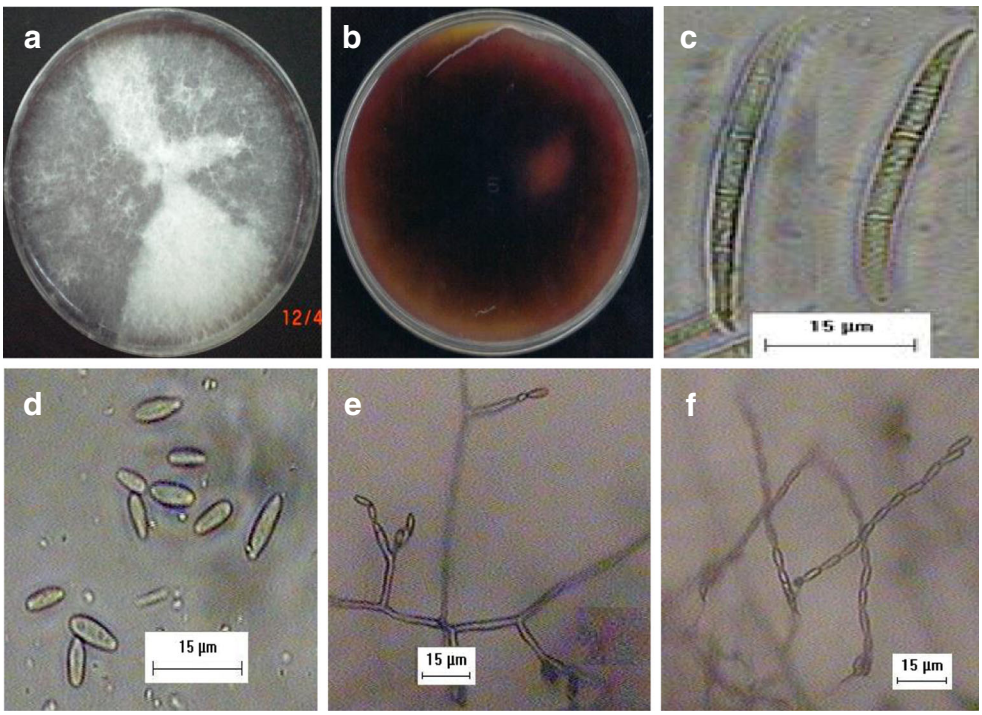

Fig. 2 Morphological characteristics of *F. fujikuroi* **a** floccose and cottony colony, **b** dark violet pigmentation on lower surface of PDA, **c** slender to relatively straight 3–4 septate macroconidia, **d** obovoid microconidia **e** mono- and polyphialides, **f** V-shaped microconidial in chains

Table 1 Host and geographic origin of *Fusarium* species used in phylogenetic analysis

Fusarium species	Collection	Host/ substrate	Origin	GenBank accession number	
				TEF-1α	β-tubulin
F. fujikuroi	Isolate 37	*Ananas comosus*	Malaysia	KC584844	KC584866
F. fujikuroi	Isolate 59	*Ananas comosus*	Malaysia	KC584845	KC584867
F. fujikuroi	Isolate 68	*Ananas comosus*	Malaysia	KC584846	KC584868
F. fujikuroi	Isolate 113	*Ananas comosus*	Malaysia	KC584847	KC584869
F. fujikuroi	Isolate 115	*Ananas comosus*	Malaysia	KC584848	KC584870
F. fujikuroi	Isolate 219	*Ananas comosus*	Malaysia	KC584849	KC584871
F. fujikuroi	Isolate 223	*Ananas comosus*	Malaysia	KC584850	KC584872
F. fujikuroi	Isolate 242	*Ananas comosus*	Malaysia	KC584851	KC584873
F. fujikuroi	NRRL 13566	*Oryza sativa*	Taiwan	AF160279	U34415
F. fujikuroi	NRRL 22174/CBS221.76	*Oryza sativa*	Japan	AB725605	AB725606
F. fujikuroi	-	*Oryza sativa*	Malaysia	KJ 025023	KM044564
F. fujikuroi	-	*Oryza sativa*	Malaysia	KJ 025025	KM044566
F. fujikuroi	-	*Oryza sativa*	Malaysia	KJ 025029	KM044570
F. fujikuroi	-	*Oryza sativa*	Malaysia	KJ 025035	KM044575
F. fujikuroi	-	*Oryza sativa*	Malaysia	KJ025039	KM044580
F. fujikuroi	-	*Oryza sativa*	Philippines	JF 699611	KM044580
F. fujikuroi	-	*Oryza sativa*	Korea	KF 604038	KM044579
F. fujikuroi	-	*Oryza sativa*	Korea	KF 604059	KM044577
F. proliferatum	NRRL 22944	*Cattleta* sp.	Germany	AF160280	U34416
F. proliferatum	156	*Ananas comosus*	Malaysia	KC571430	KC571366
F. proliferatum	151	*Ananas comosus*	Malaysia	KC571429	KC571363
F. proliferatum	183	*Ananas comosus*	Malaysia	KC584808	KC571312
F. ananatum	MRC 8165	*Ananas comosus*	South Africa	DQ282167	DQ282174
F. ananatum	MRC 8166	*Ananas comosus*	South Africa	DQ282171	DQ282178
F. ananatum	MRC 8167	*Ananas comosus*	South Africa	DQ282169	DQ282176
F. guttiforme	MRC 6782	*Ananas comosus*	Brazil	DQ282170	DQ282177
F. guttiforme	MRC 6783	*Ananas comosus*	Brazil	DQ282166	DQ282173
F. guttiforme	MRC 7539	*Ananas comosus*	Brazil	DQ282165	DQ282172
F. globosum	NRRL 26131	Unknown	USA	KF466417	U61557
F. concentricum	NRRL 25181	*Musa sapientum*	Costa Rica	AF160282	U61548
F. ramigenum	NRRL 25208	*Ficus carica*	USA	AF160267	U61554
F. lactis	NRRL 25200	*Ficus carica*	USA	AF160272	U61551
F. thapsinum	NRRL 22045	*Sorghum bicolor*	South Africa	AF160270	U34418
F. phyllophilum	NRRL 13617	*Dracaena deremensis*	Italy	AF160274	U34432
F. subglutinans	NRRL 22016	*Zea mays*	USA	HM057336	U34417
F. bulbicola	NRRL 13618	*Nerine bowdenii*	Netherlands	AF160294	U61546
F. oxysporum	NRRL 20433	*Ficia faba*	Germany	AF008479	AF331804
F. napiform	NRRL 13604	*Pennisetum typhoides*	South Africa	AF160266	U34428
F. acutatum	NRRL 13308	Unknown	India	AF 160276	U34431
F. udum	NRRL 22949	Unknown	Germany	AF 160275	U34433
F. begoniae	NRRL 25300	*Begonia elatior*	Germany	AF 160293	U61543
F. bactridioides	NRRL 20476	*Cronartium conigenum*	USA	AF 160290	U34434
F. succisae	NRRL 13613	*Succusa pratensis*	Germany	AF 160291	U34419
F. anthophilum	NRRL 13602	*Hippeastrum* sp.	Germany	AF 160292	U61541
F. circinatum	NRRL 25331	*Pinus radiata*	USA	AF 160295	U61547
F. fractiflexum	NRRL 28854	*Cymbidium* sp.	Japan	AF 333932	AF333948
F. semitectum	NRRL 31160	Human lung	Texas	GQ915510	GQ915444

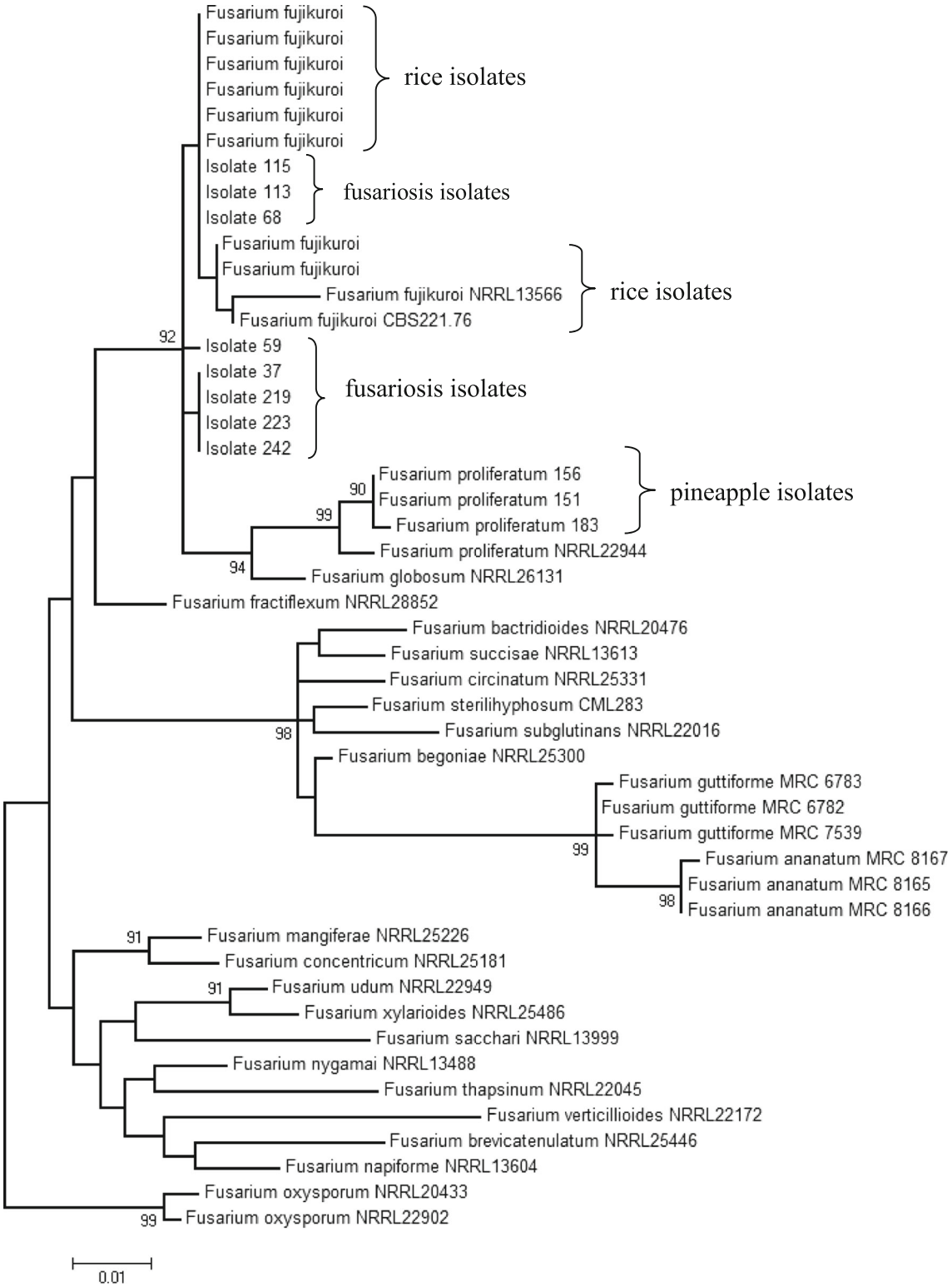

Fig. 3 Maximum likelihood tree inferred from combined *TEF* and β-tubulin sequences showing the placement of *F. fujikuroi* isolates from pineapple fusariosis. The bootstrap support values higher than 85 % from 1000 replicates are shown at the nodes. Sequences of *F. oxysporum* NRRL20433 and NRRL22902 were used to root the tree

present study indicate that in addition to *F. guttiforme* and *F. ananatum*, *F. fujikuroi* is also associated with pineapple fusariosis.

A pathogenicity test was conducted using four representative isolates (isolates 37, 59, 68 and 219). The test was conducted on Gandul, Josapine and Moris varieties using a pricking technique

for pineapple fruits and an agar plug technique for pineapple leaves (Dianese et al. 1981). Detached matured fruits and leaves were surface sterilized using 1 % sodium hypochlorite for 3 min and then rinsed with sterile distilled water. For the inoculation test on fruits, the skin was wounded with a sterile tooth pick before a colonized tooth pick with mycelia was inserted into the wounded area. All of the tooth picks were left intact until the end of the experiment. On leaves, a fine sterile needle was used to wound the healthy leaf surface and a 5 mm mycelia plug (from 7-day-old culture) was placed in the wounded area. Control fruits were pricked with a sterile tooth pick without inoculum while control leaves were inoculated with a PDA plug without inoculum. All of the inoculated fruits were arranged in a container that contained moist sterile filter paper to maintain moisture and then covered with a plastic wrap (Cling Wrap). Inoculated leaves were also covered with plastic wrap for one week to maintain moisture content. Symptom development was observed one week after inoculation.

Two weeks after inoculation, the fruits were cut vertically and brown lesions around the inoculated areas were observed as well as mycelia growth in some of the inoculated areas (Fig. 4a). On leaves, a small necrotic spot appeared on the inoculated leaves which turned brown and grew into a dark brown lesion after eight weeks incubation (Fig. 4b). All inoculated fruits and leaves of Gandul, Josapine and Moris varieties showed fusariosis symptoms similar to those observed on infected pineapple plant in the field. The *F. fujikuroi* isolates were re-isolated from brown lesions on the infected fruit tissues as well as from black and brown necrotic spots on leaves, thus completing Koch's postulates.

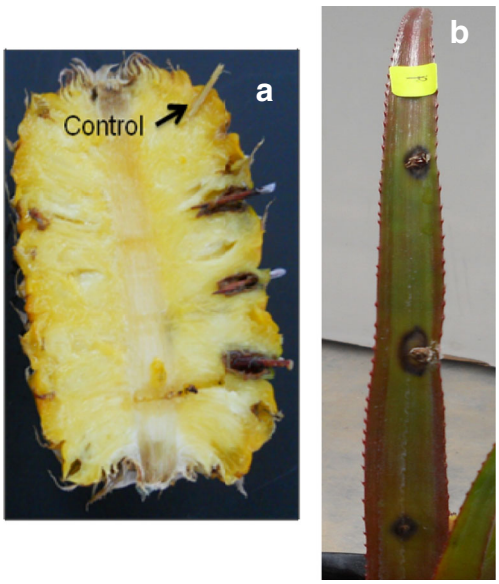

Fig. 4 Pathogenicity test on pineapple fruit and leaf. **a** brown to black lesions on pineapple fruit. **b** necrotic lesions on pineapple leaf

The present study revealed that *F. fujikuroi* isolates caused pineapple fusariosis in Peninsular Malaysia. Although morphologically, *F. fujikuroi* was similar with *F. proliferatum*, in the present study *F. fujikuroi* isolates produced colony with cottony appearance and formation of short chain microconidia which were minor morphological differences that can be used to differentiate both species (Gerlach and Nirenberg 1982). Moreover, in phylogenetic analysis isolates of *F. fujikuroi* and *F. proliferatum* were clearly grouped into separate clades. *Fusarium fujikuroi* is a well-known pathogen of bakanae disease of rice. Besides rice, *F. fujikuroi* has been reported as saprophytic or endophytic colonisers of vanilla (Pinaria et al. 2010) and was isolated from human skin (O'Donnell et al. 2010). According to Slippers et al. (2005), fungi are more likely to undergo plant host jumps as a result of human movement and weather changes. Movement of pineapple plants for trading and exchange of planting materials between plantations and farmers within Peninsular Malaysia may have introduced and spread the pathogen into new areas. To our knowledge, this is the first report of *F. fujikuroi* associated with pineapple fusariosis in Peninsular Malaysia.

Acknowledgment This work was supported by the Exploratory Research Grant Scheme (203/PBIOLOGI/6730053), Minstry of Higher Education, Malaysia.

References

Dianese JC, Bolkan HA, da Silva CB, Couto FAA (1981) Pathogenicity of epiphytic *Fusarium moniliforme* var. *subglutinans* to pineapple. Phytopathology 71:1145–1149

Gerlach W, Nirenberg HI (1982) The genus *Fusarium* – a pictorial atlas. Parey, Berlin

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with PCR to amplify conserved genes from filamentaous ascomycetes. Appl Environ Microbiol 61:1323–1330

Leslie JF, Summerell BA (2006) The *Fusarium* laboratory manual. Iowa, USA, Blackwell Publishing, Ames

Malaysian Pineapple Industry Board (2015) What is the variety of pineapple which is planted in Malaysia? http://mpib.gov.my/en/soalan-lazim. Accessed 17 July 2015

O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol Phylogenet Evol 7:103–116

O'Donnell K, Cigelnik E, Nirenberg HI (1998) Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90:465–493

O'Donnell K, Sutton DA, Rinaldi MG, Sarver BA, Balajee SA, Schroers HJ, Summerbell RC, Robert VA, Crous PW, Zhang N, Aoki T, Jung K, Park J, Lee YH, Kang S, Park B, Geiser DM (2010) Internet-accessible DNA sequence database for identifying fusaria from human and animal infections. J Clin Microbiol 48:3708–3718

Pinaria AG, Liew ECY, Burgess LW (2010) *Fusarium* species associated with vanilla stem rot in Indonesia. Australas Plant Pathol 39:176–183

Ploetz RC (2006) *Fusarium*-induced disease of tropical, perennial crops. Phytopathology 96:648–652

Rohrbach KG and Schmitt DP (1998) Fusariosis. In: Compendium of Tropical Fruit Diseases RC Ploetz, GA Zentmyer, WT Nishijima, KG Rohrbach and HD Ohr (eds). pp. 49

Slippers B, Stenlid J, Wingfield MJ (2005) Emerging pathogens: fungal host jumps following anthropogenic introduction. Trends Ecol Evol 20:420–421

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol Biol Evol 28:2731–2739