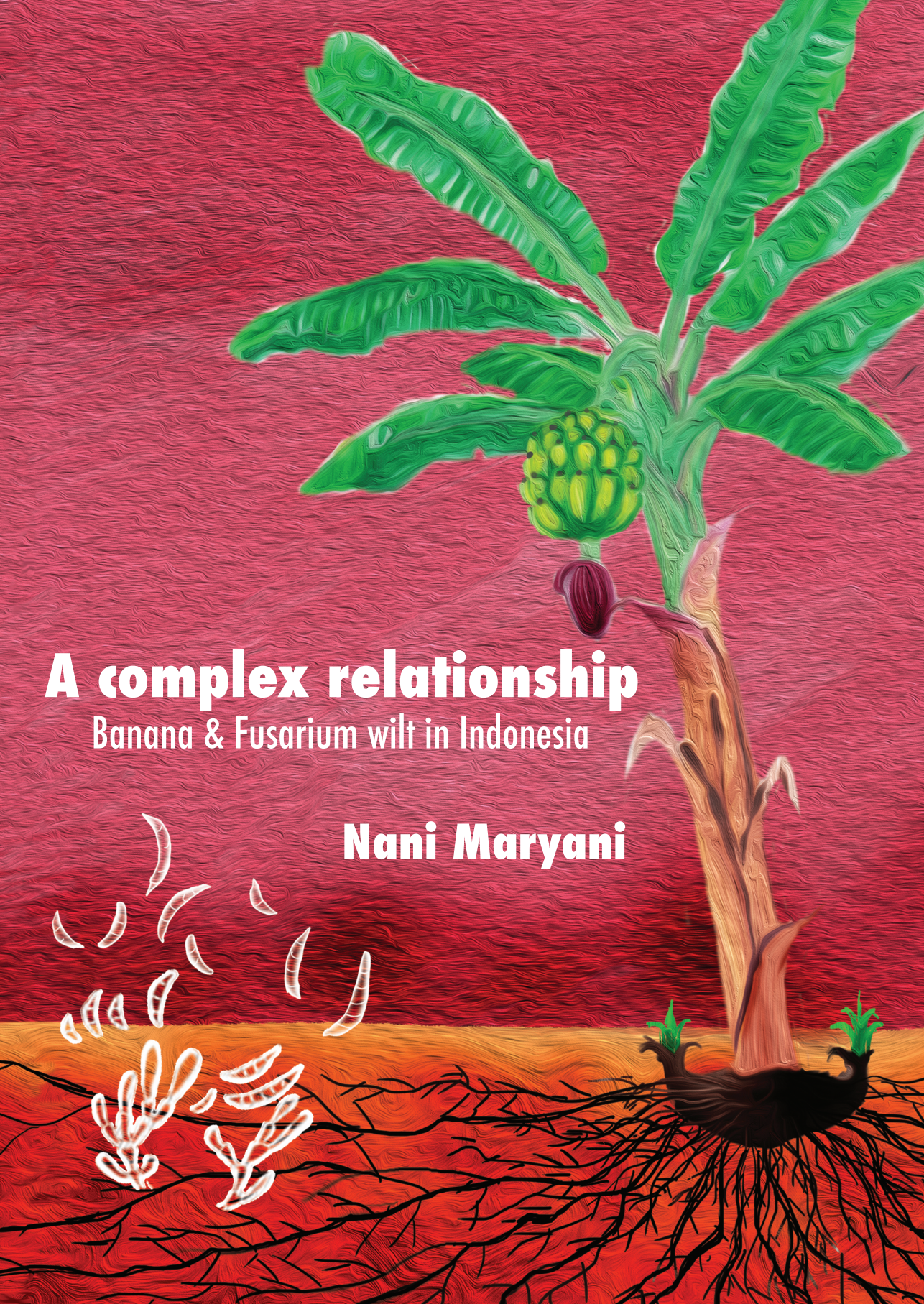

A complex relationship

Banana & Fusarium wilt in Indonesia

Nani Maryani

Propositions

1. The taxonomy of the *Fusarium oxysporum* species complex is outdated and requires a thorough revision.
   (this thesis)

2. The future of banana research lies in genetics, which also requires the phenotyping of many isolates to understand the banana – *Fusarium* spp. interaction.
   (this thesis)

3. Complexity guarantees durability and denounces monocultures as utter simplicity.

4. Collaboration in science diminishes skills.

5. Even the best politicians and scientists only flourish through logic, grammar and rhetoric.

6. Indonesian players excel in badminton because of their tactic and individuals skills to win the game.

Propositions belonging to the thesis, entitled
"A complex relationship: Banana & Fusarium wilt in Indonesia"

Nani Maryani
Wageningen, 29 October 2018

A complex relationship

Banana & Fusarium wilt in Indonesia

Nani Maryani

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This research was conducted under the auspices of the Graduate School of Experimental Plant Sciences

A complex relationship

Banana & Fusarium wilt in Indonesia

Nani Maryani

Thesis

submitted in fulfilment of the requirements for the degree of doctor
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in the presence of the
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"Verily! In the creation of the heavens and the earth,
and in the alternation of night and day,
there are indeed signs for men of understanding"

Q. 3: 190

Untuk Mama dan Bapa

CONTENTS

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[1] Narated from Abu Hurayra, in the Kitab Sunan Abu Dawud, The Book of Manners: Regarding Giving Thanks in Return for a favor.

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Chapter 1

General introduction

The global human population increases exponentially. However, agricultural progress lags behind and hence, food security for the predicted 9.8 billion people by 2050 is a main concern (Population Reference Bureau, www.prb.org). Thomas Robert Malthus already predicted in his essay *On the Principle of Population* (1798), that a catastrophic food scarcity will become apparent as human populations grow geometrically (i.e. doubling with each cycle) while food production grows at an arithmetic rate (i.e. by the repeated addition of a uniform increment). He, however, overlooked the impact of advanced technology in agriculture and considered subsistence farming as the only source of food production. Through plant breeding, fertilizers, irrigation and mechanization - to name a few - food supply can outpace population growth. Nevertheless, the Malthusian view is not passed, as contemporary agriculture faces many challenges, not the least by new and expanding plant diseases (Bebber *et al*. 2014).

Plant diseases have dramatic impacts and destabilizing effects on societies. They can change the course of history, due to social-economic and political upheaval. One of the most destructive plant disease epidemics ever recorded in human history is Fusarium wilt of banana, also known as Panama disease (Simmonds 1962, Stover 1962). The impact of Fusarium wilt on food security, as banana is an important part of local diets, is not well documented. The disease has caused huge economic losses and has had a massive social impact due to unemployment in many banana exporter countries in Latin America (Koeppel 2009). The disease devastated one of the most important fruits and staple food crops which is grown in more than 135 countries in the (sub)tropics (FAO 2018, http://www.fao.org/). In 2016, the global banana production reached 78.8 million tonnes, with 18.6 million tonnes accounting for exports, mainly from Central and South America (representing 30 % of the global productions) (Fruit Trop 2017).

Currently, the threat of Fusarium wilt to banana is recurring, thereby alerting producers but also importers and particularly consumers, as they may see their beloved fruit go extinct. However, in the centre of origin of banana, which from the co-evolutionary standpoint is also the centre of origin of major pathogens threatening the crop (Ploetz & Pegg 1997), hundreds of excelling varieties are grown. This thesis discovers and describes the diversity of the Fusarium wilt pathogen of banana in its centre of origin, Indonesia.

Bananas: history and early domestication

Nowadays, banana is one of the most popular fruits in the world. People consume more than 100 billion bananas each year, whether as staple food or fruit, which make its one of the largest crops produced after wheat, rice, and corn (FAO 2018, http://www.fao.org/). Banana is one of the earliest plants to be cultivated by humans, which started approximately, 7000 BC in South-East Asia and Papua New Guinea, marking the early history of tropical agriculture (De Langhe & De Maret 1999). Bananas were most likely introduced to Africa via

Madagascar in the ancient time by Indonesian immigrants (Vérin 1981). However, the dispersal of bananas was mainly by Arab traders in the first millennia to Mesopotamia, Palestine, and Egypt, and became popular in that area as it is mentioned in the Koran as the "fruit of paradise" (Kervegant 1935, Koeppel 2007). The linguistic evidence supports this notion, that the first scientific term given to banana is *Musa paradisiaca* Linn (Linnaeus 1753) that means "fruit from paradise" (*Musa,* mauz-Arabic = fruit; *paradisiaca,* firdaws-Arabic = paradise) (Koeppel 2007). It took until the 15th century for Portuguese traders to introduce bananas to the New World, i.e. South America and Caribbean islands, which in the present day supplies most of the commercially produced bananas to the western world, i.e Europe and North America (Stover & Simmonds 1987).

Bananas are herbaceous monocotyledon perennial plants belonging to the family of *Musaceae* that includes the genera *Musa* and *Ensete*. The section *Eumusa* in the genus *Musa* includes wild and edible bananas that we eat today (Simmonds & Shepherd 1955). Generally, wild bananas are seeded, less pulpous and found wild in the forest or in abandoned areas, whereas edible bananas are seedless, pulpy fruits and grown in home gardens or plantations (Fig. 1). *Musa acuminata* Colla (2n = 22, AA) and *Musa balbisiana* Colla (2n = 22, BB) are the ancestors of all edible bananas. Both wild banana species originate from the Indo-Malayan region, with *M. acuminata* being the most diverse sub-species found in South-East Asia, and *M. balbisiana* occurring in the Indian subcontinent and also in South-East Asia (Valmayor *et al.* 1999, Perrier *et al.* 2011; Fig. 1F). Both wild species hybridized and diversified into various cultivated varieties (cultivars) whose genomic composition and ploidy level were derived from these two wild diploid parents. The edible cultivars can be found as diploid AA and AB groups, triploid AAA, AAB, ABB, and BBB groups, and occasionally tetraploid, AAAA, AAAB, and ABBB groups. The terms plantain, cooking banana and banana have been used interchangeably, but academically "plantain" has been used to refer to the starchy banana AAB group that is eaten after cooking, "cooking banana" has been used to refer to the starchy banana ABB group, and "dessert banana" has been used to refer to the sweet banana, that is eaten fresh upon ripening (Valmayor *et al.* 1999). However, in the place where bananas are indigenous, "banana" refers to any type of the above-mentioned terms. The term "Pisang", which means "banana" in Bahasa and Indo-Malayan language, followed by local names is used to describe cultivated varieties in the Indo-Malayan region, especially in Indonesia.

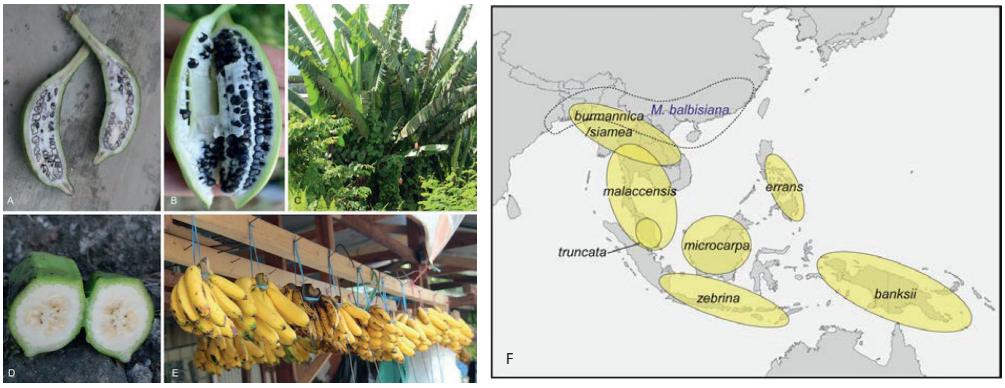

Fig. 1. A. Seeded wild banana species *Musa acuminata* var. *microcarpa*. **B.** *Musa acuminata* var. *alasensis* (Photo by F. Ahmad). **C.** *Musa bornensis* growing as wild massive herbs in the indigenous tropical forest of Kalimantan, Indonesia. **D.** Seedless cultivated banana, Pisang Kepok. **E.** Local banana cultivars for sale in a traditional open market stand. **F.** Geographical distribution of *Musa balbisiana* and subspecies of *Musa acuminata*, the wild ancestors of cultivated bananas (Perrier *et al*. 2011).

Indonesia is the homeland of bananas. They play a central role in the Indonesian culture and heritage (Kennedy 2009). Hundreds of local varieties are grown across the Indonesian archipelago, making it the number one fruit commodity in the country (http://pusdatin.setjen.pertanian.go.id). Indonesia is the primary gene centre for banana, the contact area between species and subspecies of wild *Musa* (Simmonds 1962, Perrier *et al.* 2011; Fig. 1F) and hosts around 71 *Musa* species, of which 15 are sub-species of *M. acuminata* (Nasution 1990; Fig. 2). The origin of *M. balbisiana* is largely outside Indonesia, primarily the Indian sub-continent, South China and The Philippines. Yet, the diversity of this wild species is high in Indonesia especially on the islands of Java, Sumatra, and Sulawesi (De Langhe *et al.* 2009, Ochse & van den Brink 1931, Ahmad 2013). The diversity of wild *Musa* species in Indonesia results in a huge diversity of approximately 200 cultivated varieties which are largely well identified and maintained in the Purwodadi Botanical Garden (Kebun Raya Purwodadi) in East Java (166 cultivars, mainly from Eastern Indonesia; Hapsari *et al.* 2015), the Research Center for Biology (Pusat Penelitian Biologi LIPI Cibinong) in West Java (191 cultivars, including 42 wild *Musa* accessions; Poerba *et al.* 2016) and the Indonesian Tropical Fruit Research Institute (BALITBU Solok) in South Sumatra (300 cultivars; Sutanto 2018, pers. comm.). In 2006, the International Network for the Improvement of Banana and Plantain (INIBAP, current name Biodiversity International) estimated that, 1000 banana cultivars occurred in tropical and subtropical regions. Based on a rough estimate, Indonesia hosts around 50 % of the total global banana diversity. It is essential to conserve banana germplasm for research and development and, most importantly, it provides an excellent source of diversity for the various banana breeding programs.

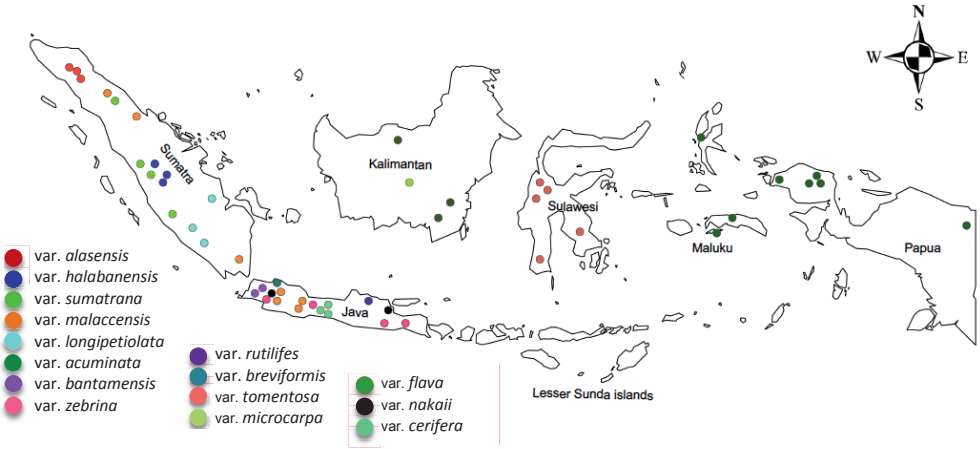

Fig. 2. Geographical distribution of 15 sub-species of the wild seeded banana, *Musa acuminata* (adopted from Nasution 1990).

Banana production

In contrast to the common knowledge that banana is an export crop, 70 % of the global banana production is aimed for domestic markets, and only 30 % for export or trade (FAO 2015). Thus, the majority of bananas is produced from smallholder farms, and is staple food in many countries in Africa, tropical America, and Asia (Ploetz *et al.* 2015). Indonesia is among the top 10 banana producers of the world and mainly supplies the domestic market (90 %) with only a small amount for export (10 %), primarily to China (53.74 %), Saudi Arabia (25.68 %), Kuwait (15.51 %) and Malaysia (3.66 %) (http://pusdatin.setjen.pertanian.go.id/). In Indonesia, and many other Asian countries banana is grown in backyards, in a mixed cropping system as well as in commercial smallholder production and corporate farms or agri-business plantations (Fig. 3). It is a cash crop that costs almost nothing, but provides a substantial income to over five million households. In 2016, >7M tons of bananas were produced from 81 812 ha, which contributes to 38 % of the national fruit production (Central Bureau Statistics, Directorate of Horticulture 2016, http://www.pertanian.go.id). The fruits are abundant in any traditional market as well as in modern supermarkets, and are highly appreciated across society as the national snack fruit and ingredient in many sweet dishes. Therefore, bananas are planted everywhere near people's livelihoods such as in yards, fields, on hills, mountains, and along rivers (Fig. 3).

Fig. 3. Types of banana cultivation in Indonesia. **A.** Backyard plantation, East Kalimantan. **B.** Monoculture commercial plantation, Lampung, South Sumatra. **C.** Intercropping in a coffee plantation, Brastagi, North Sumatra. **D.** Mixed cropping with cassava, Central Java. E. Bananas grow as weeds by the roadside, Maumere, Flores.

Fusarium wilt of banana

Despite its great importance, global banana cultivation faces significant problems due to diseases. Fusarium wilt or Panama disease is one of the most devastating plant diseases ever recorded in the history of an agricultural crop. In the beginning of the 1960's, around 40,000 ha of Gros Michel plantations were abandoned in Latin America because of this disease. Initially, the cause of the disease was a big mystery, until Smith (1910) isolated a purple fungal culture which produced both macro- and microconidia, and demonstrated that the fungus was the cause of a Cuban banana plant disease, to which he assigned the name *Fusarium cubense*. Brandes (1919) for the first time provided a formal description of the fungus, conducted pathogenicity tests and concluded that the fungus was host-specific. In later studies, Waite & Stover (1960) determined that the Gros Michel (AAA) and Silk (AAA) varieties were susceptible to Race 1, and that the Bluggoe (ABB) variety was susceptible to Race 2. In 1940, Snyder & Hansen proposed the name *Fusarium oxysporum* Schlecht f. sp. *cubense* (Foc) for this pathogen, which then was widely adopted by most plant pathologists. However, "Panama disease" became notorious after the first big epidemic in Panama, and Stover (1962) used this name for the first time in Jamaica. Later on, the name "Fusarium wilt disease" was preferred and widely used in agreement with other crop diseases caused by *Fusarium oxysporum*.

Even though the first identification of the disease was in Latin America, the pathogen was thought to come from the host origin in the Indo-Malayan region (Stover 1962). In general, pathogens evolve with the hosts since the start of agriculture, during domestication and selection for cultivation by humans (Stukenbrock & McDonald 2008). Thus, there is no doubt that Indonesia, as the centre of origin and diversity of banana also hosted its pathogens. The first recognition of Fusarium wilt of bananas in Indonesia was in 1916, in a banana plantation on the island of Java (Rijks 1915, in Stover 1962). At that time, there was a massive migration of people from Java to Suriname, which could account for the international dissemination of the disease (Malefijt 1963). Gros Michel affected by Fusarium wilt was recognised very early in Suriname (1904), where it was likely intercropped with cocoa, as many of the immigrant workers from Java worked in cocoa plantations (Stover 1962). Such records trace back the origin of the pathogen that likely disseminated along with its host from South-East Asia. This hypothesis was later substantiated by O'Donnell *et al.* (1998) who suggested that South-East Asia is the place of co-evolution between the Fusarium wilt pathogen and banana.

The banana industry in Latin America was saved by the introduction of the resistant Cavendish variety. However, two decades later, a new strain called Race 4 was identified in Cavendish plantations in Taiwan (Su *et al.* 1986). This new strain was virulent to many banana varieties in tropical areas, and was later called Tropical Race 4 (TR4) (Gerlach *et al*. 2000, Ploetz 2006). The dissemination of TR4 around the globe is recurring to many banana producing countries, for both export as well as for staple food products. To date the TR4 epidemic spans three continents (Asia, Australia, and Africa), affecting of a total of 15 countries (http//: www.fusariumwilt.org; Fig. 4) and it seems a repetition of history once this pathogen disseminates to the American continent.

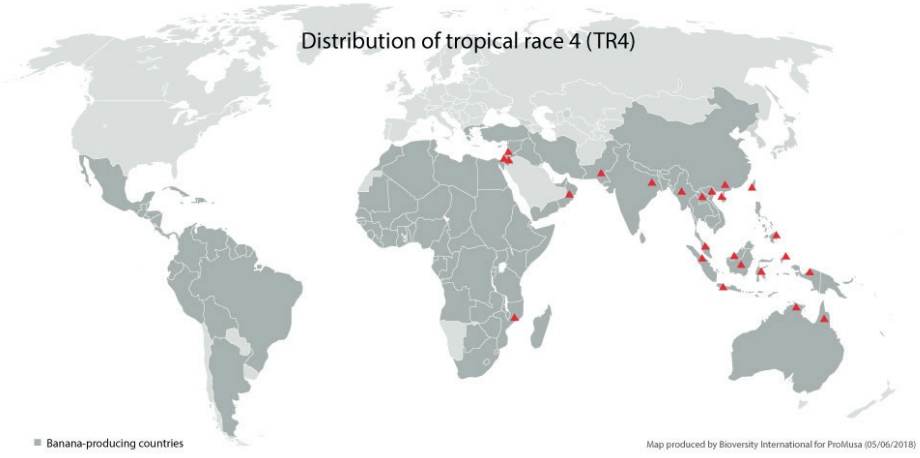

Fig. 4. Global dissemination of Tropical Race 4 (TR4).

In Indonesia, Fusarium wilt is recognized by local people as “penyakit layu” with major symptoms of wilting and yellowing of the leaves, collapsing of leaf petioles, and systemic infection causing longitudinal splitting of the lower portion of the pseudostem (Fig. 5). The clearest symptom to distinguish the disease from wilting caused by other biotic or abiotic agents is the discoloration of the pseudostem and corm (Moore *et al.* 1995; Fig. 5). The disease already spread from the Western part (Sumatra) to the Eastern part (Papua) of Indonesia (Nasir *et al.* 1999) and TR4 has devastated thousands of hectares of Cavendish plantations in South and North Sumatra (Jumjunidang *et al.* 2012). Moreover, many local popular varieties succumbed to the disease (Hermanto *et al.* 2009).

Fig. 5. Symptoms of Fusarium wilt of bananas. **A.** Wilting and yellowing of the leaves. **B.** Discoloration of the pseudostem. **C.** Discoloration of the corm.

To date, there is no effective control to manage the epidemic due to the persistence of chlamydospores that can survive in contaminated soils for decades. The above-mentioned impact of the disease on a global and local scale affects food security in many countries. Finding a resistant variety is the best solution to stop the epidemic as demonstrated by the resistant Cavendish that quenched the previous epidemic which was caused by Race 1 strains. Despite the fact that Cavendish has maintained its resistance against Race 1 for more than 50 years in areas prone to Fusarium wilt, neither the genetics of resistance nor the genes responsible for pathogenicity were explored and understood. Therefore, striving for resistance to Fusarium wilt requires elucidation of resistance as well as deciphering the interaction between Foc and banana. The race concept in Foc is only based on a limited set of varieties which consequently overshadows details of diversity in Foc populations (Cunha *et al.* 2015, Karangwa *et al.* 2017). Hence, the resistance of the host and pathogenicity of the pathogen are overly simplified. However, to unveil the interaction of the host and pathogen, both the degree of resistance and pathogenicity are very important keys to clarify the type of interaction that occurs in this particular pathosystem. It is of foremost importance to scrutinize resistance to Fusarium wilt in banana (D’Hont *et al*. 2012, Dale *et al*. 2017, Zuo *et al.* 2018). The identification of resistance to TR4 in *Musa acuminata* var. *malaccensis* is an

obvious example of a resource in a wild variety from the centre origin of bananas. Moreover, many cultivated varieties are also sources of resistance (Handayani *et al.* 2017). Thus, the diversity of both wild and cultivated varieties needs to be explored in the quest for resistance to Foc. In this thesis, we explored diversity in *Fusarium* spp. affecting banana and studied the interaction with some banana varieties of Indonesian origin. Therefore it provides a basis for improving the understanding of the banana-Foc pathosystem.

***Fusarium oxysporum* f. sp. *cubense*: taxonomy, diversity and co-evolution**

Fusarium oxysporum is an ascomycete fungus that is commonly found as a soil inhabitant worldwide. *Fusarium oxysporum* f. sp. *cubense* (Foc), together with many other pathogenic isolates from a wide range of important crops, is part of the *Fusarium oxysporum* species complex (FOSC) (Baayen *et al.* 2000, O'Donnell *et al.* 2004). Due to the lack of a sexual life cycle, the taxonomy of FOSC was traditionally defined by morphological characters, and delimited by asexual structures (Wollenweber & Renking 1935, Booth 1971, Nelson *et al.* 1983). With a more pragmatic and broad definition, especially for plant pathologists, Snyder & Hansen (1940) subdivided this complex by introducing *formae speciales* (ff. spp), thus strains infect particular plant hosts but are not pathogenic to others. *Formae speciales* are further sub-divided into races, which refer to host cultivar specificity (Armstrong & Armstrong 1981). However, molecular phylogeny showed that many *formae speciales* are polyphyletic (Baayen *et al.* 2000). Therefore, *formae speciales* and races are considered as physiological classifications, without taxonomic significance, but facilitate communication among plant pathologists (Gordon 2017). As molecular data are continually accumulated, many members of the FOSC are subject to taxonomical revisions and Foc is no exception.

The phylogeny and diversity of Foc have been studied using various morphological, physiological and molecular genetic tools (O'Donnell *et al.* 1998, Groenewald *et al.* 2006, Fourie *et al.* 2009, Ordonez *et al.* 2015, Mostert *et al.* 2017). While all results suggest co-evolution of Foc and banana in South East Asia, little is known about the diversity of the pathogen in the centre of origin of the host, which is likely to represent its full diversity. In this thesis, we explore, exploit and analyse the diversity of Foc from natural ecosystems in Indonesia where hundreds of local banana varieties are grown. This knowledge is of great importance for sustaining global banana production through advanced breeding programs.

Outline of the thesis

The first chapter lays out the history and current knowledge of the diversity of banana and the fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (Foc), the situation of Fusarium wilt in Indonesia and at the global perspective of the disease. It culminates in the importance and urgency of a diversity study of Foc in the centre of origin of banana in Indonesia.

The second chapter is the first of four experimental chapters and describes the exploration of Fusarium wilt of bananas across six main islands of Indonesia: Java, Flores, Kalimantan, Papua, Sumatra and Sulawesi (details of geographical locations, ecology, and host identity were recorded). More than 200 *Fusarium* isolates from many local varieties were isolated and Multi Locus Sequence Typing (MLST) was used for molecular identification and characterization. Multi-gene phylogenetic analyses were used for identification of new *Fusarium* spp. to replace Foc lineages in the FOSC. New species names were assigned and formal taxonomic descriptions were given. Furthermore, pathogenicity tests were conducted to further characterize new phylogenetic species.

The third chapter describes a population study using genotyping-by-sequencing (Diversity Array Technology [DArTseq]) as a technique for diversity analyses of the Indonesian isolates comprising *Fusarium* species identified in **chapter 2**. Polymorphic DArTseq markers were used for hierarchical clustering and genotype identification in each *Fusarium* species. Association between species, genotype, and physiological race were also analysed. The advantage of DArTseq and its robustness to assess genotypic diversity in the *Fusarium* spp. causing wilt in banana are discussed.

The fourth chapter provides an insight into the pathology of the *Fusarium* spp. – banana pathosystem. The knowledge on the diverse genotypes of Indonesian isolates in **chapter 3** was used to challenge wild and cultivated banana varieties with *Fusarium* diversity. Phenotyping assays were conducted in well-controlled greenhouse experiments that took almost two consecutive years to collect qualitative and quantitative disease data.

The fifth chapter describes the discovery of other *Fusarium* species which were isolated from Fusarium wilt diseased samples discussed in **chapter 2**. Twenty isolates were identified as members of three other *Fusarium* species complexes, namely the *Fusarium fujikuroi* species complex (FFSC), the *Fusarium incarnatum-equiseti* species complex (FIESC) and the *Fusarium sambucinum* species complex (FSSC). Multi-gene phylogenetic analyses of each complex were used for phylogenetic species recognition. In addition, pathogenicity tests were conducted to check host specificity of these species. **The last chapter** provides a general discussion of the thesis. The primary findings are discussed in a broader context of banana production and additional and required future research is suggested.

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Chapter 2

Phylogeny and genetic diversity of the banana Fusarium wilt pathogen *Fusarium oxysporum* f. sp. *cubense* in the Indonesian centre of origin

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Abstract

Fusarium oxysporum f. sp. *cubense* (Foc), the causal agent of Fusarium wilt or Panama disease on banana, is one of the major constraints in banana production worldwide. Indonesia is the centre of origin for wild and cultivated bananas, which likely co-evolved with Foc. This study explored the widest possible genetic diversity of Foc by sampling across Indonesia at 34 geographically and environmentally different locations in 15 provinces at six islands. This resulted in a comprehensive collection of ~200 isolates from 40 different local banana varieties. Isolates were identified and assessed using sequence analysis of the translation elongation factor-1alpha (*tef1*), the RNA polymerase II largest subunit (*rpb1*), and the RNA polymerase II second largest subunit (*rpb2*). Phylogenetic analyses of these genes allowed the identification of 180 isolates of *Fusarium oxysporum* f. sp. *cubense* (Foc), and 20 isolates of the *Fusarium fujikuroi* species complex (FFSC), the *Fusarium incarnatum-equiseti* species complex (FIESC), and the *Fusarium sambucinum* species complex (FSSC). Further analyses, incorporating a worldwide collection of Foc strains, revealed nine independent genetic lineages for Foc, and one novel clade in the *Fusarium oxysporum* species complex (FOSC). Selected isolates from each lineage were tested on the banana varieties Gros Michel and Cavendish to characterise their pathogenicity profiles. More than 65 % of the isolates were diagnosed as Tropical Race 4 (TR4) due to their pathogenicity to Cavendish banana, which supports the hypothesis that TR4 is of Indonesian origin. Nine independent genetic lineages for Foc are formally described in this study. This biodiversity has not been studied since the initial description of Foc in 1919. This study provides a detailed overview of the complexity of Fusarium wilt on banana and its diversity and distribution across Indonesia.

Key words: morphology, new species, panama disease, pathogenicity, Tropical Race 4, 11 new taxa

Taxonomic novelties: New species: *Fusarium cugenangense* N. Maryani, L. Lombard, Kema & Crous*; F. duoseptatum* N. Maryani, L. Lombard, Kema & Crous*; F. grosmichelii* N. Maryani, L. Lombard, Kema & Crous*; F. hexaseptatum* N. Maryani, L. Lombard, Kema & Crous*; F. kalimantanense* N. Maryani, L. Lombard, Kema & Crous*; F. odoratissimum* N. Maryani, L. Lombard, Kema & Crous*; F. phialophorum* N. Maryani, L. Lombard, Kema & Crous; *F. purpurascens* N. Maryani, L. Lombard, Kema & Crous; *F. sangayamense* N. Maryani, L. Lombard, Kema & Crous*; F. tardichlamydosporum* N. Maryani, L. Lombard, Kema & Crous; *F. tardicrescens* N. Maryani, L. Lombard, Kema & Crous

INTRODUCTION

Indonesia is one of the main centres of origin for banana in South-East Asia (Valmayor 1999). Edible banana cultivars are descendants from two ancestral wild *Musa* species, *Musa acuminata* Colla (AA, 2n = 22) and *Musa balbisiana* Colla (BB, 2n = 22) (Simmonds 1962). These diversified into various edible varieties comprising diploids (AA, BB), triploids (AAA, AAB, ABB) and tetraploids (ABBB). Indonesia is the main contact area between species and subspecies of wild banana in sub-centres of diversity (Perrier *et al*. 2011) and, therefore, represents the primary gene centre for banana, resulting in a huge phenotypic and genotypic diversity. Indonesia is among the top 10 banana producing countries (FAOSTAT 2017) with over 200 varieties that are presently grown in almost every region of the Indonesian archipelago (Nasution 1993). The actual number of identified cultivated banana varieties could easily surpass 500. Banana is one of Indonesia's primary fruit commodities (BPS 2017), with most production supplying the domestic market.

Despite this great diversity and high popularity of bananas, there are some constraints on production. The most important of these is fungal diseases, including Fusarium wilt, also known as Panama disease (Stover 1962a). Fusarium wilt is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc), which first appeared in the 1900s in a banana plantation on Java (Stover 1962a) and thereafter disseminated to other banana production areas in Indonesia and beyond. This devastating agent of wilt on banana was first reported in the literature from samples collected in a Cuban banana plantation, and it subsequently gained notoriety as *Fusarium cubense* (Smith 1910).

The history of Fusarium wilt on banana goes back to the 20th century when this disease eliminated thousands of hectares of the favoured Gros Michel banana in Central America. The outbreak evolved into one of the worst epidemics of plant disease of all times. The discovery of resistant Cavendish bananas eventually quenched the epidemic and the variety was so successful that it was disseminated around the world until it attained its current predominance in the global banana trade. The resistance of Cavendish bananas to the so-called Race1 strains, which caused the epidemic in Gros Michel is unique and durable. The risk of global monocultures is evident and problems surfaced again once other pathogenic *Fusarium oxysporum* strains appeared that were able to cause Fusarium wilt in Cavendish plantations. A harmful strain was initially reported from Taiwan, from whence it spread further into South-East Asia, and recently to the Indian subcontinent, the Middle East and Africa (Ordóñez *et al*. 2015). The ongoing epidemic in Cavendish bananas is caused by a unique genotype, Vegetative Compatibility Group (VCG) 01213, of Foc and is called Tropical Race 4 (TR4). It has caused significant losses in commercial and subsistence production areas of Taiwan, Malaysia, and the northern territories of Australia (Su *et al*. 1986, Gerlach *et al*. 2000, Hermanto *et al*. 2009). In Indonesia, Nasir *et al.* (1999) reported that Fusarium wilt occurred from the Aceh province of Sumatra in the far west, to the far eastern Papua province.

Losses in export Cavendish plantations in southern Sumatra have exceeded 70 %. In Northern Sumatra over 1 000 ha of plantations were destroyed within 3 yr after the appearance of the disease in this area (Nasir *et al.* 1999). Not only was Cavendish affected, but also many local popular varieties named in Bahasa Indonesia with 'Pisang' (= 'banana') variety names, such as Pisang Raja Bulu, P. Raja Sereh, P. Ambon, P. Mas and P. Barangan, were damaged. The affected varieties are very important for the local markets (Hermanto *et al*. 2009).

To date, no control method has yet been identified or successfully implemented to effectively manage TR4. This is further complicated by the soil-borne nature of Foc and its ability to produce persistent chlamydospores that contaminate soils for decades (Booth 1971). Essentially, there are presently no control methods, except prevention by using pathogen-free tissue culture plants planted in non-infested soil (Ploetz 1994), and the adoption of quarantine strategies. However, these practices are mostly applied in large commercial plantations, but not in smallholder settings. Evidently, the development of new resistant banana cultivars would be the most effective control strategy to follow, and therefore research on the diversity of this pathogen is essential, particularly since it has been shown to be polyphyletic (O'Donnell *et al*. 1998). It is therefore essential to acquire a better understanding of the differences between the genetic lineages for developing control strategies, and for effective resistance breeding.

In *Fusarium* systematics, Foc belongs to the *Fusarium oxysporum* species complex (FOSC). Four clades of FOSC have been identified using translation elongation factor 1-alpha (*tef1)* and mitochondrial subunit rDNA (*mtssu*), with Foc isolates clustering as basal lineage (O'Donnell *et al*. 2004). The incorporation of Foc isolates from native host populations, especially those from indigenous ecosystems, will be of great importance for diversity studies of this complex species.

Diversity studies of Foc isolates were conducted by using various physiological and molecular methods, which included Vegetative Compatibility Groups (VCGs; Moore *et al*. 1993), random amplified polymorphic DNA markers (RAPDs; Bentley *et al*. 1995), restriction fragment length polymorphisms (RFLPs; Koenig *et al*. 1997), amplified fragment length polymorphism (AFLP; Groenewald *et al*. 2006) and DNA sequence analyses (O'Donnell *et al*. 1998). These studies showed that the South-East Asian population of this fungus exhibits a high degree of variation, suggesting that Foc lineages co-evolved with their hosts in South-East Asia (Ploetz & Pegg 1997). However, these studies used Foc isolates from various disconnected geographical areas and lacked evidence on genetic diversity from the gene centre of banana diversity, which is likely also the origin of the co-evolving Foc (Buddenhagen 2007). It has alternatively been suggested that Foc has multiple independent evolutionary origins, both within and outside the *Musa* genetic centre (Bentley *et al.* 1998). Using the phylogenetic genealogical approach, O'Donnell *et al.* (1998) identified five independent genetic lineages of Foc in a global population. Using a similar approach and additional data,

Fourie *et al.* (2009) found three additional lineages. However, neither of these studies included Indonesian populations, and hence only limited information is available on the diversity of Foc in the centre of origin of banana.

Here, we explore the genetic diversity among Indonesian Foc strains that were isolated from local banana varieties in various different ecosystems across the country. This overview of the complexity of Fusarium wilt of banana enables us to greatly improve our knowledge of the taxonomic and phylogenetic position of Foc in the FOSC.

MATERIALS AND METHODS

Isolates

A comprehensive survey of Fusarium wilt of banana was undertaken in Indonesia. In total, 34 locations in 15 provinces were visited, representing the main banana-producing regions in Java, Sumatra, Kalimantan, Sulawesi, Papua, and Nusa Tenggara (Table 1, Fig. 1). Sampling expeditions to the former three islands were undertaken in 2014, whereas the other islands were sampled in 2015. Sampling locations were identified in two to three different regions in each province. Diagnostic specimen was collected from diseased banana plants displaying typical Fusarium wilt symptoms: yellowing of older leaf margins, collapsed leaves at the petioles, and pseudostem discolouration and splitting. The pseudostems of the diseased plants were cut and discoloured vascular strands were sampled and placed on sterile filter paper to dry, and were eventually packed in a paper envelope. Global positioning coordinates were recorded and ecological parameters, including soil pH, light intensity and vegetation of the sampling area were collected at each site. For each banana plant sampled, the youngest (cigar) leaf was taken for ploidy identification of the germplasm by flow-cytometry analyses and morphological characterisation following Valmayor (1999) and Simmonds & Shepherd (1955), as well as *in-situ* comparisons with local banana varieties in the *Musa* collection at the Indonesian Institute of Sciences (LIPI) Cibinong, Bogor, Indonesia.

Isolation

The dried pseudostem samples were cut into pieces of 2 x 3 cm and plated on Komada medium (Komada 1975). After approximately 2 d, fungal colonies resembling *Fusarium* were transferred to potato dextrose agar (PDA) plates (Leslie & Summerell 2006). Axenic cultures were derived by streaking a small amount of conidia, collected with the tip of an inoculation needle, on water agar (WA) plates, which allowed conidia to separate. After 24 h of incubation, plates were observed under a dissection microscope at 50× magnification and single germinating conidia were collected and transferred to PDA. Monospore isolates were either maintained on PDA or in 20 % (v/v) glycerol at –80 °C. All isolates were deposited in the Indonesian Culture Collection (InaCC) Cibinong, Indonesia. Twenty-four Foc isolates,

representing the known VCG's (Ordóñez *et al*. 2015) in the global Foc collection were included for phylogenetic analyses.

Table 1. Names and geographical details of 34 sampling locations in Indonesia for building the Indonesian *Fusarium oxysporum* f. sp. *cubense* collection.

Province	District	GPS		
		Long.	Lat.	Alt. (m)
East Kalimantan	Kutai Timur	117.62	0.68	57
	Benajam	116.77	-1.62	21
Central Kalimantan	Kapuas Timur	114.48	-3.10	16
	Katingan	113.42	-1.71	35
	Palangkaraya	114.02	-2.43	18
South Kalimantan	Kota Baru	116.22	-2.58	118
	Tanah Bumbu	115.74	-3.63	13
	Banjar	115.03	-3.41	34
West Kalimantan	Kubu Raya	109.29	-0.06	8
	Pontianak	109.34	-0.04	17
West Java	Bogor	107.10	-6.68	657
	Cianjur	107.10	-7.02	875
	Sukabumi	106.79	-7.01	263
Central Java	Kendal	110.35	-7.20	794
	Semarang	110.59	-7.00	9
	Demak	110.74	-7.06	21
East Java	Lumajang	113.11	-8.08	637
	Bondowoso	113.94	-8.09	379
	Purwodadi	112.75	-7.82	491
	Jember	113.68	-8.24	39
Aceh	Jantho Aceh Besar	95.63	5.35	133
North Sumatra	Karo	98.25	3.00	NA
	Brastagi	98.51	3.19	NA
West Sumatra	Bukit Tinggi	100.38	-0.29	NA
	Padang	100.35	-0.94	NA
South Sumatra	Ogan Ilir	104.70	-3.29	27
	Palembang	104.75	-2.99	NA
Lampung	Way Jepara	105.54	-5.56	NA
Papua	Sentani Jayapura	140.83	-2.65	NA
South Sulawesi	Barru	119.62	-4.08	8
	Bone	120.02	-4.62	101
	Maros	119.63	-5.10	48
	Sidreng Rappang	119.69	-3.93	165
East Nusa Tenggara	Sikka Flores	122.37	-8.61	20

DNA isolation, amplification and analyses

Total genomic DNA was extracted from axenic isolates grown for 7 d on PDA, using the DNA Wizard Magnetic DNA Purification System for Food kit (Promega, USA) following the protocols provided by the manufacturer. Partial gene sequences were determined for the RNA polymerase largest subunit gene (*rpb1*) using primers RPB1-Fa & RPB1-G2R (O'Donnell *et al.* 2010), the RNA polymerase second largest subunit gene (*rpb2*) using primers RPB2-5f2 & RPB2-7cr (O'Donnell *et al*. 2010), and the translation elongation factor 1-alpha gene (*tef1*) using primers EF1 & EF2 (O'Donnell *et al*. 1998). Amplicons were sequenced in both directions

using the same primer pairs as were used for amplification to ensure integrity of the sequences.

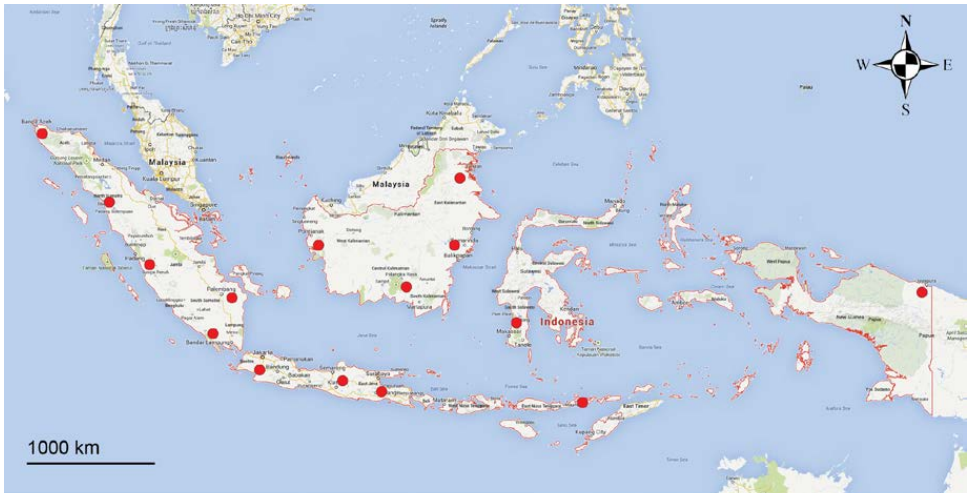

Fig. 1. Map of sampling collection in 2014–2015 on the island of Java, Sumatra, Kalimantan, Sulawesi, Papua, and Flores.

Consensus sequences were determined and assembled using MEGA v. 6 (Tamura *et al.* 2013) and compared to representative sequences from previous studies (O'Donnell *et al.* 1998, Fourie *et al.* 2009, Ordóñez *et al*. 2015). Subsequent alignments for each individual locus were generated using MAFFT v. 7.110 (Katoh & Standley 2013) and manually corrected if necessary. The individual sequences generated in this study were compared with those maintained in the *Fusarium* MLST database (http://www.westerdijkinstitute.nl/fusarium/) and the NCBI's GenBank, and relevant sequences were included in the subsequent phylogenetic inference. Phylogenetic congruencies of the three loci were tested using a 70 % reciprocal bootstrap criterion (Mason-Gamer & Kellogg 1996).

Phylogenetic inference in this study was based on Maximum Likelihood (ML) and Bayesian Inference (BI). The ML analysis was performed using RAxML v. 8. (randomised accelerated (sic) maximum likelihood for high performance computing) (Stamatakis 2014) through RAxML BlackBox (http://embnet.vital-it.ch/raxml-bb/index.php). Bootstrap support (BS) was determined automatically by the software to assess the robustness of the analyses. The BI analysis was performed using MrBayes v. 3.2 (Ronquist *et al*. 2012). A Markov Chain Monte Carlo (MCMC) algorithm of four chains was initiated in parallel from a random tree topology with a heating parameter set at 0.3. The MCMC analyses lasted until the average standard deviations of split frequencies were below 0.01 with phylogenies saved every 1 000 generations. The first 25 % of saved phylogenies were discarded as the "burn-in" phase and posterior probabilities (PP) were determined from the remaining phylogenies. All the

sequences generated in this study were deposited in the European Nucleotide Archive (ENA) and the alignments in TreeBASE.

Morphology

All Foc isolates were grown on carnation leaf agar (CLA; Fisher *et al*. 1982), synthetic low-nutrient agar (SNA; Nirenberg 1981) and PDA to induce sporulation under continuous light (Osram L18W/840 Cool White) for 7 d at 25 °C. Growth rates of all isolates were determined after 7 d incubation at 25 °C in the dark on PDA. Colony colours were determined using the mycological colour charts of Rayner (1970). Gross morphological characters, including microconidia, macroconidia, chlamydospores and conidiophores, were examined (50×) after mounting fungal structures in sterile water and observed using light microscopy at 1000× magnification. For each taxonomically informative structure, the extremes are provided, but for conidia we calculated the 95 % confidence intervals and provide extremes in parentheses. All descriptions, illustrations and nomenclatural data were deposited in MycoBank (Crous *et al*. 2004).

Pathogenicity assays

Isolates of Foc clustering in different clades based on the MLST analyses were selected for pathogenicity assays. The TR4 reference strain FocII5-NRRL5006 (Ordóñez *et al*. 2015) was included as a positive control, and negative controls were treated with water. For all assays, we followed the inoculum production, inoculation and diseases assessment protocols developed by Garcia-Bastidas *et al*. (2018, in prep.) using 2–3-mo-old Cavendish and Gros Michel plants. Prior and post-inoculation greenhouse conditions were adjusted to a constant day temperature of 25 °C (ambient light until max. 16 h), a night temperature of 23 °C, and a relative humidity of ≥ 75 %. After 7 wk, disease severities were evaluated by scoring external foliage and internal corm symptoms.

RESULTS

Isolates

Symptoms characteristic of Fusarium wilt were observed in most of the sampling locations on a diverse suite of banana varieties in typical backyards and in a Cavendish industrial plantation (Fig. 2). In total, 40 local banana varieties showed Fusarium wilt symptoms and were sampled (Table 2, Fig. 3). However, wild banana species, including *Musa acuminata* var. *bantamensis* in West Java, *M. acuminata* var. *rutilifes* in the forest of East Java, and *M. acuminata* var. *microcarpa* and *M. bornensis* in Kalimantan, and the *Musa*-related species, *Ensete glaucum* in Flores, were consistently free of external Fusarium wilt symptoms. In total, 203 isolates were obtained from the symptomatic banana plants (Table 3).

Fig. 2. Symptoms of Fusarium wilt on banana. **A.** External wilting symptom on leaves in a monoculture plantation in Lampung, Sumatra. **B.** External wilting symptom on leaves in a backyard home plantation in Cianjur, West Java. **C.** Splitting of the pseudostem. **D.** Internal symptoms, discoloration of the pseudostem. **E**. discoloration of the corm.

Phylogenetic analyses

Approximately 632 bp were determined for *tef1*, 864 bp for *rpb2* and 1444 bp for the *rpb1* gene regions. The congruency analyses revealed no conflicts in tree topologies, with only minor differences in branch support. Therefore, the sequences of the three loci were combined in a single dataset for subsequent analyses. For the BI and ML analyses, a GTR+I+G model was selected for all three gene regions and incorporated into the analyses. The ML tree topology confirmed the tree topologies obtained from the BI analyses, and therefore, only the ML tree is presented.

The combined *tef1, rpb1* and *rpb2* sequences dataset included 244 ingroup taxa and *F. dimerum* (NRRL 36140) as outgroup taxon. This dataset consisted of 2 909 characters, which yielded a single best ML tree with −lnL = -9286.260647 (Fig. 4). The BI lasted for 11 M generations, and the consensus tree, with posterior probabilities, was calculated from 8 251 trees left after 2 750 trees were discarded as the “burn-in” phase.

Table 2. List of 40 susceptible local banana varieties at six Indonesian islands from which samples were taken to isolate *Fusarium oxysporum* f. sp. *cubense* strains.

Islands	Banana varieties				Genome[1]
	Local name	Popular name	International name	Scientific name[1]	
Sumatra	Pisang Ayam	Pisang Barangan	Lakatan	*Musa acuminata*	AAA
	P. Wak	P. Awak	Awak	*Musa* sp.	ABB
	P. Abe	P. Kepok	Saba	*Musa* sp.	ABB
	P. Talon	P. Raja	Raja	*Musa* sp.	AAB
	P. Barangan	P. Barangan	Lakatan	*Musa acuminata*	AAA
	P. Tanduk Bawen	P. Tanduk	Horn	*Musa* sp.	AAB
	P. Mas	P. Mas	Sucrier	*Musa acuminata*	AA
Kalimantan	P. Sanggar/ Manurun/ Nipah	P. Kepok	Saba	*Musa* sp.	ABB
	P. Awak/ Pulau Pinang	P. Awak	Awak	*Musa* sp.	ABB
	P. Ambon	P. Ambon Hijau	Cavendish	*Musa acuminata*	AAA
	P. Susu	P. Raja Sereh	Silk	*Musa* sp.	AAB
	P. Hawa	P. Awak	Awak	*Musa* sp.	ABB
	P. Gelobok	P. Awak	Awak	*Musa* sp.	ABB
	P. Talas	P. Talas	NA	*Musa acuminata*	AA
	P. Selendang	NA	NA	*Musa acuminata*	AAA
	Dwarf Cavendish	P. Kapal	Dwarf Cavendish	*Musa acuminata*	AAA
	P. Raja	P. Raja Buluh	Raja	*Musa* sp.	AAB
	P. Kepok	P. Kepok	Saba	*Musa* sp.	ABB
Java	P. Mas Kirana	P. Mas Kirana	Sucrier	*Musa acuminata*	AA
	P. Embuk	NA	NA	*Musa* sp.	AAB
	P. Kongkong	NA	NA	*Musa acuminata*	AAA
	P. Susu	P. Raja Sereh	Silk	*Musa* sp.	AAB
	P. Glintung	NA	NA	-	NA
	P. Ambon	P. Ambon Kuning	Gros Michel	*Musa acuminata*	AAA
	P. Ambon Lumut	P. Ambon Hijau	Cavendish	*Musa acuminata*	AAA
	Cau Langadai	P. Siem	NA	*Musa* sp.	ABB
	Cau Apu	P. Siem	NA	*Musa* sp.	ABBB
	P. Jimbluk	P. Siem Jumbo	NA	*Musa* sp.	ABBB
	P. Uli	P. Uli	NA	*Musa acuminata*	AA
	P. Raja Nangka	P. Nangka	Laknau	*Musa acuminata*	AAA
	P. Cavendish	P. Ambon Hijau	Cavendish	*Musa acuminata*	AAA
	P. Kepok Pipik	P. Kepok Putih	NA	*Musa* sp.	ABB
	P. Raja	P. Raja Buluh	Raja	*Musa* sp.	AAB
Papua	P. Tanduk	P. Tanduk	Horn	*Musa* sp.	AAB
	P. Raja	P. Raja Buluh	Raja	*Musa* sp.	AAB
Sulawesi	P. Kepok	P. Kepok	Saba	*Musa* sp.	ABB
	P. Ambon	P. Ambon Hijau	Cavendish	*Musa acuminata*	AAA
	P. Cere	NA	NA	*Musa acuminata*	AAA
East Nusa Tenggara	P. Kepok	P. Kepok	Saba	*Musa* sp.	ABB
	P. Barangan	P. Barangan	Lakatan	*Musa acuminata*	AAA

[1] https://www.crop-diversity.org/mgis/taxonomy

Fig. 3. Local Indonesian banana varieties. **A.** Pisang Raja Bulu (AAB). **B.** Pisang Awak (ABB). **C.** Pisang Ambon Hijau (AAA). **D.** Pisang Udang (ABB). **E.** Left, Pisang Raja Manten (AAB), right, Pisang Barangan (AAA). **F.** Pisang Mas Lampung (AA). **G.** Pisang Tanduk (AAB). **H.** Pisang Susu (AAB). **I.** Pisang Kepok (ABB). **J.** Pisang Jarum (AA).

Phylogenetic inference of the three gene regions placed all isolates recovered from the symptomatic samples in the genus *Fusarium* (Fig. 4). Of these, 180 isolates clustered in the FOSC clade, one isolate clustered in the *Fusarium sambucinum* species complex (FSSC) closely related to *F. longipes*, 11 isolates clustered in the *Fusarium incarnatum-equiseti* species complex (FIESC), and eight isolates clustered in the *Fusarium fujikuroi* species complex (FFSC). The highest phylogenetic support was obtained using the *tef1* and *rpb1* gene regions. The *rpb2* gene region displayed less resolution of the isolates, between the various *Fusarium* species complexes and within each complex. The clades representing FIESC and FSSC resolved in this study were highly supported (BS = 100 %; PP = 1). The FFSC resolved FOSC and other members of the FFSC into two highly supported clades (BP = 100 %; PP = 1 & BP = 97 %; PP = 1, respectively).

In the FOSC, using the single gene analyses of *tef1*, and after incorporation of the dataset of O'Donnell *et al*. (2004) and Fourie *et al*. (2009), two clades were resolved as in the previous study (O'Donnell *et al*. 2004; Fig. 5). None of the Indonesian isolates resided in Clade 3. A single isolate, representing FocCNPMF.R1 (Dita *et al.* 2010), clustered in the FOSC Clade 4. The phylogeny, however, revealed one new clade in the FOSC (BP = 100 %, PP = 1.0), assigned to FOSC Clade 5, comprising five isolates that were isolated from Pisang Kepok (ABB, 2n = 33) and Pisang Ambon (AAA, 2n = 33) in Central and South Kalimantan.

Further analyses of the Foc phylogeny using the combined *tef1, rpb1* and *rpb2* dataset included 216 ingroup taxa and *F. fujikuroi* (CBS 221.76.) as an outgroup taxon (Fig. 5). The majority of Indonesian isolates clustered in Clade 1, including eight previously established Foc lineages (Fig. 5; O'Donnell *et al*. 1998, Fourie *et al*. 2009), and the overall phylogeny revealed nine independent clonal lineages (Fig. 6). The Indonesian Foc isolates were equally distributed across the nine lineages except for L9 that did not include any Indonesian isolate. We did not identify significant correlation between the origin of the isolates and host genotypes.

Taxonomy

Based on phylogenetic inference and morphological observations, several novel *Fusarium* taxa could be identified in this study, and these are described below.

Foc Lineage L1

Fusarium odoratissimum N. Maryani, L. Lombard, Kema & Crous, **sp. nov.** MycoBank MB826800. Figs 7, 8.

Etymology: Name refers to the strong odour associated with older cultures.

Macroconidia abundant on CLA, less abundant on PDA and SNA, formed on sporodochia on CLA and on aerial conidiophores on SNA and PDA, falcate, (44–)59–75(–79) × 6–8 µm (av. 67 × 7 µm), 0–6-septate, with apical cells papillate, basal cells foot-shaped. *Conidiogenous cells* mono- or polyphialidic on sporodochia, or formed directly on hyphae (lateral phialides), 12–28 × 4–8 µm. *Microconidia* abundant on PDA and SNA, less frequent on CLA, oval to ellipsoid, (6–)8–16(–23) × (4–)6(–8) µm (av. 12 × 5 µm), 0–3-septate, arranged in false heads on branched conidiophores carried on hyphae. Aerial conidiophores rare on CLA and SNA but formed abundantly on PDA, branched sparsely or formed laterally. *Chlamydospores* globose to subglobose, formed intercalarily or terminally, single or in pairs, (7–)9–13(–14) × (7–)8–11(–12) µm, rarely produced on SNA after 7 d, rough-walled.

Table 3. Details of strains included in the phylogenetic analyses.

Species name	Accession number[1]	Identification[2]	f. sp	Country	Host	GenBank/ENA accession[3]		
						rpb1	*rpb2*	*tef1*
Fusarium cugenangense	[9]InaCC F983	7	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479559	LS479307	LS479756
	InaCC F984	7	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479560	LS479308	LS479757
	NRLL 36118	7	*cubense*	Thailand	*Musa* sp. var. Pisang Kepok	LS479477	LS479221	LS479669
	NRRL 25433	7	*vasinvectum*		*Gosypium* sp.	LS479462	LS479202	LS479648
F. dimerum	NRRL 36140				*Citrus* sp.	HM347203	HM347218	HM347133
F. duoseptatum	[4,5]FocMal43	5	*cubense*	Malaysia	*Musa* sp. var. Pisang Rastali	-	LS479207	LS479653
	InaCC F828	5	*cubense*	Indonesia	*Musa* sp. var. Pisang Rastali	LS479520	LS479266	LS479715
	InaCC F829	5	*cubense*	Indonesia	*Musa* sp. var. Pisang Rastali	LS479528	LS479274	LS479723
	InaCC F831	5	*cubense*	Indonesia	*Musa* sp. var. Pisang Rastali	LS479538	LS479285	LS479734
	InaCC F835	5	*cubense*	Indonesia	*M. acuminata* var. Dwarf Cavendish	LS479567	LS479315	LS479764
	InaCC F911	5	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	-	LS479234	LS479683
	InaCC F915	5	*cubense*	Indonesia	*Musa* sp. Pisang Raja	LS479494	LS479238	LS479687
	[8]InaCC F916	5	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479495	LS479239	LS479688
	InaCC F920	5	*cubense*	Indonesia	*Musa* sp. var. Pisang Hawa	LS479499	LS479244	LS479693
	InaCC F921	5	*cubense*	Indonesia	*Musa* sp. var. Pisang Hawa	LS479500	LS479245	LS479694
	InaCC F975	5	*cubense*	Indonesia	*Musa* sp. var. Pisang Awak	LS479549	LS479296	LS479745
	InaCC F976	5	*cubense*	Indonesia	*Musa* sp. var. Pisang Awak	LS479550	LS479297	LS479746
	InaCC F977	5	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479551	LS479298	LS479747
	InaCC F978	5	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479552	LS479299	LS479748
	[8]InaCC F979	5	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479553	LS479300	LS479749
	InaCC F980	5	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479554	LS479301	LS479750
	Indo80	5	*cubense*	Indonesia	*Musa* sp. var. Pisang Hawa	LS479619	LS479387	LS479829
	NRRL 36115	5	*cubense*	Malaysia	*M. acuminata* var. Pisang ambon	LS479475	LS479218	LS479666
	NRRL 36116	5	*cubense*	Malaysia	*Musa* sp. var. Pisang Keling	-	LS479219	LS479667
F. grosmichelii	[8]InaCC F820	4	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	-	LS479364	LS479810

Table 3. (Continued).

	InaCC F832	4	*cubense*	Indonesia	*Musa* sp. var. Pisang Awak	LS479542	LS479289	LS479738
	[8]InaCC F833	4	*cubense*	Indonesia	*Musa* sp. var. Pisang Awak	LS479548	LS479295	LS479744
	[8]InaCC F848	4	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479588	LS479338	LS479786
	InaCC F849	4	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479589	LS479339	LS479787
	InaCC F850	4	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	-	LS479340	LS479788
	[8]InaCC F851	4	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	-	LS479341	LS479789
	[8]InaCC F852	4	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon Lumut	-	LS479342	LS479790
	InaCC F853	4	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon Lumut	-	LS479343	LS479791
	InaCC F854	4	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon Lumut	LS479591	LS479345	LS479793
	InaCC F855	4	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon Lumut	LS479592	LS479346	LS479794
	InaCC F859	4	*cubense*	Indonesia	*M. acuminata* var. Cavendish	LS479596	LS479350	LS479796
	InaCC F861	4	*cubense*	Indonesia	*M. acuminata* var. Cavendish	LS479597	LS479351	LS479797
	InaCC F862	4	*cubense*	Indonesia	*M. acuminata* var. Cavendish	LS479598	LS479352	LS479798
	InaCC F863	4	*cubense*	Indonesia	*Musa* sp. var. Pisang Siem Jumbo	LS479599	LS479353	LS479799
	InaCC F867	4	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon Kuning	-	LS479360	LS479806
	InaCC F868	4	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon Kuning	-	LS479361	LS479807
	InaCC F884	4	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479616	LS479382	LS479824
	InaCC F887	4	*cubense*	Indonesia	*Musa* sp. var. Pisang Siem Jumbo	LS479620	LS479388	LS479830
	InaCC F888	InaCC F889	*cubense*	Indonesia	*Musa* sp. var. Pisang Siem Jumbo	LS479621	LS479389	LS479831
F. hexaseptatum	[8]InaCC F866	8	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon Kuning	-	LS479359	LS479805
F. incarnatum-equiseti	NRRL 45997	FIESC			*Poaceae*	-	GQ505850	GQ505672
F. kalimantanense	[9]InaCC F917	FOSC Clade 5 Nov.	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479497	LS479241	LS479690
	InaCC F918	FOSC Clade 5 Nov.	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	-	LS479242	LS479691
	InaCC F922	FOSC Clade 5 Nov.	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	-	LS479246	LS479695
F. longipes	NRRL 20695	FSSC				-	GQ915493	GQ915509

Table 3. (Continued).

F. mangiferae	UMA F0924	FFSC			*Mangifera indica*	KP753435	KP753442	KP753402
F. odoratissimum	[7]FocII5=NRRL 54006	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Manurung	LS479459	LS479198	LS479644
	InaCC F816	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479485	LS479228	LS479677
	[7]InaCC F817	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479556	LS479304	LS479753
	InaCC F818	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479584	LS479333	LS479782
	InaCC F819	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479600	LS479354	LS479800
	InaCC F821	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Raja	LS479609	LS479374	LS479818
	[7]InaCC F822	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Raja	LS479618	LS479386	LS479828
	[7]InaCC F824	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479486	LS479229	LS479678
	InaCC F825	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Raja	LS479496	LS479240	LS479689
	[7]InaCC F836	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Mas Kirana	LS479577	LS479325	LS479774
	InaCC F837	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Mas Kirana	LS479578	LS479326	LS479775
	InaCC F838	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Mas Kirana	LS479579	LS479327	LS479776
	InaCC F839	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Mas Kirana	LS479580	LS479328	LS479777
	InaCC F840	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Embuk	-	LS479329	LS479778
	InaCC F841	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Embuk	LS479581	LS479330	LS479779
	[7]InaCC F846	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	-	LS479336	LS479785
	InaCC F847	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479587	LS479337	-
	[7]InaCC F856	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Siem	LS479593	LS479347	-
	InaCC F857	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Siem	LS479594	LS479348	LS479795
	InaCC F858	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Siem	LS479595	LS479349	-
	InaCC F864	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Siem	-	LS479356	LS479802
	InaCC F865	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Siem	-	LS479358	LS479804
	InaCC F870	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479602	LS479363	LS479809
	InaCC F871	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	-	LS479365	LS479811
	InaCC F873	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479604	LS479369	LS479814

Table 3. (Continued).

InaCC F874	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479606	LS479371	-
InaCC F875	1	*cubense*	Indonesia	*M. acuminata* var. Cavendish	LS479607	LS479372	LS479816
InaCC F876	1	*cubense*	Indonesia	*M. acuminata* var. Cavendish	LS479608	LS479373	LS479817
InaCC F877	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479610	LS479375	LS479819
InaCC F878	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479611	LS479376	-
InaCC F879	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479612	LS479377	LS479820
InaCC F880	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	-	LS479378	LS479821
InaCC F881	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479613	LS479379	-
InaCC F882	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479614	LS479380	LS479822
InaCC F883	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479615	LS479381	LS479823
InaCC F885	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Raja	-	LS479384	LS479826
InaCC F890	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479623	LS479392	-
[7]InaCC F891	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Glitung	-	LS479393	LS479833
InaCC F892	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479624	LS479394	LS479834
InaCC F893	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479625	LS479395	LS479835
InaCC F894	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479626	LS479396	LS479836
InaCC F896	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Wak	LS479629	LS479399	LS479839
InaCC F897	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479630	LS479400	LS479840
InaCC F898	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479631	LS479401	LS479841
[7]InaCC F899	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479632	LS479402	LS479842
InaCC F900	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479633	LS479403	LS479843
InaCC F901	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479634	LS479404	LS479844
InaCC F902	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Talon	LS479635	LS479405	LS479845
InaCC F903	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479636	LS479406	LS479846
InaCC F904	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479637	LS479407	LS479847
InaCC F905	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479638	LS479408	LS479848
InaCC F906	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479639	LS479409	LS479849
InaCC F907	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Tanduk	LS479487	LS479230	LS479679
[7]InaCC F908	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Tanduk	LS479488	LS479231	LS479680

Table 3. (Continued).

[7]InaCC F909	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Mas	LS479489	LS479232	LS479681
InaCC F910	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Mas	LS479490	LS479233	LS479682
InaCC F912	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479491	LS479235	LS479684
InaCC F919	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Awak	LS479498	LS479243	LS479692
InaCC F923	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Raja	LS479501	LS479247	LS479696
InaCC F924	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Raja	LS479502	LS479248	LS479697
InaCC F925	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Raja	LS479503	LS479249	LS479698
InaCC F926	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Raja	LS479504	LS479250	LS479699
[7]InaCC F927	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Raja	LS479506	LS479252	LS479701
InaCC F928	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Raja	LS479507	LS479253	LS479702
InaCC F929	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Tanduk	LS479508	LS479254	LS479703
InaCC F930	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Tanduk	LS479509	LS479255	LS479704
[7]InaCC F931	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Tanduk	LS479510	LS479256	LS479705
InaCC F932	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Tanduk	LS479511	LS479257	LS479706
InaCC F933	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479512	LS479258	LS479707
InaCC F934	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479514	LS479260	LS479709
InaCC F935	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479515	LS479261	LS479710
[7]InaCC F936	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479516	LS479262	LS479711
InaCC F937	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479517	LS479263	LS479712
InaCC F938	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479518	LS479264	LS479713
InaCC F939	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479519	LS479265	LS479714
InaCC F942	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479521	LS479267	LS479716
InaCC F943	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479522	LS479268	LS479717
InaCC F944	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479523	LS479269	LS479718
InaCC F945	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479524	LS479270	LS479719
InaCC F946	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479525	LS479271	LS479720
InaCC F947	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479526	LS479272	LS479721
InaCC F948	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479527	LS479273	LS479722
InaCC F953	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479529	LS479275	LS479724

Table 3. (Continued).

	InaCC F954	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479530	LS479276	LS479725
	InaCC F955	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479531	LS479277	LS479726
	InaCC F973	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479547	LS479294	LS479743
	InaCC F985	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479562	LS479310	LS479759
	InaCC F986	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479563	LS479311	LS479760
	[7]InaCC F988	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479565	LS479313	LS479762
	InaCC F989	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479566	LS479314	LS479763
	InaCC F990	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok Pipik	LS479568	LS479316	LS479765
	InaCC F994	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Mas Kirana	LS479569	LS479317	LS479766
	[7]InaCC F997	1	*cubense*	Indonesia	*M. acuminata* var. Cavendish	LS479572	LS479320	LS479769
	[7]InaCC F998	1	*cubense*	Indonesia	*M. acuminata* var. Cavendish	LS479573	LS479321	LS479770
	InaCC F999	1	*cubense*	Indonesia	*M. acuminata* var. Cavendish	LS479574	LS479322	LS479771
	InaCC F1000	1	*cubense*	Indonesia	*M. acuminata* var. Cavendish	LS479575	LS479323	LS479772
	Indo4	1	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479590	LS479344	LS479792
	Indo51	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Siem	LS479601	LS479355	LS479801
	Indo53	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Siem	-	LS479357	LS479803
	Indo61	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	-	LS479366	LS479812
	Indo62	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	-	LS479367	-
	Indo66	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479605	LS479370	LS479815
	Indo77	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok Pipik	LS479617	LS479383	LS479825
	Indo89	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Wak	LS479627	LS479397	LS479837
	Indo204	1	*cubense*	Indonesia	*Musa* sp. var. Pisang Uli	LS479561	LS479309	LS479758
	Indo222	1	*cubense*	Indonesia	*M. acuminata* var. Cavendish	LS479576	LS479324	LS479773
	[4]JV11	1	*cubense*	Jordan	*M. acuminata* var. Cavendish	LS479465	LS479205	LS479651
	[4]Leb1.2C	1	*cubense*	Lebanon	*M. acuminata* var. Cavendish	LS479466	LS479206	LS479652
	NRRL 36102	1	*cubense*	China	*M. acuminata* var. Cavendish	LS479468	LS479209	LS479655
	[4]Pak1.1A	1	*cubense*	Pakistan	*M. acuminata* var. Cavendish	LS479479	LS479223	LS479671
	[4]Phi2.6C	1	*cubense*	Philippines	*M. acuminata* var. GCTCV218	LS479480	LS479224	LS479672

Table 3. (Continued).

F. oxysporum	CAV794	FOSC Clade 1	*cubense*	Indonesia	*Musa* sp. var. Pisang Rubus	-	-	FJ664922
	CAV300	FOSC Clade 1	*cubense*	Indonesia	*M. acuminata* var. Valery	-	-	FJ664932
	CAV1107	FOSC Clade 1	*cubense*	Vietnam	*Musa* sp. var. Cuoi Xiem	-	-	FJ664950
	CAV299	FOSC Clade 1	*cubense*	Nigeria	*M. acuminata* var. Gros Michel	-	-	FJ664946
	CAV602	FOSC Clade 2	*cubense*	Australia	*M. acuminata* var. Lady Finger	-	-	FJ664957
	CAV189	FOSC Clade 2	*cubense*	Malawi	*Musa* sp. var. Harare	-	-	FJ664956
	CAV194	FOSC Clade 2	*cubense*	Indonesia	*Musa* sp. var.Pisang Siem	-	-	FJ664955
	[4,6,8]FocCNPMF-R1	FOSC Clade 4	*cubense*	Brazil	*Musa* sp. var. Silk	LS479457	LS479196	LS479642
	NRRL 34936	FOSC Clade 3	*lycopersici*		*Solanum lycopersicum*	LS479460	LS479200	LS479646
	NRRL 26406	FOSC Clade 3	*melonis*		*Cucumis melo*	LS479461	LS479201	LS479647
	NRRL 54002	FOSC Clade 3			Soil	LS479455	LS479194	LS479640
	NRRL 26381	FOSC Clade 3	*lycopersici*		*S. lycopersicum*	LS479456	LS479195	LS479641
	NRRL 25603	FOSC Clade 1	*cubense*		*M. acuminata*	-	-	AF008487
	NRRL 22550	FOSC Clade 1	*pernicosum*		*Albizia julibrissin*	-	-	AF008506
	NRRL 25357	FOSC Clade 1			Soil	-	-	AF008481
	NRRL 26035	FOSC Clade 1	*canariensis*		*Phoenix canariensis*	-	-	AF008485
	NRRL 20433	FOSC Clade 2	*inflexum*		*Viciba faba*	-	-	AF008479
	NRRL 25607	FOSC Clade 2	*cubense*		*M. acuminata* X *M. balbisiana*	-	-	AF008489
	NRRL 25609	FOSC Clade 2	*cubense*		*M. acuminata* X *M. balbisiana*	-	-	AF008490
	NRRL 26022	FOSC Clade 2	*cubense*		*M. acuminata* X *M. balbisiana*	-	-	AF008491
	NRRL 25598	FOSC Clade 2	*glycines*		*Glycine sp.*	-	-	AF008496
	NRRL 26178	FOSC Clade 2	*melonis*		*Cucumis melo*	-	-	AF008503
	NRRL 25420	FOSC Clade 2	*vasinvectum*		*Gossypium hirsutum*	-	-	AF008512
	NRRL 25369	FOSC Clade 2			*Terminalia ivorensis*	-	-	AF008482
	NRRL 26406	FOSC Clade 3	*melonis*		*C. melo*	-	-	AF008504
	NRRL 26379	FOSC Clade 3	*radicis-lycopersici*		*S. esculentum*	-	-	AF008508
	NRRL 22549	FOSC Clade 3	*passiflorae*		*Passiflora edulis*	-	-	AF008505
	NRRL 26033	FOSC Clade 3	*radicis-lycopersici*		*S. esculentum*	-	-	AF008507

Table 3. (Continued).

	NRRL 26574	FOSC Clade 3	*erythroxily*		*Erythroxylum coca*	-	-	AF008495
	NRRL 26383	FOSC Clade 3	*lycopersici*		*S. esculentum*	-	-	AF008502
	NRRL 26380	FOSC Clade 3	*lycopersici*		*S. esculentum*	-	-	AF008509
	NRRL 26029	FOSC Clade 3	*cubense*		*M. acuminata* X *M. balbisiana*	-	-	AF008493
	NRRL 22555	FOSC Clade 3	*tuberosi*		*S. tuberosum*	-	-	AF008511
	NRRL 26203	FOSC Clade 3	*lycopersici*		*S. esculentum*	-	-	AF008501
	NRRL 26374	FOSC Clade 3			*Homo sapiens*	-	-	AF008483
	NRRL 25594	FOSC Clade 4	*batatas*		*Ipomoea batatas*	-	-	AY337717
	NRRL 26360	FOSC Clade 4				-	-	AY527522
F. phialophorum	[4,5]FocIndo25	3	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon	LS479464	LS479204	LS479650
	[4,5]FocST4.98	3	*cubense*	Spain	*M. acuminata* var. Dwarf Cavendish	LS479484	LS479227	LS479676
	InaCC F826	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Wak	LS479505	LS479251	LS479700
	InaCC F827	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Wak	LS479513	LS479259	LS479708
	InaCC F830	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479536	LS479282	LS479731
	InaCC F834	3	*cubense*	Indonesia	*M. acuminata* var. Pisang Selendang	LS479557	LS479305	LS479754
	InaCC F842	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Embuk	LS479582	LS479331	LS479780
	InaCC F843	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Embuk	LS479583	LS479332	LS479781
	[8]InaCC F844	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479585	LS479334	LS479783
	InaCC F845	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479586	LS479335	LS479784
	InaCC F869	3	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon Kuning	-	LS479362	LS479808
	InaCC F889	3	*cubense*	Indonesia	*M. acuminata* var. Pisang Ambon Kuning	LS479622	LS479391	LS479832
	InaCC F969	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Wak	LS479543	LS479290	LS479739
	InaCC F970	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Wak	LS479544	LS479291	LS479740
	[8]InaCC F971	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Wak	LS479545	LS479292	LS479741
	InaCC F972	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Wak	LS479546	LS479293	LS479742
	InaCC F980	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479555	LS479302	LS479751
	InaCC F981	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	-	LS479303	LS479752

Table 3. (Continued).

	InaCC F982	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479558	LS479306	LS479755
	InaCC F987	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479564	LS479312	LS479761
	InaCC F995	3	*cubense*	Indonesia	*M. acuminata* var. Pisang Kongkong	LS479570	LS479318	LS479767
	[8]InaCC F996	3	*cubense*	Indonesia	*M. acuminata* var. Pisang Kongkong	LS479571	LS479319	LS479768
	Indo64	3	*cubense*	Indonesia	*Musa* sp. var. Pisang Susu	LS479603	LS479368	LS479813
	NRRL 36101	3	*cubense*	Australia	*Musa* sp. var. Mons Mari	LS479467	LS479208	LS479654
	NRRL 36103	3	*cubense*	Philippines	*M. acuminata* var. Cavendish	LS479469	LS479210	LS479656
	NRRL 36109	3	*cubense*	Australia	*Musa* sp. var. SH 3142	LS479471	LS479214	LS479661
	NRRL 36110	3	*cubense*	Australia	*Musa* sp. var.Mons	-	-	LS479662
	NRRL 36112	3	*cubense*	South Africa	*M. acuminata* var. Cavendish	LS479473	LS479216	LS479664
	[4,6]FocRace1.0124	3	*cubense*	Cuba		LS479483	-	LS479675
F. proliferatum	NRRL 62905	FFSC				KU171687	KU171707	KU171727
F. purpurascens	ATCC76244	2	*cubense*	USA	*M. acuminata* var. Apple	-	LS479199	LS479645
	InaCC F823	2	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479628	LS479398	LS479838
	[8]InaCC F886	2	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	-	LS479385	LS479827
	InaCC F913	2	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479492	LS479236	LS479685
	InaCC F914	2	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479493	LS479237	LS479686
	[8]InaCC F966	2	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479539	LS479286	LS479735
	InaCC F967	2	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479540	LS479287	LS479736
	InaCC F968	2	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479541	LS479288	LS479737
	NRRL 36107	2	*cubense*	Honduras	*Musa* sp. var. Maqueno	-	LS479213	LS479659
F. sacchari	NRRL 13999	FFSC				-	-	AF160278
F. sangayamense	[9]InaCC F960	FOSC Clade 5 Nov.	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	LS479537	LS479283	LS479732
	InaCC F961	FOSC Clade 5 Nov.	*cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	-	LS479284	LS479733
F. tardichlamydosporum	[4,6]FocCNPMF-R2	6	*cubense*	Brazil	*Musa* sp. var. Monthan	LS479458	LS479197	LS479643
	InaCC F956	6	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479532	LS479278	LS479727
	InaCC F957	6	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479533	LS479279	LS479728
	[8]InaCC F958	6	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479534	LS479280	LS479729

Table 3. (Continued).

	InaCC F959	6	*cubense*	Indonesia	*M. acuminata* var. Pisang Barangan	LS479535	LS479281	LS479730
	NRRL 36105	6	*cubense*	Honduras	*Musa* sp. var. Bluggoe	LS479470	LS479211	LS479657
	NRRL 36106	6	*cubense*	Australia	*M. acuminata* var. Lady finger	-	LS479212	LS479658
	NRRL 36108	6	*cubense*	Tanzania	*Musa* sp. var. Ney Poovan	-	-	LS479660
	NRRL 36111	6	*cubense*	Australia	*Musa* sp. var. Bluggoe	LS479472	LS479215	LS479663
	NRRL 36117	6	*cubense*	Malaysia	*Musa* sp. var. Pisang awak legor	LS479476	LS479220	LS479668
F. tardicrescens	NRRL 36113	9	*cubense*	Malawi	*Musa* sp. var. Harare	LS479474	LS479217	LS479665
	NRRL 37622	9	*pisi*		*Cicer* sp.	LS479463	LS479203	LS479649
	NRRL 54005	9	*raphani*		*Raphanus* sp.	LS479482	LS479226	LS479674
	NRRL 54008	9	*conglutinans*		*Raphanus* sp.	LS479481	LS479225	LS479673
F. verticilloides	NRRL 20956	FFSC			*Zea mays*	-	-	FN552074
Fusarium sp.	InaCC F872	FFSC		Indonesia	*Musa* sp. var Pisang Raja Nangka	-	LS479850	LS479441
	InaCC F940	FIESC		Indonesia	*M. acuminata* var. Pisang Cere	-	LS479855	LS479443
	InaCC F941	FIESC		Indonesia	*M. acuminata* var. Pisang Cere	-	LS479856	LS479444
	[9]InaCC F950	FFSC		Indonesia	*Musa* sp. var. Pisang Kepok	LS479870	LS479852	-
	InaCC F951	FFSC		Indonesia	*Musa* sp. var. Pisang Kepok	LS479871	LS479853	-
	InaCC F952	FFSC		Indonesia	*Musa* sp. var. Pisang Kepok	LS479872	LS479854	-
	InaCC F962	FFSC		Indonesia	*M. acuminata* var. Pisang Talas	-	LS479868	LS479453
	InaCC F963	FIESC		Indonesia	*Musa* sp. var. Pisang Awak	LS479875	LS479859	LS479445
	InaCC F964	FIESC		Indonesia	*Musa* sp. var. Pisang Awak	LS479876	LS479860	LS479446
	InaCC F965	FIESC		Indonesia	*M. acuminata* var. Pisang Talas	LS479877	LS479863	LS479448
	[9]InaCC F974	FSSC		Indonesia	*Musa* sp. var. Pisang Awak	LS479880	LS479866	LS479451
	InaCC F991	FFSC		Indonesia	*Musa* sp. var. Pisang Kepok	LS479881	LS479867	LS479452
	[9]InaCC F992	FFSC		Indonesia	*M. acuminata* var. Pisang Mas Kirana	LS479882	LS479869	LS479454
	InaCC F993	FFSC		Indonesia	*M. acuminata* var. Pisang Mas Kirana	-	LS479851	LS479442
	Indo161	FIESC		Indonesia	*M. acuminata* var. Pisang Talas	LS479873	LS479857	-
	Indo167	FIESC		Indonesia	*Musa* sp. var. Pisang Kepok	LS479874	LS479858	-
	Indo 174	FIESC		Indonesia	*Musa* sp. var. Pisang Awak	-	LS479861	-

Table 3. (Continued).

	Indo175	FIESC	Indonesia	*M. acuminata* var. Pisang Talas	-	LS479862	LS479447
	Indo186	FIESC	Indonesia	*Musa* sp. var. Pisang Kepok	LS479878	LS479864	LS479449
	Indo188	FIESC	Indonesia	*Musa* sp. var. Pisang Awak	LS479879	LS479865	LS479450

[1]InaCC: Indonesian Culture Collection, Research Center for Biology, Indonesian Institute of Sciences (LIPI) Cibinong, Indonesia; Indo: Collection of N. Maryani, Wageningen Plant Research, Wageningen University, The Netherlands; NRRL: Agricultural Research Service Culture Collection, USA; ATCC: American Type Culture Collection, U.S.A.; CAV: Forestry Agricultural Biotechnology Institutre (FABI), University of Pretoria South Africa; CBS: The Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; NRRL: Agricultural Research Service Culture Collection, USA; UMAF: Microbiology and Plant Pathology Laboratory Collection, University of Malaga, Spain.

[2] Foc lineage/ FOSC clade/ *Fusarium* species complex.

[3]*rbp1*: RNA polymerase II largest subunit; *rpb2*: RNA polymerase II second largest subunit; *tef1*: translation elongation factor-1a.

[4]Collection of Wageningen Plant Research, Wageningen University, The Netherlands.

[5] Ecosciences Precinct, Brisbane Australia.

[6]Embrapa Cassava & Tropical Fruits, Brazil.

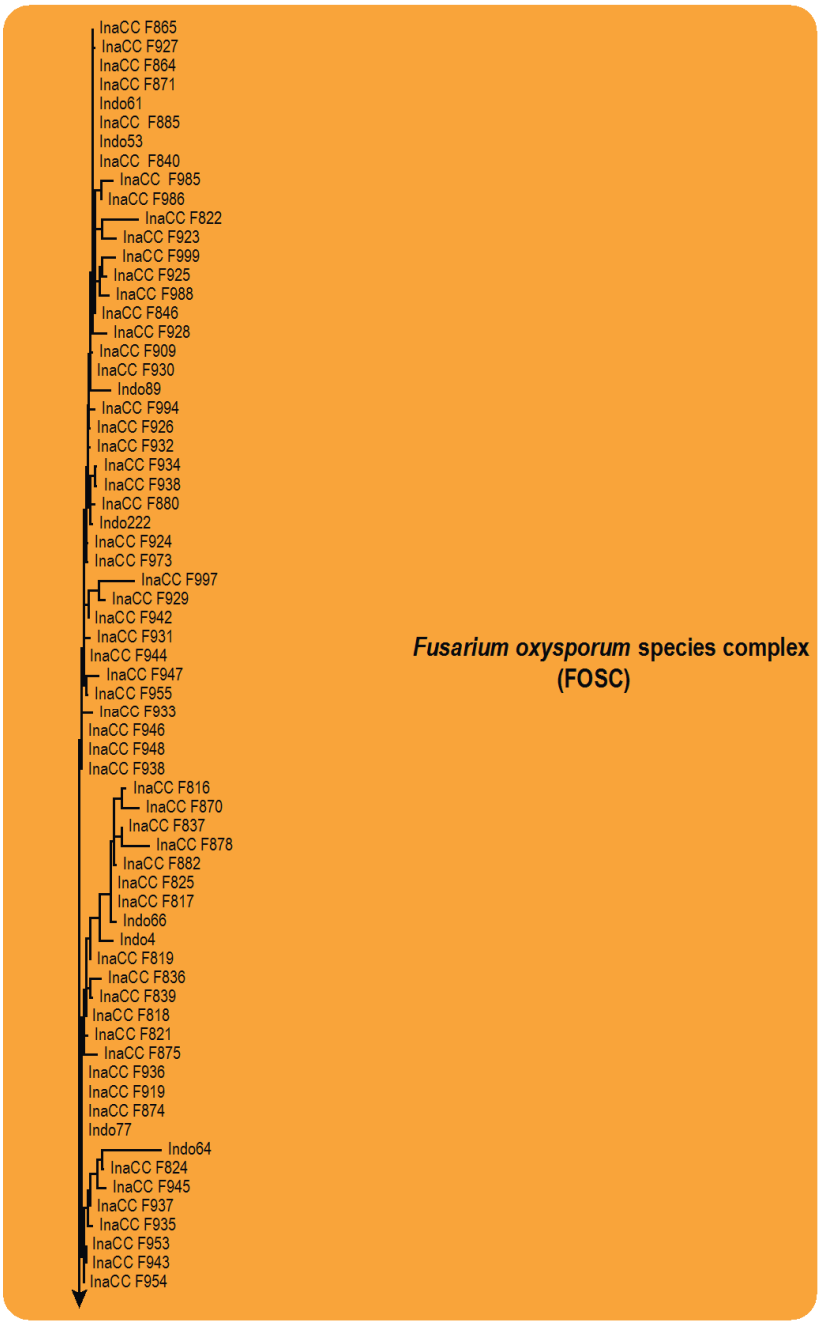

Fig. 4. Maximum likelihood tree inferred from the combined *rpb1, rpb2* and *tef1* genes sequence data sets of 244 isolates. The bootstrap support values (BP) and Bayesian posterior probabilities (PP) are given at nodes. Coloured blocks indicate the various *Fusarium* species complexes included. The tree is rooted to *Fusarium dimerum* (NRRL 36140).

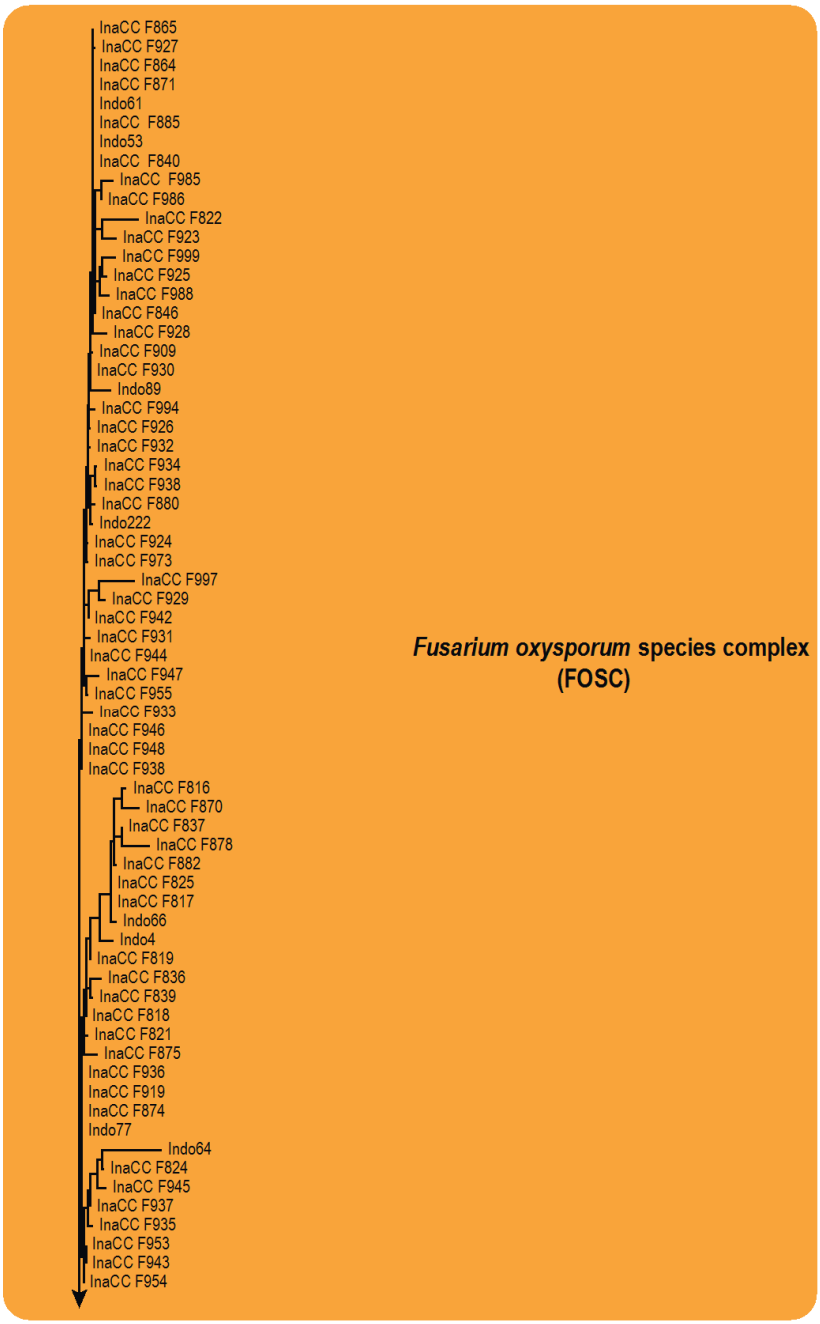

Fig. 4. (Continued).

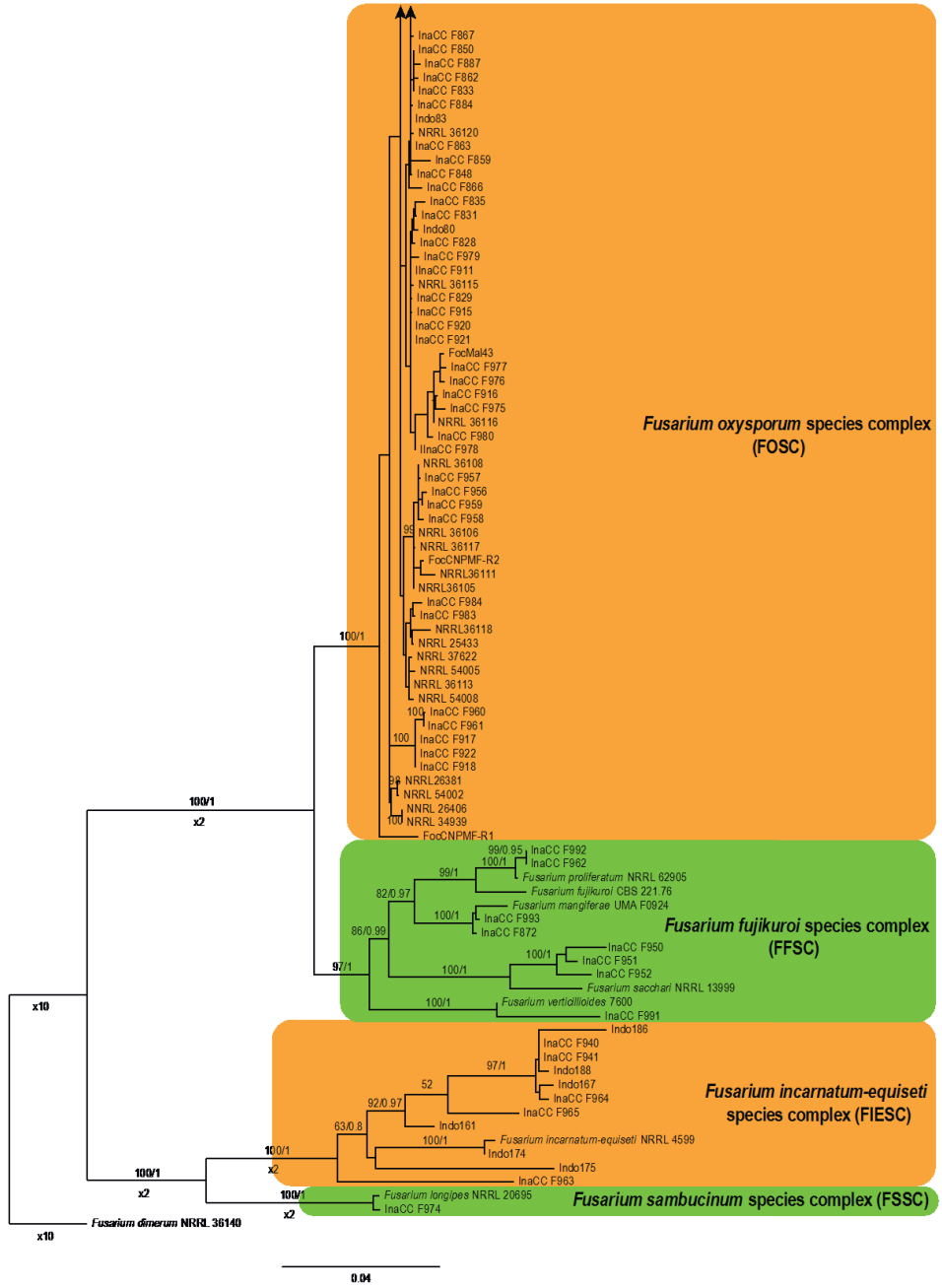

Fig. 4. (Continued).

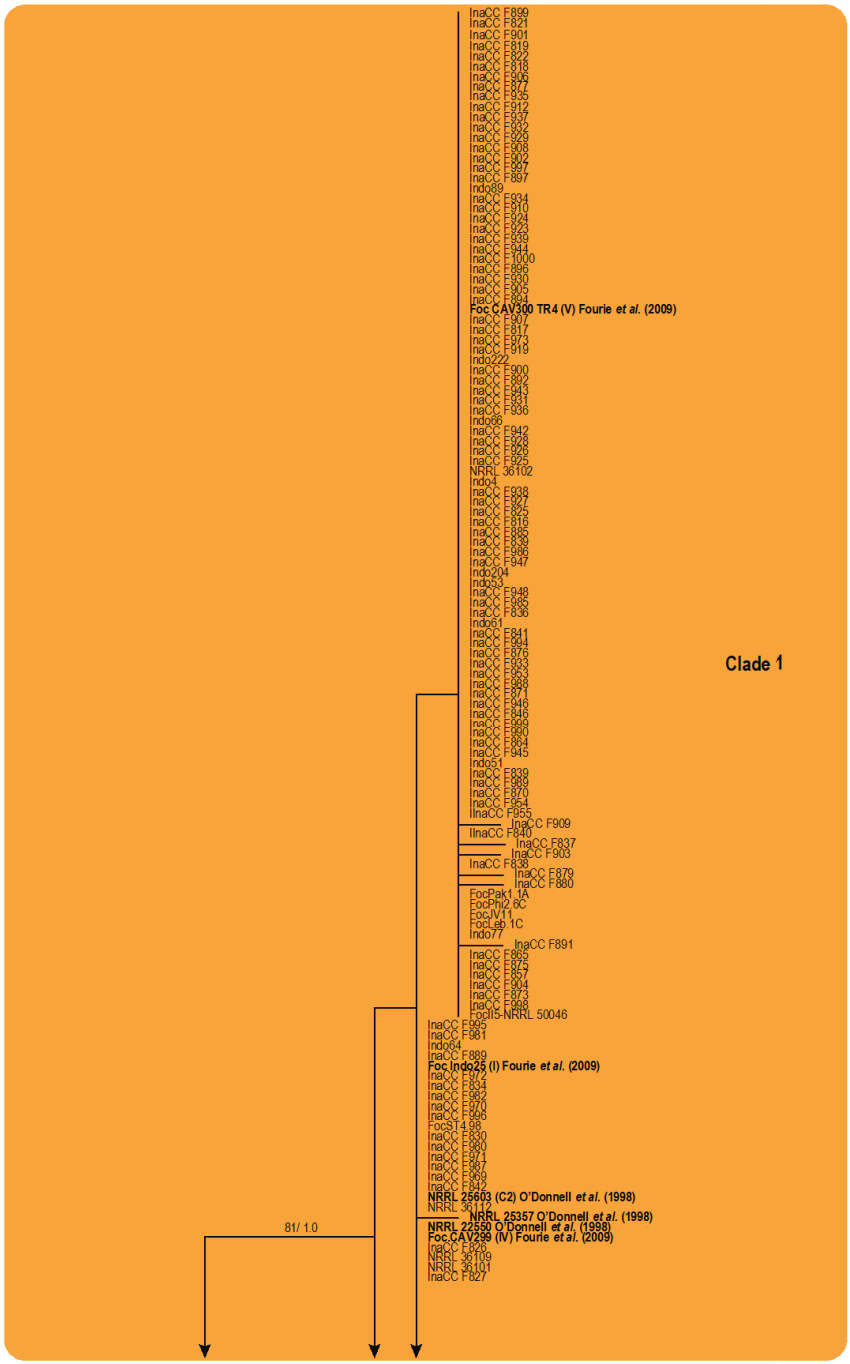

Fig. 5. Maximum likelihood tree inferred from the *tef1* gene sequence data set of 183 Indonesian isolates in the FOSC clade. Included are representatives of the studies by O'Donnell *et al.* (1998, 2004) and Fourie *et al.* (2009), indicated in **bold**. The bootstrap support values >70 % (BS) and Bayesian posterior probabilities >0.95 (PP) are given at nodes. The tree is rooted to *Fusarium fujikuroi* (CBS 221.76).

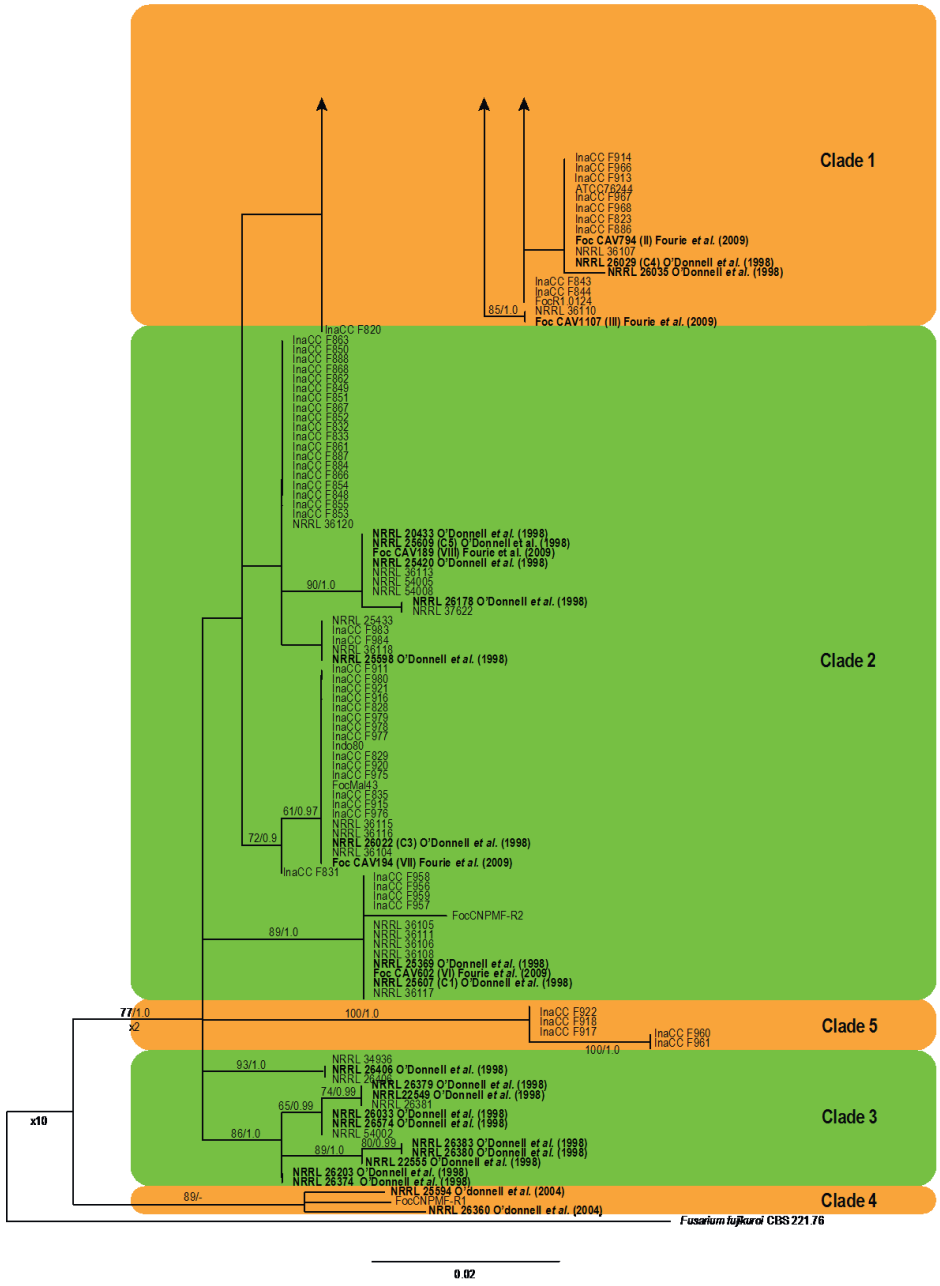

Fig. 5. (Continued).

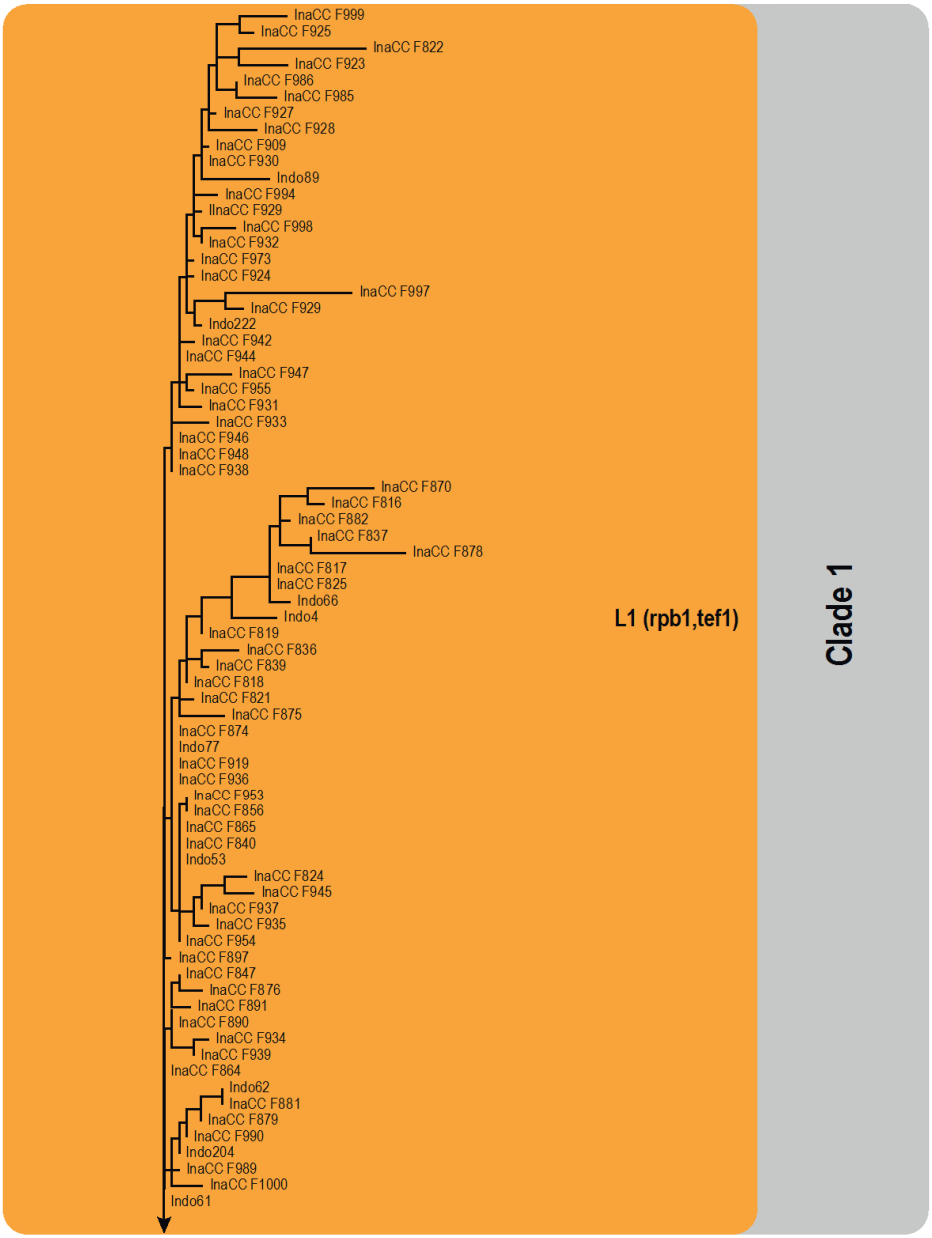

Fig. 6. Maximum likelihood tree inferred from the combined *rpb1*, *rpb2* and *tef1* genes sequence data sets. The bootstrap support values >70 % (BS) and Bayesian posterior probabilities >0.95 (PP) are given at nodes. Foc lineages are numbered based on the consensus from single and combined gene data sets represented by the coloured blocks. The tree is rooted to *Fusarium fujikuroi* (CBS 221.76).

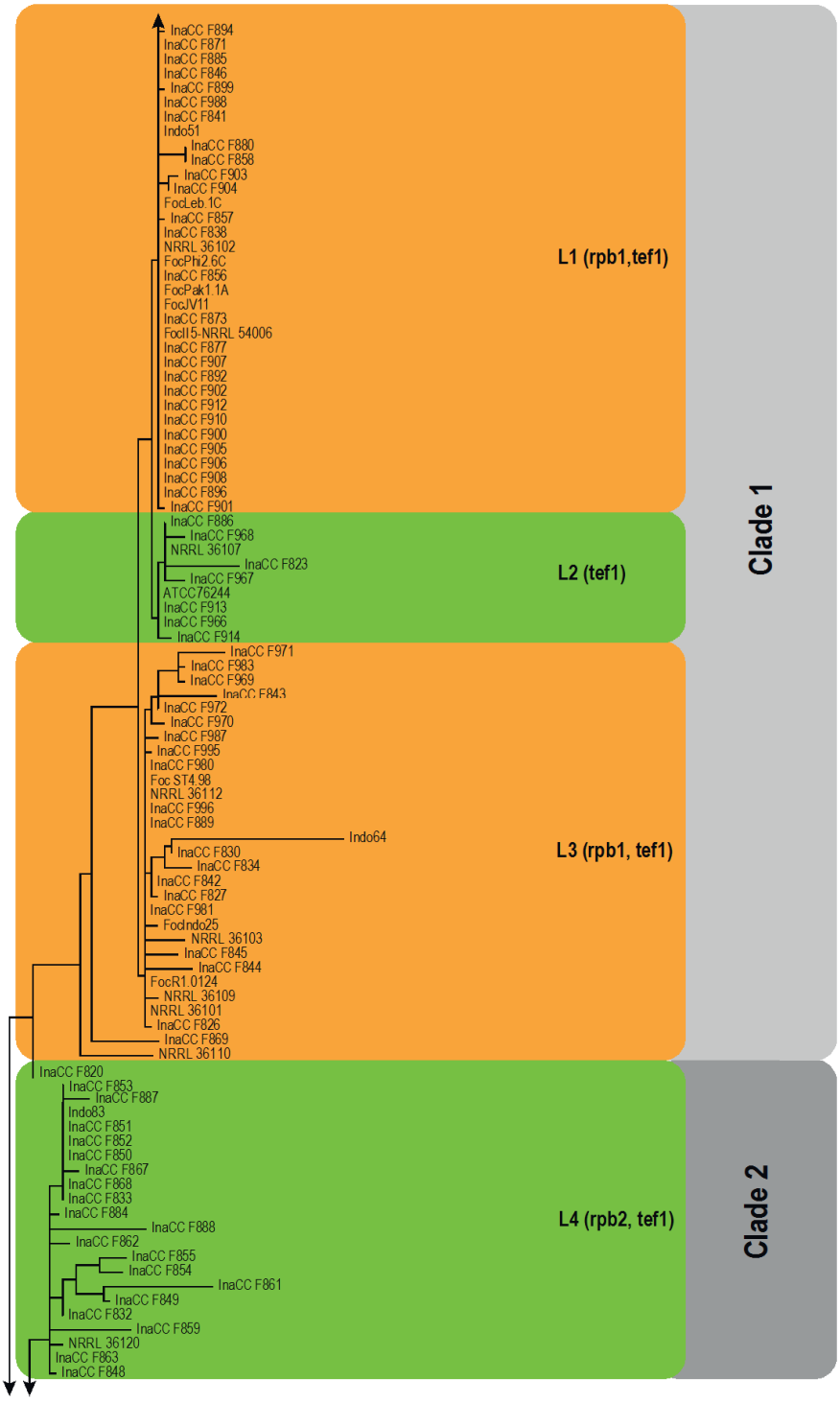

Fig. 6. (Continued).

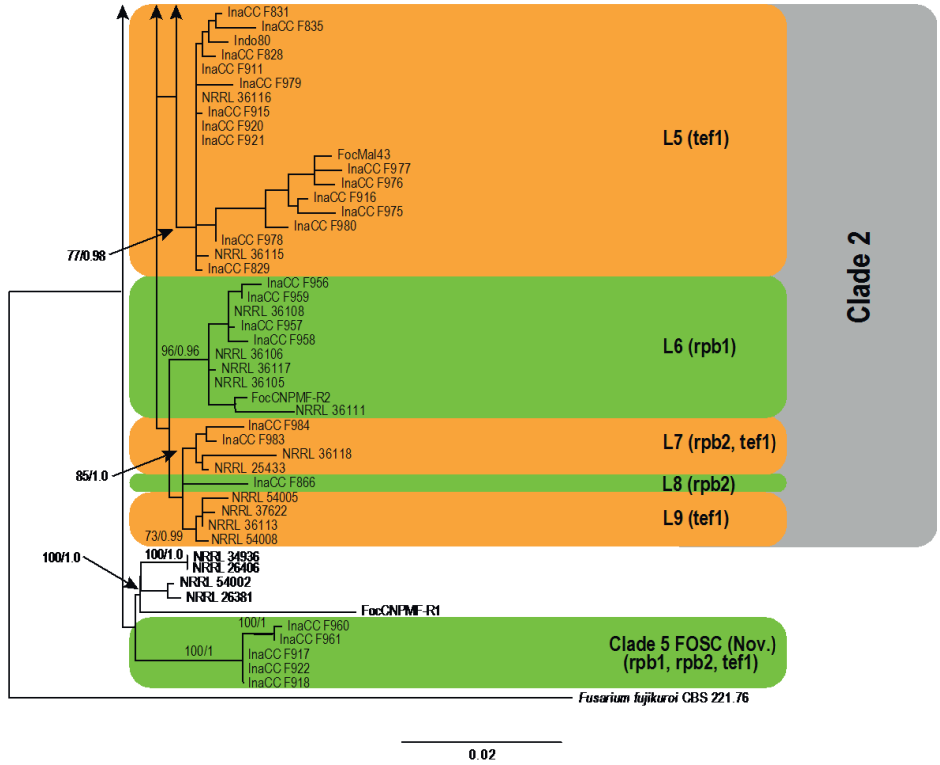

Fig. 6. (Continued).

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 4.5–5.0 mm/d. Colony reverse, uniformly white and unpigmented. Colony surface dry, cottony, white, with filamentous margin. No exudates observed. Aerial mycelium abundant, cottony, with abundant sporulation. Sporodochia formed abundantly on CLA after 7 d, pale orange.

Geography and host: Kutai Timur, East Kalimantan, *Musa* sp. var. Pisang Kepok (ABB).

Pathogenicity: Pathogen on Gros Michel (AAA) and Cavendish (AAA).

Material examined: **Indonesia**, Kampung Salak Martadinata, Kutai Timur, East Kalimantan (117°26'850"E and 0°11'590"N), on infected pseudostem of *Musa* sp. var. Pisang Kepok (ABB), 16 Jun. 2014, N. Maryani, (**holotype** preserved as metabolically inactive culture, InaCC F822).

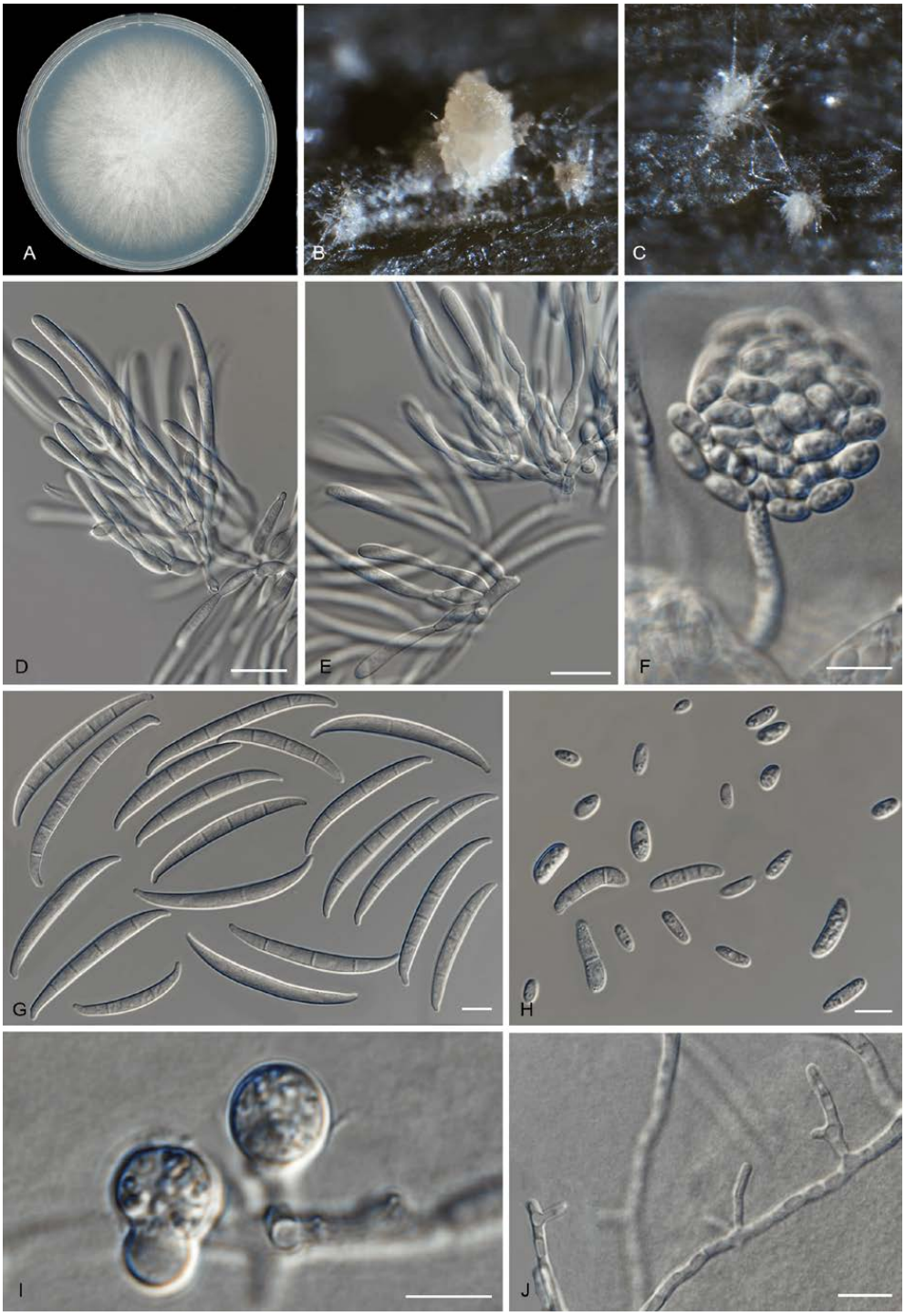

Fig. 7. *Fusarium odoratissimum* (InaCC F817). **A.** Culture grown on PDA. **B–C.** Sporodochia on carnation leaves. **D–E.** Sporodochial branched conidiophores with monophialides. **F.** False head. **G.** Falcate-shaped macroconidia. **H.** Microconidia. **I.** Chlamydospores. **J.** Polyphialides. Scale bars D–J= 10μm.

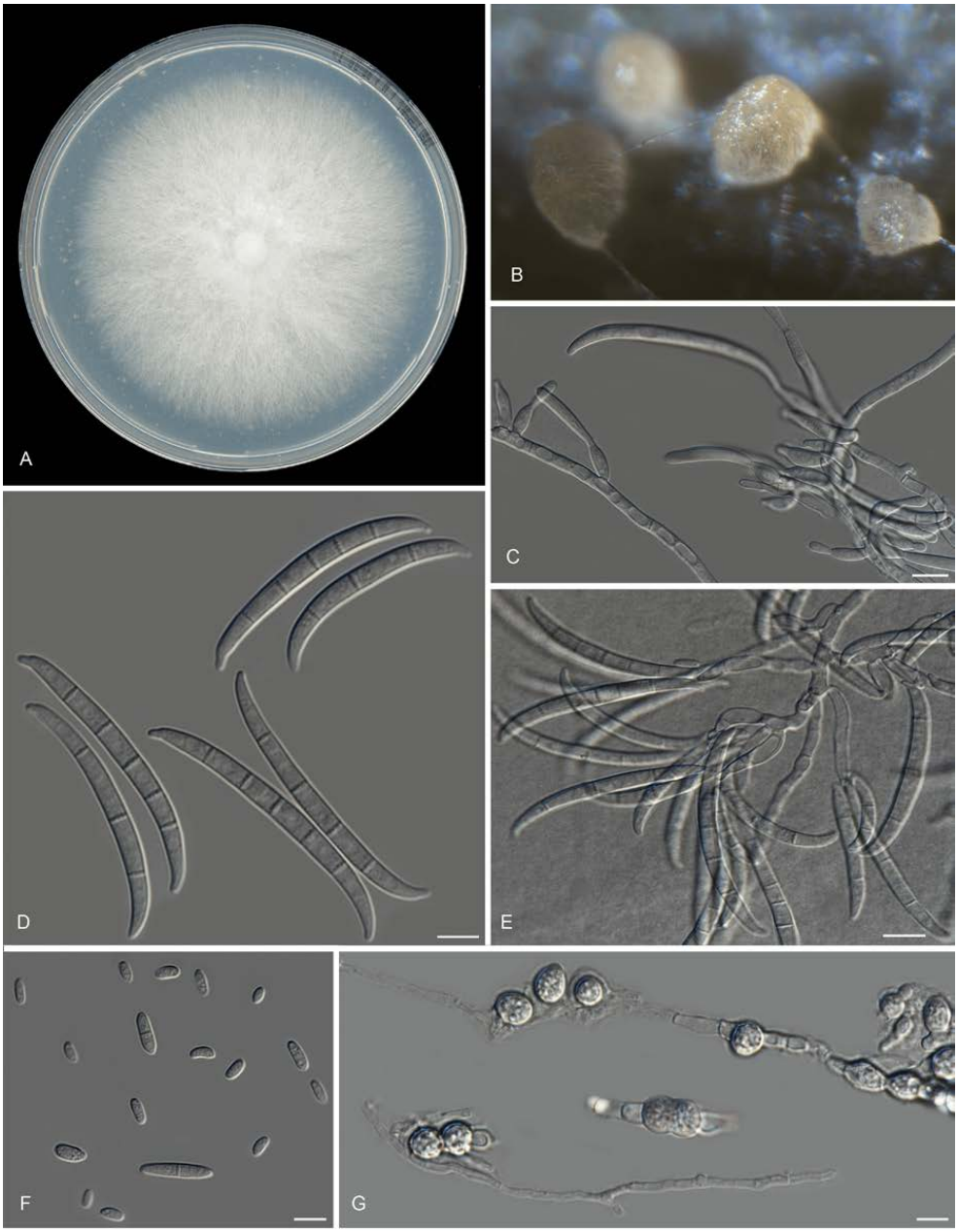

Fig. 8. *Fusarium odoratissimum* typed species (ex-type InaCC 822). **A.** Culture grown on PDA. **B.** Sporodochia on carnation leaves. **C.** Monophialides with initial conidia being formed. **D.** Falcate-shaped macroconida. **E.** Branched conidophores. **F.** Eliptical microconidia. **G.** Thick-walled chlamydospores. Scale bars C–G= 10μm.

Notes: Fusarium odoratissimum formed a small cryptic clade within the L1 cluster (Fig. 6), and can be distinguished by the septation of its macroconidia (0–6-septate) and microconidia (0–3-septate), characteristics not common for *F. oxysporum* (Leslie & Summerell 2006). This

species also produces chlamydospores relatively more rapidly than was observed for other *Fusarium* isolates examined in this study. *F. odoratissimum* and all isolates in L1 produce a strong peculiarly stale odour in mature cultures, of which the causal volatiles remain to be characterised. Pathogenicity tests showed that *F. odoratissimum* and all isolates in lineage 1 were able to infect Cavendish and Gros Michel bananas. Isolates in this lineage were thus classified as TR4.

Foc Lineage L2

Fusarium purpurascens N. Maryani, L. Lombard, Kema & Crous, **sp. nov.** MycoBank MB 826801. Fig. 9

Etymology: Name reflects the purple pigmentation which was observed when cultivated on potato dextrose agar.

Macroconidia abundant on CLA, less abundant on PDA and SNA, formed on sporodochia on CLA and on aerial conidiophores on SNA and PDA, falcate (50–)55–63(–67) × (4–)6–7(–9) µm (av. 59 × 7 µm), 3–5-septate, with apical cells papillate, basal cells foot-shaped. *Conidiogenous cells* mono- or polyphialidic on sporodochia, or formed directly from hyphae (lateral phialides), 5–45 × 3–8 µm. *Microconidia* abundant on PDA and SNA, less frequent on CLA, ovoid to ellipsoid, (8–)18(–37) × (3–)5(–6) µm (av. 12 × 4 µm), 0–1-septate, arranged in false heads on branched conidiophores carried on hyphae. Aerial conidiophores rare on CLA and SNA, and formed abundantly on PDA, branched sparsely or formed laterally. *Chlamydospores* not observed.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 4.4–4.8 mm/d. Colony reverse, livid purple. Colony surface dry, cottony, white, filamentous in the centre and livid purple towards the margin, forming exudate droplets. Aerial mycelium abundant, cottony, with moderate sporulation. Sporodochia formed abundantly on CLA after 7 d, pale orange.

Geography and host: Kutai Timur, East Kalimantan, *Musa* sp. var. Pisang Kepok (ABB).

Pathogenicity: Pathogen on Gros Michel (AAA).

Material examined: **Indonesia**, Kampung Salak Martadinata, Kutai Timur, East Kalimantan (117°26’684”E, 0°26’684”N), on infected pseudostem of *Musa* sp. var. Pisang Kepok (ABB), 17 Jun. 2014, N. Maryani, (**holotype** preserved as metabolically inactive culture, InaCC F886).

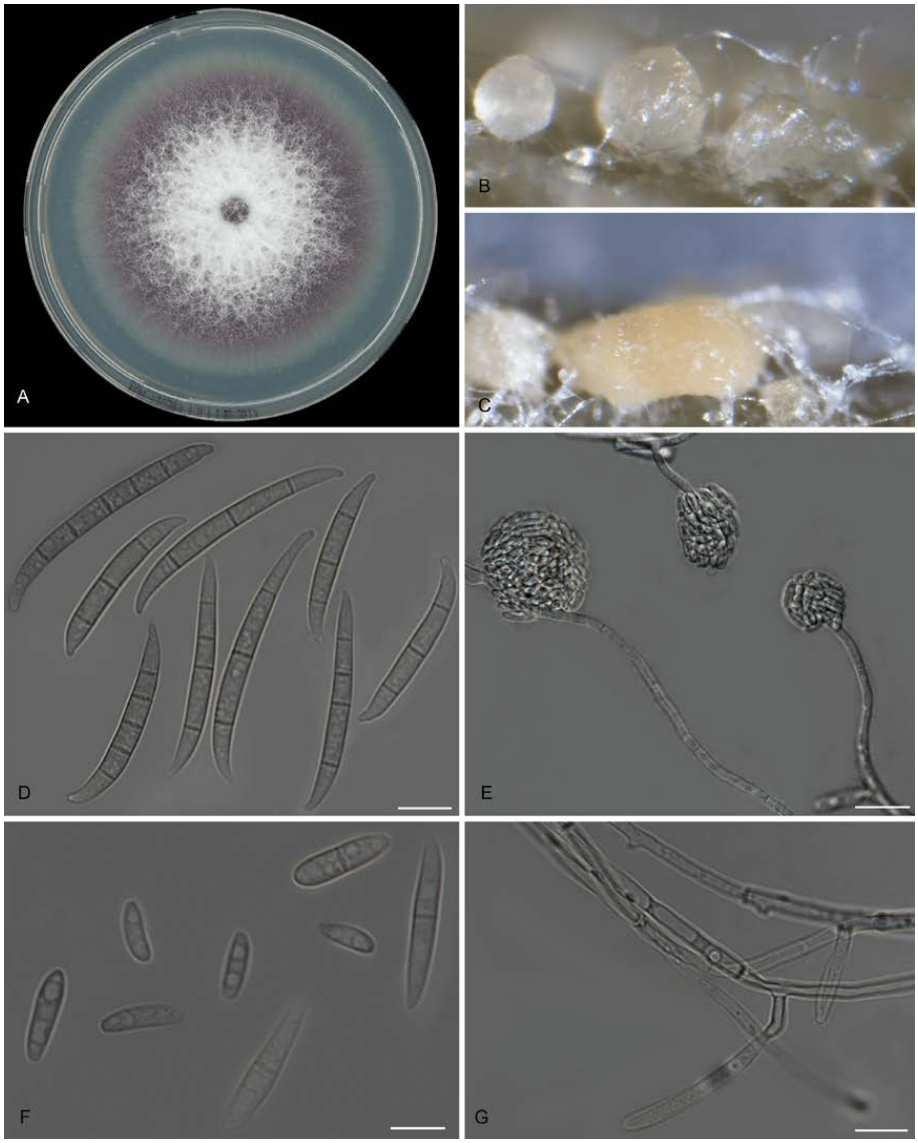

Fig. 9. *Fusarium purpurascens* (ex-type InaCC F886). **A.** Culture grown on PDA. **B–C.** Sporodochia grown on carnation leaves. **D.** Falcate-shaped macroconidia. **E.** False head. **F.** Microconidia. **G.** Monophialides. Scale bars D–G= 10µm.

Notes: *Fusarium pupurascens* exhibits the strongest purple colony colour on PDA of all the isolates with purple colonies. It is relatively slow-growing compared to other isolates clustered in lineage L1. No chlamydospores were observed for this species, in contrast to other L1 members, which readily produce chlamydospores in culture. Furthermore, *F. purpurascens* produces exudate droplets, something not observed among other L1 isolates. Older cultures

become pigmented, a distinctive phenomenon rarely seen in L1. *F. purpurascens* and other isolates in this lineage were able to infect Gros Michel, and were therefore classified as Race1.

Foc Lineage L3

Fusarium phialophorum N. Maryani, L. Lombard, Kema & Crous, **sp. nov.** MycoBank MB826802. Fig. 10.

Etymology: Name refers to its elongated phialidic collarettes observed in culture.

Macroconidia abundant on CLA, less abundant on PDA and SNA, formed on sporodochia on CLA and on aerial conidiophores on SNA and PDA, falcate, (50–)54–60(–62) × (3–)4–5(–7) µm (av. 57 × 7 µm), 2–5-septate, with apical cells papillate, basal cells foot-shaped. *Conidiogenous cells* monophialidic on sporodochia, or formed directly from hyphae (lateral phialides) with elongated collarettes, 7–41 × 3–7 µm. *Microconidia* abundant on PDA, less frequent on CLA, ovoid to ellipsoid, (6–)7–16(–24) × (3–)4(–6) µm (av. 12 × 5 µm), 0–1-septate, arranged in false heads on branched or lateral conidiophores carried on hyphae. Aerial conidiophores rare on CLA and SNA and formed abundantly on PDA, branched sparsely or forming short lateral conidiophores. *Chlamydospores* globose to subglobose, formed terminally, single or in pairs, (8–)9–12(–13) × (9–)10(–11) µm, rarely produced on SNA after 7 d, rough-walled.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 4.9–5.2 mm/d. Colony reverse, uniformly white and unpigmented. Colony surface dry, cottony, white, filamentous margin. No exudates observed. Aerial mycelium abundant, cottony, with high sporulation. Sporodochia formed abundantly on CLA after 7 d, pale orange to orange.

Geography and host: Tanah Bumbu, South Kalimantan, *Musa* sp. var. Pisang Awak (ABB).

Pathogenicity: Pathogen on Gros Michel (AAA).

Materials examined: **Indonesia,** Kampung Betung, Tanah Bumbu, South Kalimantan (115°37’477”E, 3°37’45”S), on infected pseudostem of *Musa* sp. var. Pisang Awak (ABB), 20 Jun. 2014, N. Maryani, (**holotype** preserved as metabolically inactive culture, InaCC F971).

Notes: *Fusarium phialophorum* has elongated phialidic collarettes, which are rarely found in other lineages. Polyphialidic conidiophores were not found, and chlamydospores were formed, but were rare. Isolates in this lineage were able to infect Gros Michel but not Cavendish, and were therefore classified as Race1.

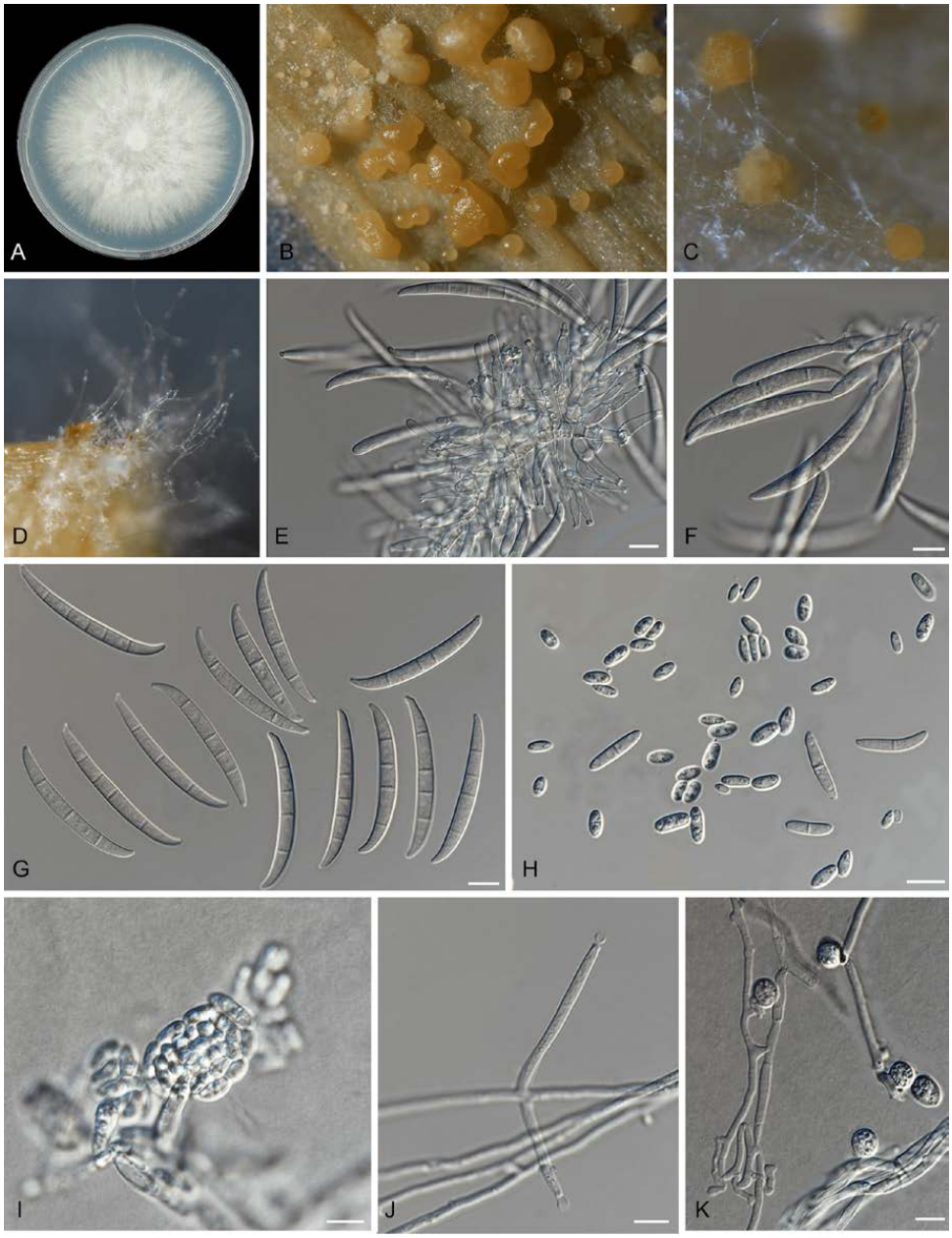

Fig. 10. *Fusarium phialophorum* (ex-type InaCC 971) . **A.** Culture grown on PDA. **B–C.** Sporodochia on carnation leaves. **D.** Aerial conidiophore on carnation leaves. **E–F.** Sporodochial phialides. **G.** Falcate-shaped macroconidia. **H.** Microconidia. **I.** False head. **J.** Lateral monophialides with long collaretes. **K.** Thick-walled chlamydospores. Scale bars E–K= 10 μm.

Foc Lineage L4

Fusarium grosmichelii N. Maryani, L. Lombard**,** Kema & Crous, **sp. nov.** MycoBank MB 826803. Fig. 11.

Etymology: Name reflects its association with the banana variety Gros Michel.

Macroconidia abundant on CLA, less abundant on PDA and SNA, formed on sporodochia on CLA and on aerial conidiophores on SNA and PDA, falcate, (47–)51–59(–64) × (5–)6–8(–9) µm (av. 55 × 7 µm), 3–5-septate, with apical cells papillate, basal cells foot-shaped. *Conidiogenous cells* mono- or polyphialidic on sporodochia, on branched conidiophores, or formed directly from hyphae (lateral phialides), (8–)16–28(–36) × (3–)4–6(–7) µm. *Microconidia* abundant on PDA and SNA, less frequent on CLA, ovoid to ellipsoid, (4–)9–17(–21) × (3–)4–6(–7) µm (av. 12 × 5 µm), 0–1-septate, arranged in false heads on branched or lateral conidiophores carried on hyphae. *Chlamydospores* globose to subglobose, formed terminally or intercalarily, single or in clumps, rarely produced on SNA after 7 d, rough-walled.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 4.7–5.0 mm/d. Colony reverse in the dark uniformly white and unpigmented. Colony surface dry, cottony white with filamentous margin. No exudates observed. Aerial mycelium abundant, cottony, with abundant sporulation. Sporodochia formed abundantly on CLA after 7 d, pale orange to orange.

Geography and host: Bogor, West Java, *Musa acuminata* var. Pisang Ambon Lumut (AAA).

Pathogenicity: Pathogen on Gros Michel (AAA).

Materials examined: **Indonesia**, Suakarya Megamendung, Bogor, West Java (106°54’214”E, 6°41’185”N), on infected pseudostem *Musa acuminata* var. Pisang Ambon Lumut (AAA), 10 Jul. 2014, N. Maryani, (**holotype** preserved as metabolically inactive culture InaCC F833); *ibid*.*,* Indo18.

Notes: *Fusarium grosmichelii* is morphologically very similar to *F. phialophorum*, but differs in having a higher number of septa in its macroconidia (3–5-septate). *F. grosmichelii* and others in this lineage are morphologically similar to *F. odoratissimum*, but *F. grosmichelii* was not able to infect Cavendish. Most of the isolates in L4 were tested on Gros Michel, and were able to cause disease, and were thus classified as Race1.

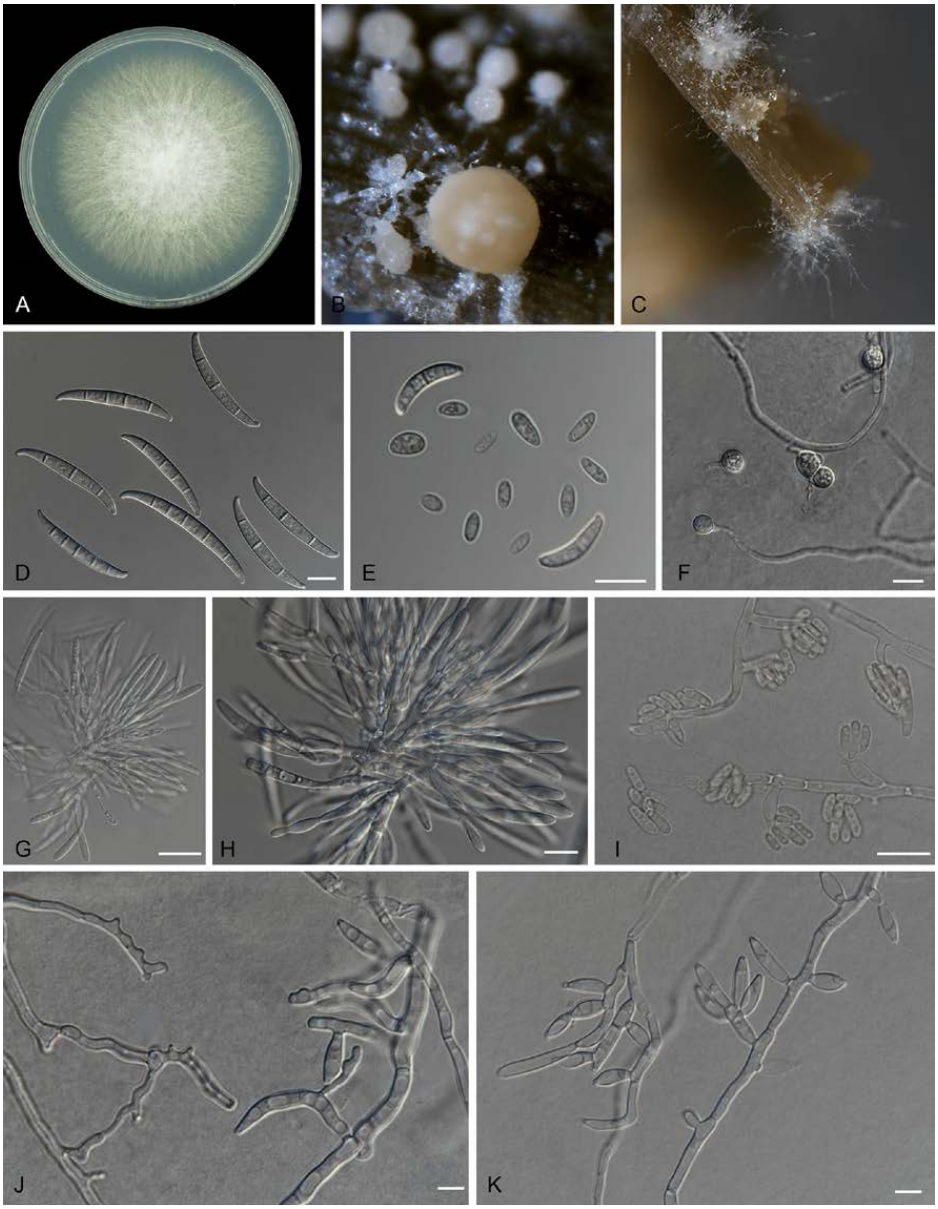

Fig. 11. *Fusarium grosmichelii* (ex-type InaCC 833). **A.** Culture grown on PDA. **B.** Sporodochia on carnation leaves. **C.** Aerial conidiophores from stereo microscope. **D.** Falcate-shaped macroconidia. **E.** Microconidia. **F.** Chlamydospores. **G–H**. Sporodochial phialides. I. False head. **J.** Polyphialides. **K.** Branched conidiophore. Scale bars D–F, H–K= 10 μm, G= 20 μm.

Foc Lineage L5

Fusarium duoseptatum N. Maryani, L. Lombard, Kema & Crous, **sp. nov.** MycoBank MB826804. Fig. 12.

Etymology: Name reflects the fact that its microconidia are frequently 2-septate.

Macroconidia abundant on CLA, less abundant on PDA and SNA, formed on sporodochia on CLA and on aerial conidiophores on SNA and PDA, falcate, (50–)53–63(–68) × (5–)6–8(–9) µm (av. 58 × 7 µm), 3–5-septate, with apical cells papillate, basal cells foot-shaped. *Conidiogenous cells* mono- or polyphialidic on sporodochia, or on aerial hyphae, or formed directly from hyphae as lateral phialides, (5–)9–25(–38) × (3–)4–7(–9) µm. *Microconidia* abundant on PDA and SNA, less frequent on CLA, ovoid to ellipsoid, (9–)21(–33) × (2–)3(–6) µm (av. 15 × 5 µm), 0–2-septate, arranged in false heads on branched conidiophores carried on hyphae. Aerial conidiophores rare on CLA and SNA, formed abundantly on PDA, branched sparsely or formed laterally. *Chlamydospores* globose to subglobose, formed laterally, intercalary or terminally, single or in pairs, (6–)8–10(–11) × (6–)7–9(–11) µm, abundantly produced on SNA after 7 d, rough-walled.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 3.8–4.1 mm/d. Colony reverse violet, mycelium becoming purple, and pigmented with age. Colony surface dry, cottony violet in the centre, and white towards the margin. No exudates observed. Aerial mycelium abundant, cottony, with moderate sporulation. Sporodochia formed abundantly on CLA after 7 d, pale orange to orange.

Geographic and host: Kapuas, Central Kalimantan, *Musa* sp. var. Pisang Kepok (ABB).

Pathogenicity: Pathogen on Gros Michel (AAA).

Material examined: **Indonesia**, Serapat tengah, Kapuas Timur, Central Kalimantan (114°28’65”E, 3°6’9”S), on infected pseudostem of *Musa* sp. var. Pisang Kepok (ABB), 22 Jun. 2014, N. Maryani, (**holotype** preserved as metabolically inactive culture InaCC F916).

Notes: *Fusarium duoseptatum* has distinctive septation in its microconidia, being 0–2-septate, thus differing from *F. gromichelii*, which is 0–1-septate. The former is relatively slow-growing compared to members of the most closely related lineage, L4, and forms pigmentation in the centre of colony that is not observed in isolates of L4. *F. duoseptatum* and most of the members of L5 were able to infect Gros Michel, and were therefore classified as Race1.

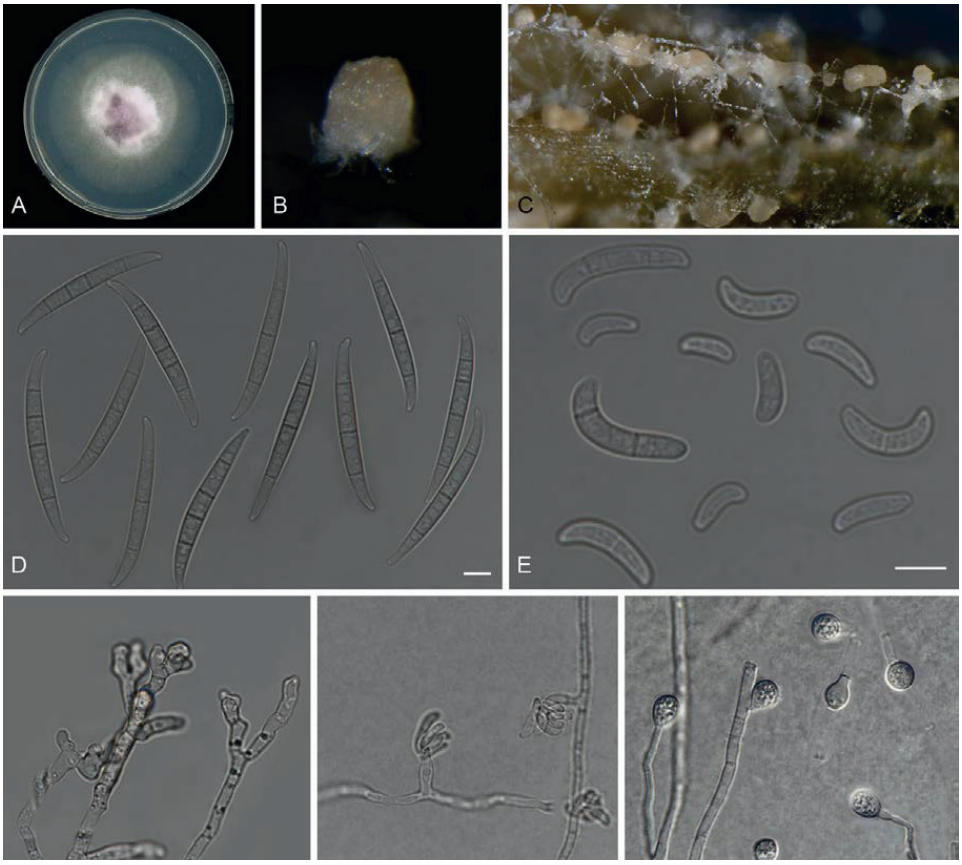

Fig. 12. *Fusarium duoseptatum* (ex-type InaCC 916). **A.** Culture grown on PDA. **B–C.** Sporodochia on carnation leaves. **D.** Falcate-shaped macroconidia. **E.** Microconidia. **F.** Polyphialidic conidiogenous cells. **G.** False head. **H.** Chlamydospores. Scale bars D–H= 10 µm.

Foc Lineage L6

Fusarium tardichlamydosporum N. Maryani, L. Lombard, Kema & Crous, **sp. nov.** MycoBank MB826805. Fig. 13.

Etymology: Name reflects the delayed chlamydospore production observed in this species.

Macroconidia abundant on CLA, less abundant on PDA and SNA, formed on sporodochia on CLA and on aerial conidiophores on SNA and PDA, falcate, (36–)37–43(–45) × (4–)5–6(–7) µm (av. 40 × 5 µm), 3–5-septate, with apical cells papillate, basal cells foot-shaped. *Conidiogenous cells* monophialidic on sporodochia, or on aerial hyphae, or formed directly on hyphae as lateral phialides, (3–)7–14(–19) × (2–)3–5(–8) µm. *Microconidia* abundant on PDA and SNA, ovoid to ellipsoid, (3–)5–9(–15) × (2–)5(–9) µm (av. 7 × 3 µm), 0–2-septate, arranged in false heads on branched conidiophores carried on hyphae. Aerial conidiophores rare on CLA and

SNA and formed abundantly on PDA, branched sparsely or formed laterally. *Chlamydospores* abundantly produced after 4 wk, globose to subglobose, (6–)7–10(–13) × (4–)6–9(–10) µm, formed terminally or intercalarily, single or in pairs, rough-walled.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 4.6–5.6 mm/d. Colony reverse sparsely dark purple in the centre, becoming white towards the margins, and purple slate, pigmented with age. Colony surface dry, cottony, with white filamentous margin, and lacking exudates. Aerial mycelium abundant, cottony, with abundant sporulation. Sporodochia formed abundantly on CLA after 7 d, pale orange to orange.

Geography and host: Sikka, Flores, *Musa acuminata* var. Pisang Barangan (AAA).

Pathogenicity: Pathogen on Gros Michel (AAA).

Materials examined: **Indonesia**, Desa Kota Uneng Kecamatan Alok, Sikka Flores, East Nusa Tenggara (112°12′16″E, 8°37′11″S), on infected pseudostem of *Musa acuminata* var. Pisang Barangan (AAA), 21 Aug. 2015, N. Maryani, (**holotype** preserved as metabolically inactive culture, InaCC F958).

Notes: Colonies of *Fusarium tardichlamydosporum* are relatively fast growing (av. 4.6–5.6 mm/d) compared to those of *F. duoseptatum* (av. 3.8–4.1 mm/d). Polyphialidic conidiophores were not observed in this species/lineage. Chlamydospores were produced, but only after 4 wk. *F. tardichlamydosporum* was able to infect Gros Michel, and is therefore classified as Race1.

Foc Lineage L7

Fusarium cugenangense N. Maryani, L. Lombard, Kema & Crous, **sp. nov.** MycoBank MB826807. Fig. 14.

Etymology: Name reflects Cugenang, the location where this species was collected in Indonesia.

Macroconidia abundant on CLA, formed on sporodochia, on aerial conidiophores or on lateral phialides, falcate, (44–)47–54(–57) × (5–)6–7(–8) µm (av. 53 × 7 µm), 3–6-septate, with apical cells papillate, basal cells foot-shaped. *Conidiogenous cells* monophialidic on sporodochia, or on aerial hyphae, or formed directly from hyphae as lateral phialides, (5–)12–31(–45) × (3–)5–7(–8) µm. *Microconidia* abundant on PDA and SNA, less frequent on CLA, ovoid to ellipsoid, (7–)8–11(–24) × (2–)7(–12) µm (av. 12 × 5 µm), 0–3-septate, arranged in false heads on

branched conidiophores carried on hyphae. Aerial conidiophores rare on CLA and SNA, and formed abundantly on PDA, branched sparsely or formed laterally. *Chlamydospores* rarely produced on SNA after 4 wk, globose to subglobose, (9–)10–14(–16) × (10–)11–14(–16) µm, formed terminally, single or in pairs, rough-walled.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 5.2–5.4 mm/d. Colony reverse purple at centre to pale viscous grey, white towards the margins, becoming purple slate with age, and pigmented. Colony surface dry, cottony, dark purple to white with filamentous margin, lacking exudates. Aerial mycelium abundant, cottony, with profuse sporulation. Sporodochia formed abundantly on CLA after 7 d, pale orange to orange.

Geography and host: Cianjur, West Java, *Musa* sp. var. Pisang Kepok (ABB).

Pathogenicity: Non-pathogenic on Gros Michel (AAA) and Cavendish (AAA).

Material examined: **Indonesia,** Cugenang, Cianjur, West Java (107°4'109"E, 6°47'867"S), on infected pseudostem *Musa* sp. var. Pisang Kepok (ABB), 10 Jul. 2014, N. Maryani, (**holotype** preserved as metabolically inactive culture, InaCC F984).

Notes: Lineage 7, including *Fusarium cugenangense* and other isolates, represents an Indonesian lineage with isolates that are closely related to other formae speciales (Fig. 6; e.g. NRRL 25433 *Fusarium oxysporum* f. sp*. vasinvectum*). Polyphialidic conidiogenous cells were not observed in this species. This species has macroconidia with unique septation (3–6-septate) and microconidia (0–3-septate), which is rather uncommon for *Fusarium oxysporum* species. This species causes a slight infection on Cavendish and Gros Michel, and testing on other cultivars such as Bluggoe (Pisang Kepok, ABB) are needed to fully classify strains as Foc-Race2.

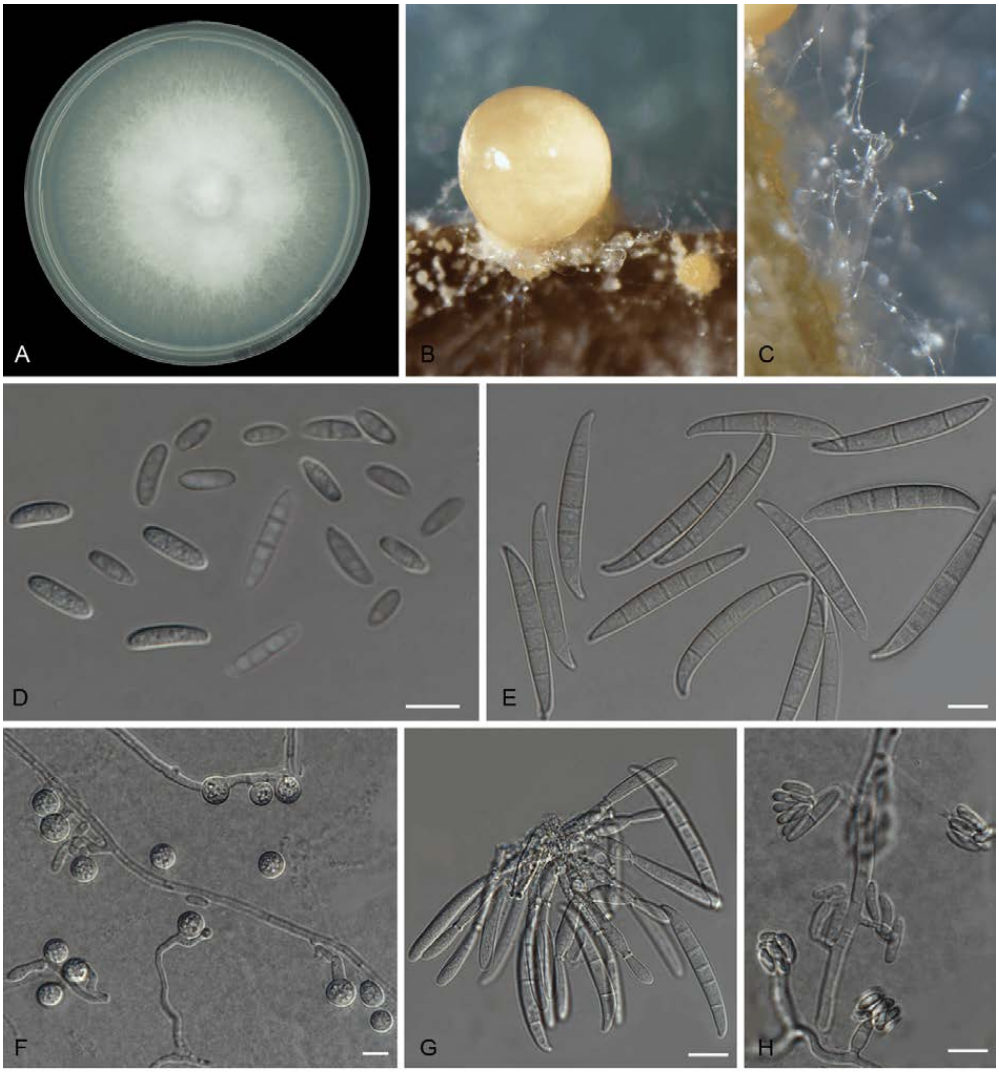

Fig. 13. *Fusarium tardichlamydosporum* (ex-type InaCC F958). **A.** Culture grown on PDA. **B.** Sporodochia on carnation leaves. **C.** Aerial conidiophore. **D.** Microconidia. **E.** Falcate-shaped macroconidia. **F.** Chlamydospores. **G.** Sporodochial phialides. **H.** False head. Scale bars D–H= 10 μm.

Foc Lineage L8

Fusarium hexaseptatum N. Maryani, L. Lombard, Kema & Crous, **sp. nov.** MycoBank MB826808. Fig. 15.

Etymology: Name reflects the six conidial septa observed in its macroconidia.

Macroconidia abundant on CLA, less so on PDA and SNA, formed on sporodochia on CLA and on aerial conidiophores on SNA and PDA, falcate, (34–)45–71(–76) × (5–)6–8(–9) μm (av. 58 ×

7 µm), 3–6-septate, with apical cells papillate, basal cells foot-shaped. *Conidiogenous cells* mono- or polyphialidic on sporodochia, or formed directly from on hyphae (lateral phialides), 7–20 × 2–6 µm. *Microconidia* abundant on PDA and SNA, rare on CLA, ovoid to ellipsoid, (4–)8–23(–29) × (2–)7(–12) µm (av. 16 × 5 µm), 0–1-septate, arranged in false heads on branched conidiophores carried on hyphae. Aerial conidiophores rare on CLA and SNA and formed abundantly on PDA, branched sparsely or formed laterally. *Chlamydospores* abundantly formed in hyphae, globose to subglobose, (5–)14(–20) × (4–)6–12(–17) µm, formed terminally or intercalarily, single or in pairs.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 4.9–5.9 mm/d. Colony reverse, in the dark, white and becoming livid purple in the centre of the colony. Colony surface with filamentous margin, dry, cottony, white becoming livid vinaceous in age. No exudates observed. Aerial mycelium abundant, cottony, with high sporulation. Sporodochia formed abundantly on CLA after 7 d, colourless to pale orange.

Geography and host: Sukabumi, West Java, Pisang Ambon Kuning (AAA).

Pathogenicity: Pathogen on Gros Michel (AAA).

Material examined: **Indonesia,** Parakan Lima, Sukabumi, West Java (106°48'674"E, 6°59'874"S), on infected pseudostem *Musa acuminata* var. Pisang Ambon Kuning (AAA), 11 Jul. 2014, N. Maryani, (**holotype** preserved as metabolically inactive culture, InaCC F866).

Notes: Fusarium hexaseptatum is the single species in L8. Macroconidia with 6 septa are abundantly observed in this lineage, whereas in L7 and L9, they are very rare. This lineage is distinguished from L7 and L9 by its ability to cause disease on Gross Michel, and therefore it was classified as Race1. *Fusarium hexaseptatum* has chlamydospores that are relatively large compared to those in other lineages (av. 9 × 9 µm).

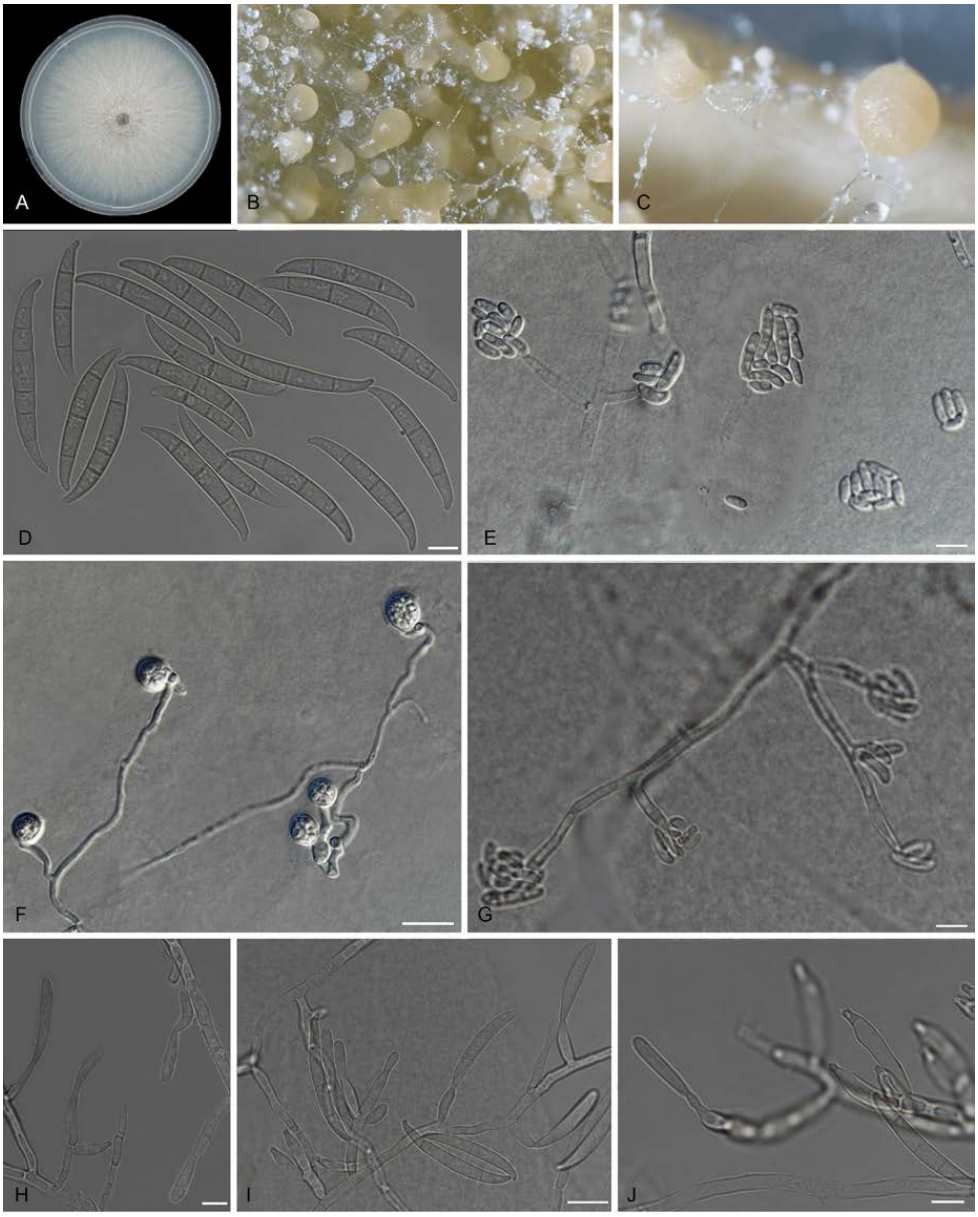

Fig. 14. *Fusarium cugenangense* (ex-type InaCC F984). **A.** Culture grown on PDA. **B–C.** Sporodochia on carnation leaves. **D.** Falcate-shaped macroconidia. **E.** Microconidia. **F.** Chlamydospores. **G.** False head. **H.** Monophialides conidiogenous cells. **I–J.** Branched conidiophores. Scale bars D–J= 10 μm.

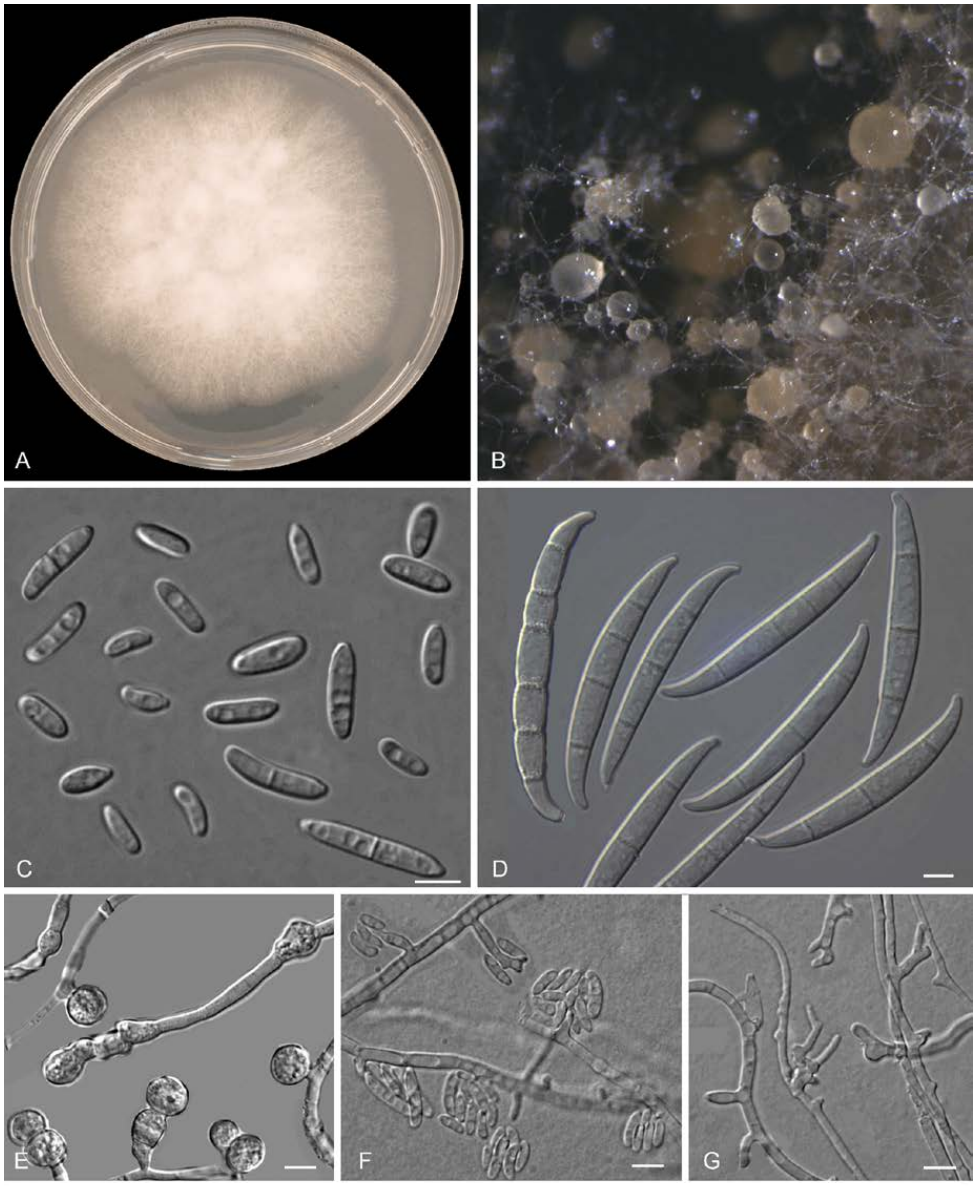

Fig. 15. *Fusarium hexaseptatum* (ex-type InaCC F866). **A.** Culture grown on PDA. **B.** Sporodochia on carnation leaves. **C.** Microconidia. **D.** Falcate-shaped macroconidia. **E.** Thick-walled chlamydospores. **F.** False head. **G.** Monophialides and polyphialides. Scale bars C–G= 10 μm.

Foc Lineage L9

Fusarium tardicrescens N. Maryani, L. Lombard**,** Kema & Crous, **sp. nov.** MycoBank MB826809. Fig. 16.

Etymology: Name reflects the slow growth rate in culture.

Macroconidia abundant on CLA and SNA, less abundant on PDA, formed on sporodochia on CLA and on aerial conidiophore on SNA and PDA, falcate, (52–)56–75(–89) × (5–)6–8(–9) µm (av. 66 × 7 µm), 2–6-septate, with apical cells papillate, basal cells foot-shaped. *Conidiogenous cells* mono- and polyphialidic on sporodochia formed directly from hyphae (lateral phialides), 7–32 × 2–6 µm. *Microconidia* abundant on PDA and SNA, less so on CLA, ovoid to ellipsoid, (7–)10–16(–20) × (2)–5(–7) µm (av. 13 × 4 µm), 0–1-septate, arranged in false heads on branched conidiophores carried on hyphae. *Chlamydospores* globose to subglobose, (5–)7–9(–10) × (5–)6–8(–10) µm, formed intercalarily or terminally, singly or in pairs, produced abundantly on SNA after 7 d, brown, rough-walled.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 2.9–3.9 mm/d. Colony reverse, in the dark, dark violet becoming dark livid and pigmented. Colony surface dry, cottony, dark purple becoming dark livid. No exudates observed. Aerial mycelium abundant, cottony, with abundant sporulation. Sporodochia formed abundantly on CLA after 7 d, pale orange to orange.

Geography and host: NA.

Pathogenicity: NA.

Material examined: **Malawi,** Karonga**,** Misuku Hills, *Musa sapientum* cv. Harare, 1989, R.C. Ploetz NRRL 36113 (**holotype** preserved as metabolically inactive culture CBS 102024).

Notes: *Fusarium tardicrescens* in L9 represents one of two lineages of *formae specialis cubense,* which clustered with other *formae speciales*. This lineage does not contain any Indonesian isolates. *Fusarium tardicrescens* is the slowest growing species (av. 2.9–3.9 mm/d). *Fusarium tardicrescens* causes moderate infection on both Cavendish and Gros Michel (Ordóñez 2018).

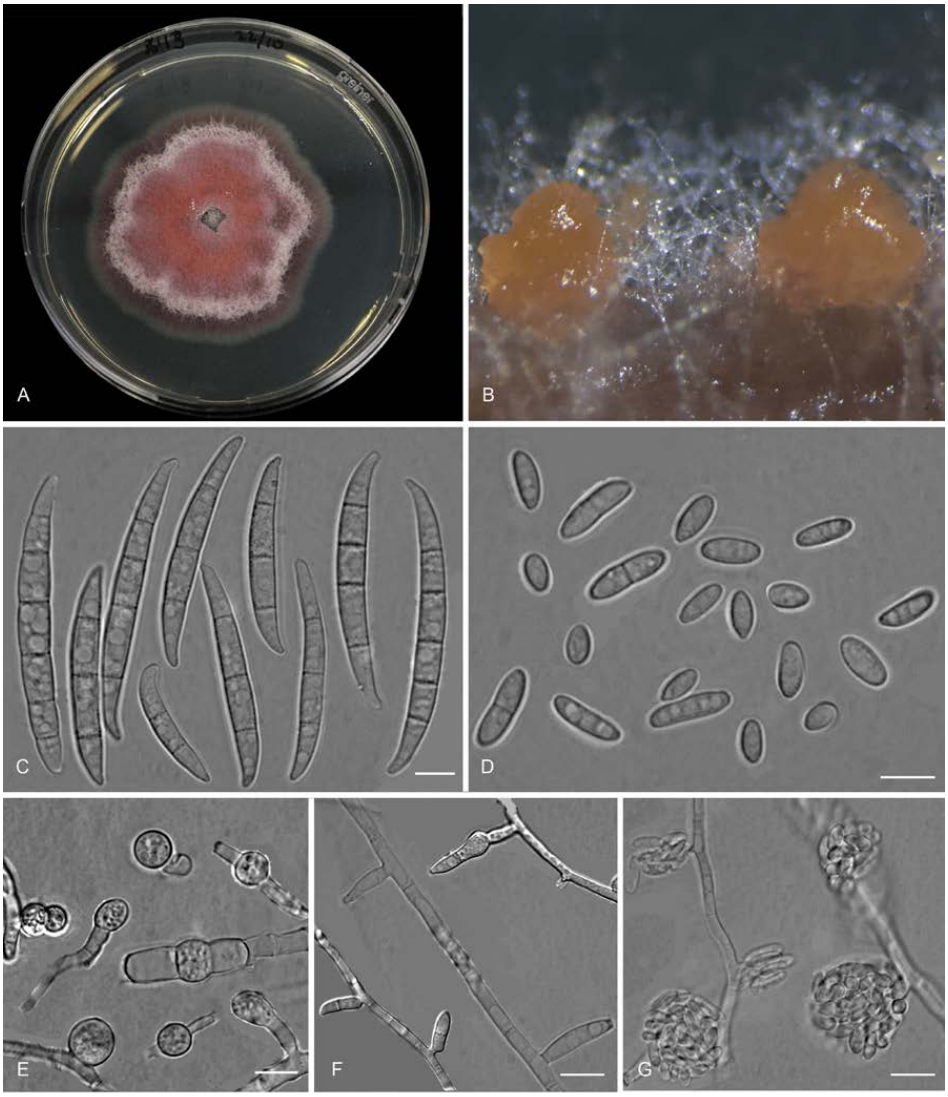

Fig. 16. *Fusarium tardicrescens* (ex-type CBS 102024). **A.** Culture grown on PDA. **B.** Sporodochia on carnation leaves. **C.** Falcate-shaped macroconidia. **D.** Microconidia. **E.** Thick-walled chlamydospores. **F.** Monophialides produce micro conidia and macroconidia. **G.** False head. Scale bars C–G= 10 μm.

Novel Clade/ Taxa in FOSC

Fusarium kalimantanense N. Maryani, L. Lombard, Kema & Crous, **sp. nov.** MycoBank MB826810. Fig. 17.

Etymology: Name reflects Kalimantan, the island in Indonesia from where this fungus was collected.

Macroconidia abundant on CLA, less abundant on PDA and SNA, formed on sporodochia on CLA and on aerial conidiophores on SNA and PDA, falcate, (52–)56–63(–65) × (5–)6–7(–8) µm (av. 59 × 7 µm), 3–5-septate, with apical cells papillate, basal cells foot-shaped. *Conidiogenous cells* monophialidic on sporodochia, or on aerial hyphae, or formed directly from hyphae as lateral phialides, (9–)11–15(–16) × (2–)3(–5) µm. *Microconidia* abundant on PDA and SNA, less frequent on CLA, ovoid to ellipsoid, (6–)8–15(–20) × (2–)3–4(–7) µm (av. 12 × 4 µm), aseptate, arranged in false heads on branched conidiophores borne on hyphae. Aerial conidiophore sparse on CLA and SNA and formed abundantly on PDA, branched sparsely or formed laterally. *Chlamydospores* rarely produced on SNA after 7 d, globose to subglobose, formed terminally or laterally, single or in pairs, (6–)7–10(–11) × (7–)8–9(–10) µm, rough-walled.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 4.8–1.2 mm/d. Colony reverse rosy buff (pinkish) to white towards the margins, becoming fuscous black and pigmented with age. Colony surface dry, cottony, rosy buff (pinkish) to white, becoming purplish grey with age, filamentous margin, and lacking exudates. Aerial mycelium abundant, cottony, with moderate sporulation. Sporodochia formed abundantly on CLA after 7 d, pale orange to orange.

Geography and host: Katingan, Central Kalimantan, *Musa acuminata* var. Pisang Ambon (AAA).

Pathogenicity: Non-pathogenic on Gros Michel (AAA) and Cavendish (AAA).

Material examined: **Indonesia**, Pulau Malam, Katingan, Central Kalimantan (113°13’333”E, 1°36’374”S), on infected pseudostem *Musa acuminata* var. Pisang Ambon (AAA), 23 Jun. 2014, N. Maryani, (**holotype** preserved as metabolically inactive culture, InaCC F917).

Notes: *Fusarium kalimantanense* represents a new clade (Clade 5) in FOSC, which was previously considered to include only four clades (Fig. 6; sensu O’Donnell *et al*. 2004). This species has relatively fast-growing colonies compared to those of other members of FOSC in this study, and has a unique character in its aseptate microconidia. *Fusarium kalimantanense* causes a slight infection on both Cavendish and Gros Michel. Further pathogenicity tests on other cultivars like Bluggoe (syn. Pisang Kepok, AAB) will be required to determine its race.

Fusarium sangayamense N. Maryani, L. Lombard, Kema & Crous, **sp. nov.** MycoBank MB826811. Fig. 18.

Etymology: Name reflects Sangayam, the location from where this species was collected in Indonesia.

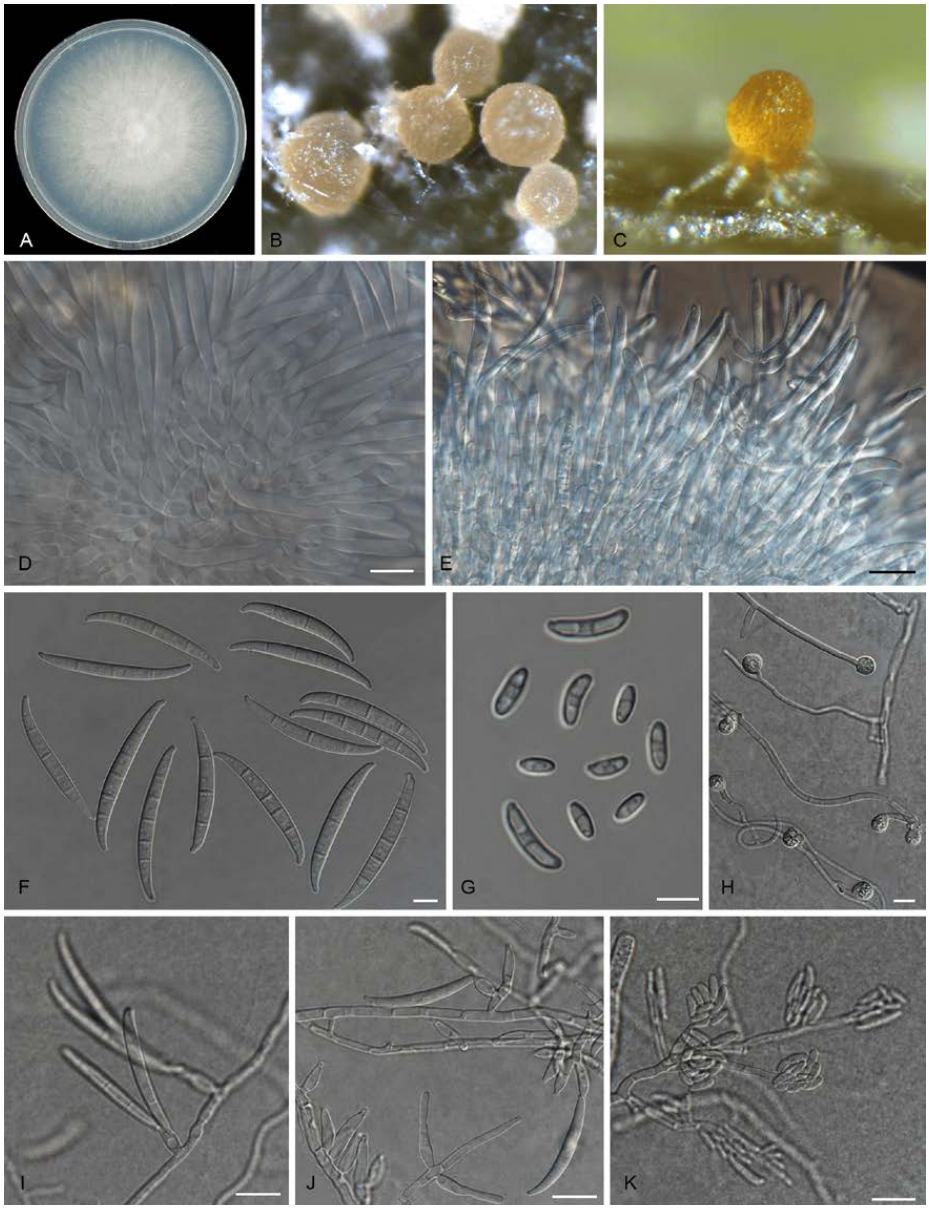

Fig. 17. *Fusarium kalimantanense* (ex-type InaCC F917). **A.** Culture grown on PDA. **B–C.** Sporodochia on carnation leaves. **D–E.** Sporodochial phialides. **F.** Falcate-shaped macroconidia. **G.** Microconidia. **H.** Thick-walled chlamydospores. **I.** Monophialides produce macroconidia. **J.** Branched conidiophores. **K.** False head. Scale bars D–K= 10 μm.

Macroconidia abundant on CLA and SNA, rare on PDA, formed on sporodochia on CLA and on aerial conidiophores on SNA and PDA, falcate, (48–)52–60(–65) × (5–)6–7(–8) µm (av. 56 × 7 µm), 2–5-septate, with apical cells papillate, basal cells foot-shaped. *Conidiogenous cells* monophialidic, similar in sporodochia and on hyphae, polyphialidic, rare, (6–)11–31(–47) × (3–)4–6(–9) µm. *Microconidia* abundant on PDA and SNA, less frequent on CLA, ovoid to ellipsoid, (8–)9–17(–24) × (3–)4–6(–7) µm (av. 13 × 5 µm), 0–1-septate, arranged in false heads on branched conidiophores borne on hyphae. Aerial conidiophores rare on CLA, and formed abundantly on SNA and PDA, sparsely branched, and formed laterally. *Chlamydospores* rarely produced on SNA after 7 d, globose to subglobose, formed terminally or intercalarily, single or in pairs, (6–)7–10(–12) × (6–)7(–9) µm, rough-walled.

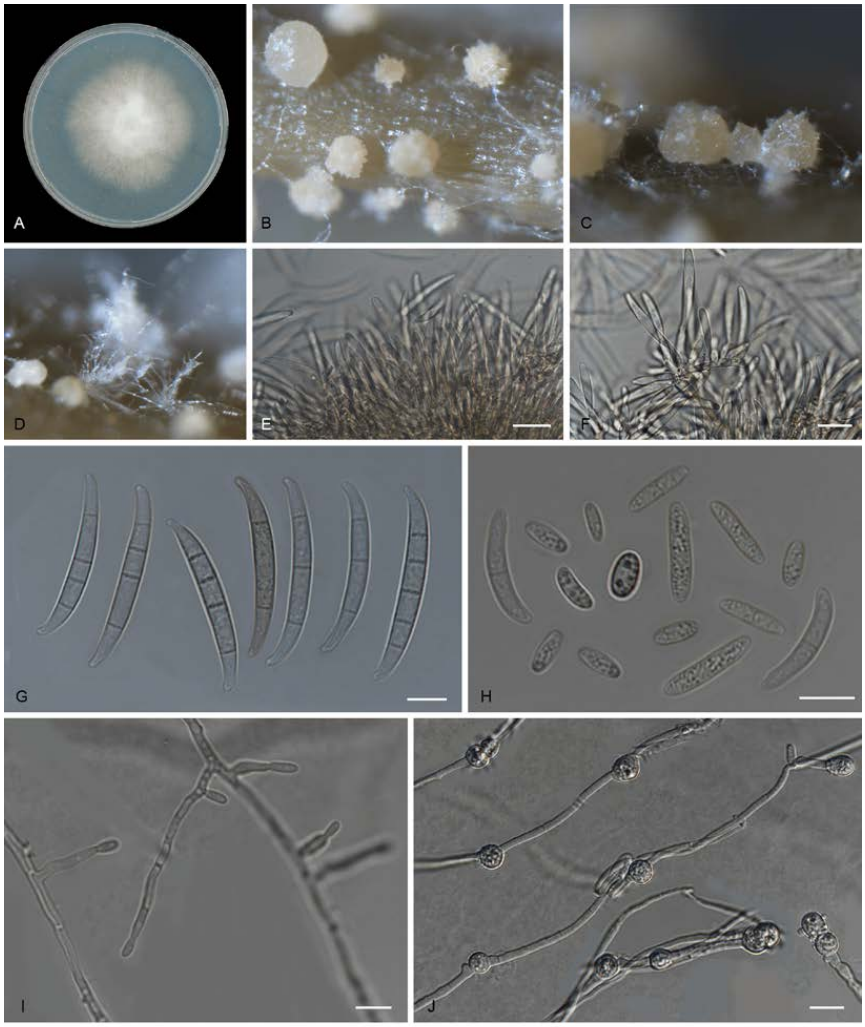

Fig. 18. *Fusarium sangayamense* (extype InaCC F960). **A.** Culture grown on PDA. **B–C.** Sporodochia on carnation leaves. **D.** Aerial conidiophore. **E–F.** Sporodochial phialides. **G.** Falcate-shaped macroconidia. **H.** Microconidia. **I.** Short monophialides. **J.** Thick-walled chlamydospores. Scale bars D–J= 10 µm.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C, with an average growth rate of 3.5–4.2 mm/d. Colony reverse uniformly white and unpigmented. Colony surface dry, cottony, white, with filamentous margin and lacking exudates. Aerial mycelium abundant, cottony, with moderate sporulation. Sporodochia formed abundantly on CLA after 7 d, pale orange.

Geography and host: Kota Baru, South Kalimantan, *Musa sp.* var. Pisang Kepok (ABB).
Pathogenicity: Non-pathogenic on Gros Michel (AAA) and Cavendish (AAA).

Material examined: **Indonesia**, Sangayam, Kota Baru, South Kalimantan (115°59'440"E, 2°20'420"S), on infected pseudostem of *Musa sp.* var. Pisang Kepok (ABB), 19 Jun. 2014, N. Maryani, (**holotype** preserved as metabolically inactive culture, InaCC F960).

Notes: Isolates of *F. sangayamense* formed a subclade in the new FOSC Clade 5 (Fig. 6) with high support (BP = 100 % and PP = 1.0). *Fusarium sangayamense* can be distinguished from *F. kalimantanense* based on the septation of its macroconidia (2–5-septate) and microconidia (0–1-septate). This species has polyphialidic conidiogenous cells, which are absent in *F. kalimantanense*. *Fusarium sangayamense* was not able to infect Cavendish or Gros Michel.

Pathogenicity assays

The pathogenicity assay showed that all collected Foc isolates were able to cause typical Fusarium wilt symptoms on either Cavendish or Gros Michel, or in both varieties (Fig. 19). The positive control isolate Foc-II5 was lethal to both varieties, whereas all negative (water) controls remained free of disease. Isolates affecting Cavendish were classified as TR4 (Su *et al*. 1986), while those only infecting Gros Michel were classified as Race1 (Waite & Stover 1960, Ploetz 1990). No fewer than 65 % of the isolates clustered in L1, which only comprised the strains that caused Fusarium wilt in Cavendish and hence, represented TR4. The rest of the isolates tested were able to infect Gros Michel and are therefore considered to be Race1 strains. Strains fitting this pathogenicity profile were equally distributed over all other lineages, except L7 and L9. L7 contains two Indonesian isolates that caused a slight discolouration of the corms of both varieties. Isolates in the new clade within FOSC were not able to cause disease in either tested banana variety. Isolates identified as other *Fusarium* species in the phylogenetic analyses were negative in all pathogenicity assays.

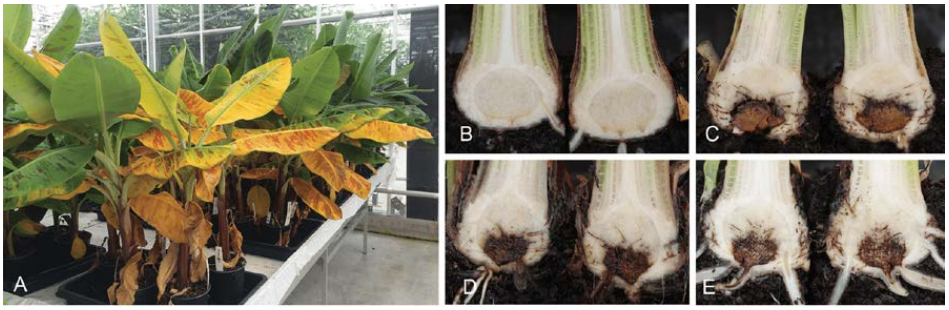

Fig. 19. Pathogenicity assays. **A.** External wilting symptoms. **B–C.** Left panel Cavendish and right panel Gros Michel, corm symptom caused by Race1, *Fusarium tardichlamydosporum*. **D–E.** Left panel Cavendish and right panel Gros Michel, corm symptom caused by TR4, *Fusarium odoratissimum*.

DISCUSSION

The *Musa* gene centre (Perrier *et al.* 2011), as with the wheat gene centre in the Middle-East (Banke *et al.* 2004, Stukenbrock *et al.* 2007) and that of potato in Central Mexico (Grünwald & Flier 2005), contains a myriad of endemic diseases that co-evolved with the host. It is therefore considered a typical hot-spot of pathogen diversity (Stukenbrock *et al.* 2008). The gene centre of *Musa* has been studied in detail since the previous century. The wild ancestor of edible banana, *Musa acuminata,* originated in South-East Asia and Melanesia, and *Musa balbisiana* originated in South Asia (Perrier *et al.* 2011), where Indonesia is the contact area between these two wild *Musa* species. Approximately 11–13 *Musa acuminata* sub-species are of Indonesian origin, found in Sumatra, Kalimantan, Java, and the Lesser Sundas (Daniells 1995, Simmonds 1962). Most of the *Musa balbisiana* sub-species found in Java, Sumatra, and Sulawesi originate from India (Ochse & van den Brink 1931, De Lange 2009). However, the genetic diversity of *Musa* pathogens in the centre of origin of *Musa* has remained virtually unsampled. Although a recent overview of Foc in Asia was published (Mostert *et al*. 2017), a need remained for a thorough taxonomic analysis of Foc in its centre of origin. Our results present the most comprehensive study of Foc in the Indonesian gene centre of banana to date. Isolates of Foc were recovered from all the samples that were collected in all areas surveyed. The results demonstrated that Fusarium wilt is widely distributed in Indonesia and could be found in every banana producing area surveyed. Past reports showing compatible results have spanned an area from Aceh province in the west to Papua province in the east (Nasir *et al.* 1999, Wibowo *et al.* 2011). In 2012, 1 700 of the 21 000 acres of cultivated banana suffered from Fusarium wilt in Indonesia, including large commercial Cavendish plantations (Jumjunidang *et al.* 2012). Factors making this disease difficult to control include traditional farming practices, limited quarantine restriction on movement of planting material, and limited knowledge on the dissemination of the pathogen(s). As a result, the disease is unwittingly distributed to new areas. Moreover, the abundant diversity of banana varieties in

Indonesia allows farmers to easily change the varieties they grow, resulting in epidemiological contact that allows the pathogen to infect new cultivars in different areas.

Demographic factors could have played a significant role in the dissemination of this disease in Indonesia. Java is the most populated island and, therefore, banana production and the available cultivated varieties are the most numerous on this island, as is the pathogen. Mass migration of people from this over-populated island to less populated islands such as Kalimantan, Sumatra, and Papua from 1980 to1990 could account for the dissemination of Fusarium wilt throughout Indonesia, since infected banana planting material was taken along (Nasir *et al.* 1999).

The high number of local banana varieties from which Foc was recovered indicate that co-evolution of this pathogen is occurring along with its host in this region. Nasir *et al.* (1999) reported that 15 local varieties in Sumatra were susceptible to Fusarium wilt, including the most popular varieties, Pisang Ambon Kuning (AAA, Gros Michel synonym), Barangan (AAA) and Pisang Raja Sereh (AAA). This finding was reconfirmed in this study. An increasing number of infected varieties was also reported by Hermanto *et al.* (2009) and Jumjunidang *et al.* (2012). Of the hundreds of banana cultivars identified in Indonesia, many appear to be resistant or partially resistant to Fusarium wilt, a prior finding that was also observed during the present survey. No wild banana or close relative surveyed in this study showed any symptoms of Fusarium wilt. In Africa, *Ensete ventricosum*, a member of the *Musaceae*, is susceptible to Foc Race2 (Ploetz 2006a). By contrast, *Ensete glaucum* growing on the outskirt forest of Flores, Indonesia, was found to be healthy. None of the wild *M. acuminata* varieties found during the surveys was susceptible to Fusarium wilt. This finding is in agreement with some reports and greenhouse experiments on the infection of Foc on wild *M. acuminata*. *M. acuminata* var. *malaccensis* from the Malaysian Peninsula was reported to be experimentally resistant (Javed *et al*. 2004), as was its sister variety *M. acuminata* var. *malaccensis* from Sumatra (Ahmad & Maryani 2017, unpubl. data). This study and our observations during surveys indicate that Indonesia is the primary gene centre of Foc, and the most likely place to find a diverse palette of disease resistance markers for Fusarium wilt in banana.

The high diversity of Foc isolates found in this study is unparalleled by the findings of any previous study (O'Donnell *et al.* 1998; Fourie *et al*. 2009) where a similar approach was used. The taking of larger numbers of samples in Indonesia inclusive of more banana cultivars, could result in an even higher diversity, as well as the discovery of yet more novel taxa belonging to FOSC. This accords with the view of Leslie & Summerell (2006), who stated that the most informative studies on the systematics and evolution of *Fusarium* species from natural ecosystems, as well as different agro-ecosystems, should incorporate native host populations, in order to allow discovery of the full existing species diversity (Leslie & Summerell 2006).

Employing rotations with alternative crops, such as corn, sugar cane, peanuts and coffee, was found to decrease disease incidence in some plantations in Sumatra, Java, and Kalimantan. However, this practice probably has allowed for other *Fusarium* species, pathogenic to the rotation crops, to become established in these plantations, explaining their recovery in this study. These species include *F. mangiferae, F. proliferatum F. sacchari* and *F. verticillioides,* which are members of the *Fusarium fujikuroi* species complex (FFSC) and are associated with several tropical crops (Marasas *et al*. 2006, Ploetz 2006b) such as mango, maize, rice and sugarcane (Hsuan *et al.* 2011). These crops were commonly found in the areas surveyed for Fusarium wilt on bananas during this study. *Fusarium proliferatum* and *F. oxysporum* have been reported from the roots of the wild banana, *Musa acuminata,* from Malaysia (Zakaria & Rahman 2011), which is closely related to several other *M. acuminata* varieties present in Sumatra and Java (Nasution 1990). This study represents the first report of both *F. longipes* and *F. incarnatum-equiseti* from banana varieties displaying symptoms of Fusarium wilt, although disease symptoms could not be induced in the pathogenicity assays undertaken here. However, both species are well-known as soil inhabitants and saprobes with a wide global distribution in tropical regions (Leslie & Summerell 2006). They could, therefore, be secondary colonisers of the decaying vascular tissue collected during the survey. The majority of the isolates that clustered outside the FOSC clade are well-known endophytes of various plant hosts, saprobes, and soil inhabitants, and are known to be non-pathogenic to banana (Waalwijk *et al*. 1996, O'Donnell *et al*. 1998, Ploetz 2006a).

In the FOSC clade, the Indonesian isolates were equally distributed throughout the two previously known clades in FOSC (*sensu* O'Donnell et al. 2004). Several of these *F. oxysporum* isolates are known as endophytes of banana (O'Donnell *et al.* 1998), and are unable to induce disease on Cavendish or Gros Michel. Isolates obtained in this study that were found to be non-pathogenic to both banana cultivars tested were distantly related to the pathogenic isolates, and were more closely related to other *formae speciales* that are pathogenic to other crops. This finding supported the observations of Gordon & Okamoto (1992), who reported that *Fusarium oxysporum* f. sp. *melonis*, pathogenic to cucurbits, is only distantly related to non-pathogenic strains. This also supports the view that Foc and other *formae speciales* of *F. oxysporum* have a polyphyletic origin (Baayen *et al.* 2000, O'Donnell *et al.* 2009).

Nine Foc lineages were revealed in this study, albeit with varying levels of statistical support, and described as new species. This conclusion was based on combinations of the genealogical approaches described by Dettmann *et al*. (2003) and Laurence *et al.* (2014), with supporting evidence from the inclusion of eight previously established lineages of FOC (O'Donnell *et al*. 1998; Fourie *et al*. 2009). A lineage is recognised as independent in this system if it is found to be concordantly supported by the majority of the loci, or is well supported by at least one locus but not contradicted by any other locus. Two previously known clades of Foc were resolved in this study (Boehm *et al.* 1994, Bentley *et al*. 1995, O'Donnell *et*

al. 1998, Fourie *et al.* 2009), with the majority of the isolates fell into in Clade1, Lineage1. This lineage, classified as TR4, was found on every island surveyed, including Papua and Flores and those that were previously thought to be free of TR4. This is in agreement with some reports on Fusarium wilt in Indonesia, which note that the majority of Foc strains isolated appeared to be TR4 (O'Neill *et al*. 2011, Jumjunidang *et al*. 2012). In terms of phylogenetic diversity, TR4 isolates were less diverse than Race1, which occurred in almost all lineages. The number of diverse banana varieties sampled could be the reason for the tremendous diversity of Race1 isolates found in this study. Many of the banana sampled belong to varieties Gros Michel (AAA) or Silk (AAB), both known to be highly susceptible to Race1 (Waite & Stover 1960).

The partial sequences of the three coding gene regions employed in this study, *tef1, rpb1* and *rpb2*, are well-known to be robust for use in molecular-based identification of *Fusarium* species (O'Donnell *et al*. 2015), but are unable to distinguish all of the 24 Vegetative Compatibility Groups (VCGs; Puhalla 1985, Ordóñez *et al*. 2015) that are known to represent the widest genetic diversity of Foc. Direct VCG identification is a relatively objective but time-consuming test, and the results indicate genetic similarity rather than genetic differences (Kistler 1997). Therefore, VCGs represent good phenotypic characters for assessing diversity within populations, but genetic relationships among VCGs need to be assessed by other molecular tools.

The high diversity found, based on the number of isolates recovered from different banana varieties and the high number of lineages resolved in this study, support the hypothesis that the pathogen(s) co-evolved with the host in the host's centre of origin (Ploetz & Pegg 2006). The unique agro-ecosystems and variety of ecological niches found where banana cultivation is practiced in Indonesia provide a conducive environment for the pathogen to evolve. As mentioned above, subsistence farming in Indonesia has allowed for the dissemination of banana varieties with varying degrees of tolerance and resistance to Fusarium wilt. This practice may have created a suitable environment for the incumbent pathogen to evolve and to adapt to newly introduced banana varieties. The dynamics of host diversity in these agro-ecosystems will continue to select for new pathogens (Stukenbrock & McDonald 2008), a process that, in this study, yielded a diversity of genotypes able to infect newly introduced banana cultivars.

Another scenario that could account for the high Foc diversity in Indonesia, irrespective of a lack of sexual reproduction, is horizontal gene transfer. *Fusarium oxysporum* has the ability to transfer specific chromosomes, sometimes containing unique pathogenicity genes, among non-pathogenic and pathogenic strains, resulting in new pathogenic lineages (Rep & Kistler 2010). This phenomenon is well recorded in *Fusarium oxysporum* f. sp*. lycopersici*, a pathogen of tomato (Ma *et al.* 2010). A recent study of the effector profile of different formae speciales of *F. oxysporum*, including Foc, indicated that these fungi have specific and unique effector profiles that reflect vertical and horizontal inheritance (van Dam *et al*. 2016). The

endophytic character of some *F. oxysporum* strains, some of which are weak soil-borne pathogens (Stover 1962b), allows for relatively easy assimilation of pathogenicity genes from related pathogenic *F. oxysporum* strains via horizontal gene transfer (Vlaardingerbroek *et al*. 2016).

It was initially thought that the origin of pathogenic Foc is from non-pathogenic root inhabitants or endophytes of various wild *M. acuminata* plants in Java and Sulawesi that became pathogenic after their introduction to foreign banana germplasm (Buddenhagen 2007). Alternatively, native Race1 isolates may have been exposed to selection pressure through exposure to newly introduced banana varieties, as Race1 is known to infect diverse varieties like Silk (AAB), Pome (AAB), and Pisang Awak (ABB) (Waite & Stover 1960, Ploetz 2006a). Isolates that clustered in the newly resolved subclade in the FOSC in this study were found to be non-pathogenic towards both Cavendish and Gros Michel. These isolates only caused initial discoloration in the corm, without any further disease development. They might be pathogenic on other germplasm, but until more banana varieties can be tested, this idea remains speculation.

Our study demonstrates that the Indonesian Foc population might be the most genetically diverse Foc ever studied. Further genetic study of this population using deeper genomic coverage should now be conducted. Pathogenicity tests using more banana varieties could be used to assess the wide range of pathogenicity.

Our study gives an insight into the complexity of Fusarium wilt on banana in Indonesia. This is very important for disease management not only in Indonesia but also worldwide. As the pathogen continues to evolve, new lineages could arise and escape Indonesia. In striving to find banana resistance to Fusarium wilt, researchers should consider the high diversity of Indonesian Foc reported here as one of the main obstacles to overcome.

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Chapter 3

Genotyping-by-sequencing reveals extensive genotypic diversity among sympatric Fusarium wilt pathogens of banana in Indonesia

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Abstract

Several *Fusarium* species in the *Fusarium oxysporum* species complex (FOSC) are known to cause Fusarium wilt on banana, a devastating disease with worldwide significance. Extensive genetic diversity of this pathogen is found in South-East Asia – the centre of origin of bananas. Here, we assessed the genetic diversity of the largest collection of *Fusarium* species causing Fusarium wilt that were isolated from local banana varieties in Indonesia using genotyping-by-sequencing through Diversity Array Technology (DArTseq). We show that this technique is appropriate to rapidly assess genetic diversity at high resolution below the species level and that it provides dense and well-distributed markers over the reference *Fusarium odoratissimum* II5 genome. By hierarchical clustering of DArTseq markers, we identified 19 DArT Genotype Group (DGGs) of the 10 *Fusarium* species included. Most isolates (55 %) belong to *F. odoratissimum* and are grouped in a single DGG and were highly pathogenic on the Cavendish banana variety Grand Naine, thus classified as Tropical Race 4 (TR4). In contrast to TR4, Race 1 isolates were genetically more diverse, being distributed over six DGG and were highly pathogenic on the variety Gros Michel. Collectively, our results provide strong evidence that *Fusarium* spp. co-evolved with banana, and thus besides being the centre of diversity for the host, Indonesia likely also represents the centre of origin of *Fusarium* species causing Fusarium wilt in banana.

Keywords: Co-evolution, DArTseq, genotype, Race 1, species, Tropical Race 4

Introduction

Several *Fusarium* species, members of *Fusarium oxysporum* species complex (FOSC), are devastating plant pathogens that causes Fusarium wilt on banana (Maryani *et al.* 2019). A wide-range of banana varieties is susceptible to these pathogens, hence banana-producing countries around the world are affected. Fusarium wilt causes major problems in banana cultivation, both in small-scale plantations for domestic consumption like in Indonesia, India and Uganda, as well as in big monoculture plantations that produce banana for export purposes like in Colombia, Ecuador and The Philippines (Ploetz *et al*. 2015). Once banana plants are infected by *Fusarium* spp., the mycelium will ramify plant tissue, resulting in reduced fruit production or plant death. Moreover, the fungus simultaneously produces persistent chlamydospores that reside in debris and infested soil and remain viable for decades (Stover & Waite 1960, Ploetz 2015).

A thorough phylogenetic study of hundreds of *Fusarium oxysporum* isolates from the centre origin of banana in Indonesia as well as from a worldwide collection revealed several phylogenetic species within the FOSC and thus new names and formal descriptions were assigned for each of these new *Fusarium* species with pathogenicity to banana (Maryani *et al*. 2019). *Fusarium* spp. and its banana hosts likely co-evolved in South-East Asia (Groenewald *et al.* 2006, Koenig *et al.* 1997, Ploetz & Pegg 1997), which is the centre of origin of wild and cultivated bananas. The two wild banana ancestors, *Musa acuminata* Colla (AA, 2n = 22) and *Musa balbisiana* Colla (BB, 2n = 22), diversified into various edible varieties that include diploid (AA, BB), triploid (AAA, AAB, ABB) and tetraploid (ABBB) genotypes (Simmonds 1962). The Indonesian archipelago represents the primary centre for banana diversity, where the majority of wild species and cultivated varieties (>500) are grown (Perrier *et al.* 2011, Nasution 1990). Therefore, pathogens are considered to have co-evolved with their hosts in such regions. Although previous of *Fusarium* population studies have been limited in size, they provided strong evidence for co-evolution with the host in South-East Asia (Koenig *et al.* 1997, Ploetz & Pegg 1997, Groenewald *et al*. 2006). However, *Fusarium* spp. also might have evolved independently in other parts of the world upon the introduction of bananas (O'Donnell *et al.* 1998, Stover 1972). This is in accord with the recent identification of eight phylogenetic species of *Fusarium* spp. from the centre origin of banana in Indonesia and one phylogenetic species from Africa (Maryani *et al.* 2019). Studying the genetic diversity of these banana infecting *Fusarium* pathogens in Indonesia is therefore essential to increase the understanding of their evolution (Ploetz 1990, Ploetz & Pegg 1997).

Genetic diversity of pathogen populations has been studied using various genetic markers. In the *Fusarium* spp. that affect banana, phenotypic markers were commonly used to characterize pathotypes and vegetative compatibility groups (VCGs). Pathotypes or races are identified by differential host responses. Previous pathotyping assays have recognized three races (1, 2 and 4) in the *Fusarium* – banana pathosystem (Stover 1962, Su *et al.* 1986).

These were, however, based on a very limited number of bananas – *Fusarium* isolates interactions and concluded that Race 1 is virulent on Gros Michel (AAA), Race 2 is virulent on Bluggoe (ABB), while Race 4 is virulent on Cavendish (AAA). Under abiotic stresses Cavendish plants are vulnerable and strains affecting it under such conditions are classified as Subtropical Race 4 (STR4). Strains that infect Cavendish irrespective of abiotic conditions are identified as Tropical Race 4 (TR4) and are also virulent on Gros Michel and Bluggoe (Ploetz 1990, Stover 1962, Su *et al.* 1986). However, pathogenicity assays to determine races are laborious, while the underlying genotypes of the tested host and pathogen accessions remain unknown (Milgroom 2015, Kistler 1997). Genetic identity or proximity is based on heterokaryon formation, which occurs between strains in the same group of isolates (Puhalla 1985), and such isolates are considered to belong to the same VCG (Leslie 1993, Puhalla 1985). Thus far, *Fusarium* isolates from banana affected with Fusarium wilt have been placed in 24 VCGs but the underlying genotypes are still unknown (Ordóñez *et al.* 2015, Ploetz 2006). Moreover, VCGs do not provide information on virulence and do not correlate with the known phylogenetic species (Maryani *et al.* 2019, Fourie *et al.* 2009, O'Donnell *et al.* 1998, Moore *et al.*1993). Therefore, although race and VCG determination are useful phenotypic markers, further genetic assessment is needed to study diversity of pathogen populations at a higher resolution (Kistler 1997).

To determine genotypic diversity of *Fusarium* isolates that affect banana, DNA markers are preferred over phenotypic markers as these are able to capture molecular variation. Many molecular techniques have been used to assess genetic diversity in *Fusarium* fungi that infect banana (Bentley *et al.* 1995, Bentley *et al.* 1998, Koenig *et al.* 1997, Groenewald *et al.* 2006). However, the results obtained from these studies were not always congruent. Groenewald *et al.* (2006) and Bentley *et al.* (1998) analysed the same fungal populations causing wilt on banana using DNA fingerprinting and amplified fragment length polymorphism (AFLP), respectively, resulting in different genotypic groups. Genome-wide, high-resolution and reproducible markers are required for observing genetic differentiation of pathogen populations (Metzker 2009, Elshire *et al.* 2011, Agrawal & Shrivastava 2014). Genotyping-by-sequencing meets these requirements and therefore, we used Diversity Array Technology sequencing (DArTseq) (Ordóñez *et al.* 2015) to simultaneously genotype hundreds of polymorphic loci across the entire genome (Cruz *et al.* 2013, Jaccoud *et al.* 2001).

Here, we discovered novel genotypic diversity among Indonesian *Fusarium* spp. causing Fusarium wilt in banana (Maryani *et al.* 2019). We analysed 10 *Fusarium* species comprising 196 isolates and discovered at least 19 genotypes across the known FOSC causing Fusarium wilt in banana. This extensive sympatric diversity is independent of the geographical origin or the affected host plant and is therefore likely driven by a banana – *Fusarium* co-evolution in Indonesia.

Materials and Methods

Fungal isolates

The pseudostems of Fusarium wilt-infected banana varieties were collected across Indonesia on the islands of Flores, Java, Kalimantan, Papua, Sulawesi and Sumatra (Table 1; Fig. 1). Phylogenetic analyses of the *Fusarium* spp. recovered from these samples revealed several phylogenetic species in the FOSC (Maryani *et al.* 2019). Additionally, isolates from a worldwide collection known to represent various races and VCGs were included in the analyses (Ordóñez *et al.* 2015) (Table 1). Monosporic isolates were obtained and maintained on Potato Dextrose Agar (PDA) as a working collection and in glycerol 20 % (stored at –80 ^{o}C) for long-term preservation (Maryani *et al.* 2019). The entire collection is maintained in the Indonesia Culture Collection (InaCC), Cibinong, Indonesia, and at Wageningen University and Research (WUR), Wageningen, The Netherlands.

DNA extraction and DArTseq markers

Monosporic cultures of each isolate were grown in Potato Dextrose Broth (PDB) and incubated under continuous shaking (125 rpm) at room temperature. After seven days of incubation, fungal biomass was collected by filtering the cultures through cheesecloth and samples were subsequently lyophilized in a 2 mL tube for 21 h. Genomic DNA of each isolate was extracted using the DNA-Kit Wizard Magnetic DNA Purification System for Food kit (Promega, USA), quantified using the Quant-iT™ PicoGreen™ dsDNA Reagent Invitrogen in a TECAN 2000 analyser (Thermo Fisher Scientific, Switzerland), and quality checked on an agarose gel. DNA samples of high quantity and quality (500 ng; 100 ng/uL) of each isolate were used for DArTseq analyses. DArTseq markers were generated by Diversity Arrays Technology Pty. Ltd. (http://www.diversityarrays.com), Canberra, Australia, using a genome complexity reduction method and sequencing with the Illumina sequencing platform (Kilian et al., 2012), an approach that has been modified for *Fusarium oxysporum* species (Ordóñez et al., 2015).

Mapping of DArTseq markers and cluster analyses

Polymorphic DArTseq markers (binary markers indicating the presence [positive/'1'] or absence [negative/'0'] of a specific marker in a respective isolate) were used as input for the cluster analyses. Isolates with less than 500 positive markers were removed from subsequent analyses. Additionally, DArTseq markers were filtered for genotype call rate (>0.66) and reproducibility (= 1). A binary distance matrix between isolates was calculated using Dice similarity coefficients. Isolates were then clustered using the distance matrix as an input for the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method. The analyses were performed in R software (http://www.R-project.org) (RDevelopment-Core-Team 2013). To determine genetic differences within the species (among isolates), DArT

Genotype Group (DGG) were defined as a group of isolates with less than 0.1 Dice distance in the cluster.

To determine the genomic distribution of the derived DArTseq markers, the nucleotide sequence of each DArT marker sequence (up to 69 nt), was mapped to the reference II5 genome assembly (http://www.broadinstitute.org/) using blastn (megablast, e-value cutoff 1e-10) (Morgulis *et al.* 2008). For each marker sequence of which ≥80 % of its sequence could be placed on the II5 genome assembly, the 'best' genomic location was reported. For each marker that could potentially be placed at more than one genomic location, we defined the 'best' location for this particular marker by considering the bitscore differences of its individual hits, i.e. a marker with a bitscore difference <5 between the two 'best' locations was not placed, while a marker with a bitscore difference ≥5 was placed at the highest scoring location. Marker distributions for the largest 20 *Fusarium odoratissimum* II5, scaffolds were visualized using the R package ggbio (Yin *et al.* 2012), and (upstream) distances (in nt) between individual marker positions were calculated and visualized using R.

RESULTS

A comprehensive collection of *Fusarium* species causing Fusarium wilt in banana across Indonesia

Surveys of Fusarium wilt in Indonesia were conducted in 2014 and 2015. As bananas in Indonesia are commonly produced in backyard home plantations, samples were obtained from mixed banana varieties (Maryani *et al.* 2019). We sampled a total of 172 Indonesian isolates from 40 local banana varieties (Table 1). Samples were collected at 34 geographically diverse sites in 15 provinces, distributed over the islands of Flores, Java, Kalimantan, Papua, Sulawesi and Sumatra (Fig. 1). We obtained most isolates from Java (74 isolates) and Kalimantan (52 isolates), followed by Sumatra (18 isolates), Flores (14 isolates), Papua (10 isolates) and Sulawesi (8 isolates) (Fig. 1). Taken together, we observed Fusarium wilt occurring on many banana accessions at every location (Maryani *et al.* 2019), indicating that the disease is well-spread across the Indonesian archipelago.

Abundance and distribution of DArTSeq markers

DArTseq analyses from a total of 196 isolates (Table 1) resulted in 13 150 polymorphic DArTSeq markers. Technical repeats (40 isolates) and biological repeats (24 isolates) of DArT assays produced the same sets of DArTSeq markers, indicating good repeatability and reproducibility of the assays. To assess the density and the distribution of the markers over the *F. odoratissimum* genome, we placed the DArTseq markers onto the genome assembly of the reference isolate II5. In total, 6 051 DArTSeq markers (~46 %) could be mapped to the II5 genome assembly with high confidence and these markers were evenly distributed across the largest 20 scaffolds (Fig. 2A and 2B). The genomic distances between the DArTseq markers

were generally small (3 308 markers have a distance <5 kb) (Fig. 2C). Thus, given the estimated genome size of the II5 assembly (46.5 MB), on average one DArTseq marker per 7.6 kb can be observed. Therefore, DArT technology delivers abundant and dense marker sets for genetic diversity assessment.

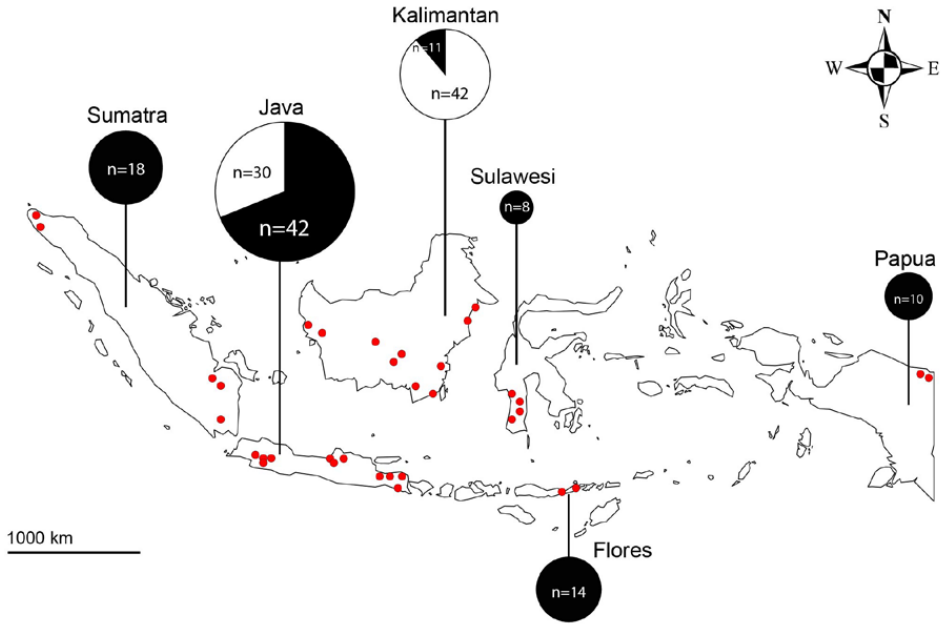

Fig. 1. Sampling sites of *Fusarium* spp. from banana plants affected by Fusarium wilt on six Indonesian islands indicated by red dots. Pie charts indicate the total number of obtained isolates and the number of *F. odoratissimum* Tropical Race 4 strains (in black).

Table 1. Overview of the *Fusarium* spp. collection that was studied and comprised 173 isolates from Indonesia. The isolates that were obtained from 40 host species at 34 location on six islands next to a reference set of 24 isolates from 14 countries, with their DArT Genotype Group (DGG), accession numbers and details of origins and hosts.

Species	DGG	Accession number	Geographical origin	Island	Country	Host (*Musa* sp. var.)	Host genotype
Fusarium odoratissimum	1	[1]InaCC F816	Kutai Timur	Kalimantan	Indonesia	Pisang Kepok	ABB
	1	[1,3]InaCC F817	Kutai Timur	Kalimantan	Indonesia	P. Kepok	ABB
	1	[1]InaCC F818	Kutai Timur	Kalimantan	Indonesia	P. Kepok	ABB
	1	[1]InaCC F819	Kutai Timur	Kalimantan	Indonesia	P. Ambon	AAA
	1	[1]InaCC F821	Kutai Timur	Kalimantan	Indonesia	P. Raja	AAB
	1	[3]InaCC F822	Kutai Timur	Kalimantan	Indonesia	P. Raja	AAB
	1	[1]InaCC F825	Kutai Timur	Kalimantan	Indonesia	P. Raja	AAB
	1	[1,3]InaCC F836	Lumajang	Java	Indonesia	P. Mas Kirana	AA
	1	InaCC F837	Lumajang	Java	Indonesia	P. Mas Kirana	AA
	1	InaCC F838	Lumajang	Java	Indonesia	P. Mas Kirana	AA
	1	InaCC F839	Lumajang	Java	Indonesia	P. Mas Kirana	AA
	1	[1]InaCC F840	Lumajang	Java	Indonesia	P. Embuk	ABB
	1	InaCC F841	Lumajang	Java	Indonesia	P. Embuk	ABB
	1	InaCC F846	Purwodadi	Java	Indonesia	P. Susu	AAB
	1	InaCC F847	Purwodadi	Java	Indonesia	P. Susu	AAB
	1	[3]InaCC F856	Cianjur	Java	Indonesia	P. Siem	ABB
	1	InaCC F857	Cianjur	Java	Indonesia	P. Siem	ABB
	1	InaCC F858	Cianjur	Java	Indonesia	P. Siem	ABB
	1	InaCC F864	Sukabumi	Java	Indonesia	P. Siem	ABB
	1	InaCC F865	Sukabumi	Java	Indonesia	P. Siem	ABB
	1	[1]InaCC F870	Kendal	Java	Indonesia	P. Susu	AAB
	1	[1]InaCC F871	Kendal	Java	Indonesia	P. Susu	AAB
	1	InaCC F873	Kendal	Java	Indonesia	P. Susu	AAB
	1	[3]InaCC F874	Kendal	Java	Indonesia	P. Susu	AAB
	1	[3]InaCC F875	Kendal	Java	Indonesia	Cavendish	AAA
	1	[1]InaCC F876	Semarang	Java	Indonesia	Cavendish	AAA

Table 1. (Continued).

	1	InaCC F877	Semarang	Java	Indonesia	P. Susu	AAB
	1	InaCC F878	Semarang	Java	Indonesia	P. Susu	AAB
	1	[1]InaCC F879	Semarang	Java	Indonesia	P. Susu	AAB
	1	InaCC F880	Semarang	Java	Indonesia	P. Ambon	AAA
	1	InaCC F881	Semarang	Java	Indonesia	P. Ambon	AAA
	1	InaCC F882	Semarang	Java	Indonesia	P. Ambon	AAA
	1	InaCC F883	Semarang	Java	Indonesia	P. Ambon	AAA
	1	InaCC F885	Demak	Java	Indonesia	P. Raja	AAB
	1	[3]InaCC F890	Kendal	Java	Indonesia	P. Kepok	ABB
	1	[3]InaCC F891	Purwodadi	Java	Indonesia	P. Glitung	-
	1	InaCC F892	Aceh Besar	Sumatra	Indonesia	P. Barangan	AAA
	1	InaCC F893	Aceh Besar	Sumatra	Indonesia	P. Barangan	AAA
	1	InaCC F894	Aceh Besar	Sumatra	Indonesia	P. Barangan	AAA
	1	InaCC F896	Aceh Besar	Sumatra	Indonesia	P. Wak	ABB
	1	InaCC F898	Aceh Besar	Sumatra	Indonesia	P. Barangan	AAA
	1	InaCC F899	Aceh Besar	Sumatra	Indonesia	P. Barangan	AAA
	1	InaCC F900	Aceh Besar	Sumatra	Indonesia	P. Kepok	ABB
	1	[3]InaCC F901	Aceh Besar	Sumatra	Indonesia	P. Kepok	ABB
	1	InaCC F902	Aceh Besar	Sumatra	Indonesia	P. Talon	AAB
	1	[3]InaCC F903	Aceh Besar	Sumatra	Indonesia	P. Kepok	ABB
	1	InaCC F904	Aceh Besar	Sumatra	Indonesia	P. Kepok	ABB
	1	InaCC F905	Ogan Ilir	Sumatra	Indonesia	P. Barangan	AAA
	1	InaCC F906	Ogan Ilir	Sumatra	Indonesia	P. Barangan	AAA
	1	[3]InaCC F907	Ogan Ilir	Sumatra	Indonesia	P. Tanduk	AAB
	1	InaCC F908	Ogan Ilir	Sumatra	Indonesia	P. Tanduk	AAB
	1	InaCC F909	Ogan Ilir	Sumatra	Indonesia	P. Mas	AA
	1	InaCC F910	Ogan Ilir	Sumatra	Indonesia	P. Mas	AA
	1	InaCC F912	Kutai Timur	Kalimantan	Indonesia	P. Ambon	AAA
	1	InaCC F919	Kutai Timur	Kalimantan	Indonesia	P. Awak	ABB
	1	InaCC F923	Jayapura	Papua	Indonesia	P. Raja	AAB
	1	InaCC F924	Jayapura	Papua	Indonesia	P. Raja	AAB
	1	InaCC F925	Jayapura	Papua	Indonesia	P. Raja	AAB

Table 1. (Continued).

1	InaCC F926	Jayapura	Papua	Indonesia	P. Raja	AAB
1	InaCC F927	Jayapura	Papua	Indonesia	P. Raja	AAB
1	InaCC F928	Jayapura	Papua	Indonesia	P. Raja	AAB
1	[3]InaCC F929	Jayapura	Papua	Indonesia	P. Tanduk	AAB
1	[1]InaCC F930	Jayapura	Papua	Indonesia	P. Tanduk	AAB
1	[3]InaCC F931	Jayapura	Papua	Indonesia	P. Tanduk	AAB
1	InaCC F932	Jayapura	Papua	Indonesia	P. Tanduk	AAB
1	InaCC F933	Barru	Sulawesi	Indonesia	P. Kepok	ABB
1	[3]InaCC F934	Barru	Sulawesi	Indonesia	P. Kepok	ABB
1	InaCC F935	Barru	Sulawesi	Indonesia	P. Ambon	AAA
1	InaCC F936	Barru	Sulawesi	Indonesia	P. Ambon	AAA
1	InaCC F937	Barru	Sulawesi	Indonesia	P. Ambon	AAA
1	InaCC F938	Barru	Sulawesi	Indonesia	P. Ambon	AAA
1	InaCC F939	Barru	Sulawesi	Indonesia	P. Ambon	AAA
1	[3]InaCC F942	Sikka	Flores	Indonesia	P. Barangan	AAA
1	InaCC F943	Sikka	Flores	Indonesia	P. Barangan	AAA
1	InaCC F944	Sikka	Flores	Indonesia	P. Barangan	AAA
1	[3]InaCC F945	Sikka	Flores	Indonesia	P. Barangan	AAA
1	[3]InaCC F946	Sikka	Flores	Indonesia	P. Barangan	AAA
1	[1]InaCC F947	Sikka	Flores	Indonesia	P. Barangan	AAA
1	InaCC F948	Sikka	Flores	Indonesia	P. Barangan	AAA
1	InaCC F953	Sikka	Flores	Indonesia	P. Kepok	ABB
1	InaCC F954	Sikka	Flores	Indonesia	P. Kepok	ABB
1	InaCC F955	Sikka	Flores	Indonesia	P. Kepok	ABB
1	InaCC F973	Kutai Timur	Kalimantan	Indonesia	P. Kepok	ABB
1	InaCC F985	Kendal	Java	Indonesia	P. Kepok	ABB
1	InaCC F986	Kendal	Java	Indonesia	P. Kepok	ABB
1	InaCC F988	Kendal	Java	Indonesia	P. Kepok	ABB
1	InaCC F989	Kendal	Java	Indonesia	P. Kepok	ABB
1	InaCC F990	Kendal	Java	Indonesia	P. Kepok Pipik	ABB
1	InaCC F994	Lumajang	Java	Indonesia	P. Mas Kirana	AA

Table 1. (Continued).

	1	[1]Indo4	Kutai Timur	Kalimantan	Indonesia	P. Ambon	AAA
	1	Indo51	Sukabumi	Java	Indonesia	P. Siem	ABB
	1	Indo53	Sukabumi	Java	Indonesia	P. Siem	ABB
	1	Indo61	Kendal	Java	Indonesia	P. Susu	AAB
	1	Indo62	Kendal	Java	Indonesia	P. Susu	AAB
	1	Indo66	Kendal	Java	Indonesia	P. Susu	AAB
	1	Indo77	Demak	Java	Indonesia	P. Kepok Pipik	ABB
	1	Indo89	Aceh Besar	Sumatra	Indonesia	P. Wak	ABB
	1	Indo204	Sukabumi	Java	Indonesia	P. Uli	AA
	1	[2,3]II5	Central Sulawesi, Luwa District	Sulawesi	Indonesia	P. Manurung	ND
	1	[2,5]JV11	Jordan Valley	ND	Jordan	Cavendish	ND
	1	[2,5]Leb1.2C	Berghliyeh	ND	Lebanon	Cavendish	ND
	1	[2,5]Pak1.1A	Baoo Pooran	ND	Pakistan	Cavendish	ND
	1	[2,5]Phi2.6C	Davao MADC Clone Trial Area	ND	Philippines	GCTCV218	ND
	2	[2,5]NRRL 36102	Taiwan	ND	China	Cavendish	ND
F. phialophorum	3	[2,5]NRRL 36103	ND	ND	Philippines	Cavendish	ND
	4	[2,5]NRRL 36110	Queensland	ND	Australia	Mons	ND
	5	InaCC F826	Tanah Bumbu	Kalimantan	Indonesia	P. Awak	ABB
	5	[1]InaCC F827	Tanah Bumbu	Kalimantan	Indonesia	P. Awak	ABB
	5	[1]InaCC F830	Kubu Raya	Kalimantan	Indonesia	P. Kepok	ABB
	5	[1]InaCC F834	Pontianak	Kalimantan	Indonesia	P. Selendang	AAA
	5	[1]InaCC F842	Lumajang	Java	Indonesia	P. Embuk	ABB
	5	[1]InaCC F843	Lumajang	Java	Indonesia	P. Embuk	ABB
	5	[4]InaCC F844	Purwodadi	Java	Indonesia	P. Susu	AAB
	5	InaCC F845	Purwodadi	Java	Indonesia	P. Susu	AAB
	5	InaCC F889	Sukabumi	Java	Indonesia	P. Ambon Kuning	AAA
	5	InaCC F969	Tanah Bumbu	Kalimantan	Indonesia	P. Awak	ABB
	5	InaCC F970	Tanah Bumbu	Kalimantan	Indonesia	P. Awak	ABB
	5	[1,4]InaCC F971	Tanah Bumbu	Kalimantan	Indonesia	P. Awak	ABB

Table 1. (Continued).

	5	InaCC F972	Tanah Bumbu	Kalimantan	Indonesia	P. Awak	ABB
	5	InaCC F980	Kubu Raya	Kalimantan	Indonesia	P. Kepok	ABB
	5	InaCC F981	Kubu Raya	Kalimantan	Indonesia	P. Kepok	ABB
	5	InaCC F982	Kubu Raya	Kalimantan	Indonesia	P. Kepok	ABB
	5	InaCC F987	Kendal	Java	Indonesia	P. Kepok	ABB
	5	InaCC F995	Lumajang	Java	Indonesia	P. Kongkong	ND
	5	[4]InaCC F996	Lumajang	Java	Indonesia	P. Kongkong	ND
	5	Indo64	Kendal	Java	Indonesia	P. Susu	AAB
	5	[2,5]NRRL 36101	Queensland	ND	Australia	Mons Mari	ND
	5	[2,5]NRRL 36109	Queensland	ND	Australia	SH 3142	ND
	5	[2,5]NRRL 36112	Burgershall.hazyview	ND	South Africa	Cavendish	ND
	5	[2,5]ST4	Canary Island	ND	Spain	Dwarf Cavendish	AAA
F. purpurascens	6	[1]InaCC F823	Kutai Timur	Kalimantan	Indonesia	P. Kepok	ABB
	6	[4]InaCC F886	Kutai Timur	Kalimantan	Indonesia	P. Kepok	ABB
	6	InaCC F913	Kutai Timur	Kalimantan	Indonesia	P. Kepok	ABB
	6	InaCC F914	Kutai Timur	Kalimantan	Indonesia	P. Kepok	ABB
	6	InaCC F966	Kutai Timur	Kalimantan	Indonesia	P. Kepok	ABB
	6	[1]InaCC F967	Kutai Timur	Kalimantan	Indonesia	P. Kepok	ABB
	6	[1]InaCC F968	Kutai Timur	Kalimantan	Indonesia	P. Kepok	ABB
	6	[2,5]NRRL 36107	ND	ND	Honduras	Maqueno	ND
F. sangayamense	7	InaCC F960	Kota Baru	Kalimantan	Indonesia	P. Kepok	ABB
	7	InaCC F961	Kota Baru	Kalimantan	Indonesia	P. Kepok	ABB
F. Kalimantanense	8	InaCC F917	Katingan	Kalimantan	Indonesia	P. Ambon	AAA
	8	InaCC F918	Katingan	Kalimantan	Indonesia	P. Ambon	AAA
	8	InaCC F922	Katingan	Kalimantan	Indonesia	P. Ambon	AAA
F. tardichlamydosporum	9	InaCC F956	Sikka, Flores	Flores	Indonesia	P. Barangan	AAA
	9	InaCC F957	Sikka, Flores	Flores	Indonesia	P. Barangan	AAA
	9	[1,4]InaCC F958	Sikka, Flores	Flores	Indonesia	P. Barangan	AAA
	9	InaCC F959	Sikka, Flores	Flores	Indonesia	P. Barangan	AAA
	10	[2,5]NRRL 36108	Tenquero Staion	ND	Tanzania	Ney Poovan	ND

Table 1. (Continued).

	11	[2,5]NRRL 36106	Queensland, Currumbin	ND	Australia	Lady finger	ND
	11	[2,5]NRRL 36105	ND	ND	Honduras	Bluggoe	ND
	11	[2,5]NRRL 36111	Queensland, South Johnstone	ND	Australia	Bluggoe	ND
	11	[2,5]NRRL 36117	Penang Island Panta Acheh	ND	Malaysia	P. Awak legor	ND
F. cugenangense	12	[2,5]NRRL 36118	North of Chiang Rai, hwy 1	ND	Thailand	Kluai nam wa	ND
	13	InaCC F983	Cianjur, West Java	Java	Indonesia	P. Kepok	ABB
	13	InaCC F984	Cianjur, West Java	Java	Indonesia	P. Kepok	ABB
F. tardicrescens	14	[2,5]NRRL 36113	Misuki Hills, Karonga,	ND	Malawi	Harare	ND
F. grosmichelii	15	InaCC F832	Pontianak	Kalimantan	Indonesia	P. Awak	ABB
	15	[1]InaCC F833	Pontianak	Kalimantan	Indonesia	P. Awak	ABB
	15	[1,4]InaCC F848	Bogor	Java	Indonesia	P. Ambon	AAA
	15	[1]InaCC F849	Bogor	Java	Indonesia	P. Ambon	AAA
	15	[1]InaCC F850	Bogor	Java	Indonesia	P. Ambon	AAA
	15	[1]InaCC F851	Bogor	Java	Indonesia	P. Ambon	AAA
	15	[1]InaCC F852	Bogor	Java	Indonesia	P. Ambon Lumut	AAA
	15	InaCC F853	Bogor	Java	Indonesia	P. Ambon Lumut	AAA
	15	InaCC F854	Bogor	Java	Indonesia	P. Ambon Lumut	AAA
	15	InaCC F855	Bogor	Java	Indonesia	P. Ambon Lumut	AAA
	15	InaCC F859	Cianjur	Java	Indonesia	Cavendish	AAA
	15	InaCC F860	Cianjur	Java	Indonesia	Cavendish	AAA
	15	[1]InaCC F861	Cianjur	Java	Indonesia	Cavendish	AAA
	15	InaCC F862	Cianjur	Java	Indonesia	Cavendish	AAA
	15	InaCC F863	Sukabumi	Java	Indonesia	P. Siem Jumbo	ABBB
	15	[1]InaCC F867	Sukabumi	Java	Indonesia	P. Ambon Kuning	AAA
	15	InaCC F868	Sukabumi	Java	Indonesia	P. Ambon Kuning	AAA
	15	InaCC F884	Semarang	Java	Indonesia	P. Ambon	AAA
	15	InaCC F887	Sukabumi	Java	Indonesia	P. Siem Jumbo	ABBB
	15	InaCC F888	Sukabumi	Java	Indonesia	P. Siem Jumbo	ABBB

Table 1. (Continued).

	15	Indo83	Bogor, West Java	Java	Indonesia	P. Kepok	ABB
	15	[2,5]NRRL 36120	Yala Prov., hwys 410 x 4063	ND	Thailand	Kluai nam wa	ND
F. duoseptatum	16	[1]InaCC F831	Kubu Raya	Kalimantan	Indonesia	P. Kepok	ABB
	16	InaCC F835	Pontianak	Kalimantan	Indonesia	Dwarf Cavendish	AAA
	17	InaCC F911	Kutai Timur	Kalimantan	Indonesia	P. Ambon	AAA
	17	[2,5]NRRL 36115	Kuching, Seman Matang, Sarawak	Kalimantan	Malaysia	P. ambon	ND
	17	[2,5]NRRL 36116	Kuching, Sarawak	Kalimantan	Malaysia	P. Keling	ND
	18	[2,5]Mal43	ND	ND	Malaysia	P. Rastali	AAB
	19	[1]InaCC F828	Katingan	Kalimantan	Indonesia	P. Awak	ABB
	19	InaCC F829	Katingan	Kalimantan	Indonesia	P. Awak	ABB
	19	InaCC F915	Kutai Timur	Kalimantan	Indonesia	P. Raja	AAB
	19	[4]InaCC F916	Kapuas	Kalimantan	Indonesia	P. Kepok	AAB
	19	InaCC F920	Katingan	Kalimantan	Indonesia	P. Hawa	ABB
	19	InaCC F921	Katingan	Kalimantan	Indonesia	P. Hawa	ABB
	19	InaCC F976	Katingan	Kalimantan	Indonesia	P. Awak	ABB
	19	InaCC F977	Katingan	Kalimantan	Indonesia	P. Susu	AAB
	19	InaCC F978	Katingan	Kalimantan	Indonesia	P. Susu	AAB
	19	[4]InaCC F979	Katingan	Kalimantan	Indonesia	P. Susu	AAB
	19	[1]InaCC F980	Katingan	Kalimantan	Indonesia	P. Susu	AAB
	19	Indo80	Katingan	Kalimantan	Indonesia	P. Hawa	ABB

[1] Isolates used for technical repeats of DArT assays.
[2] Isolates used for biological repeats of DArT assays.
[3] Highly pathogenic on Cavendish, TR4 isolate (Maryani et al. 2018).
[4] Highly pathogenic on Gros Michel, Race1 isolate (Maryani et al. 2019).
[5] Pathogenicity tests by Ordóñez 2018.

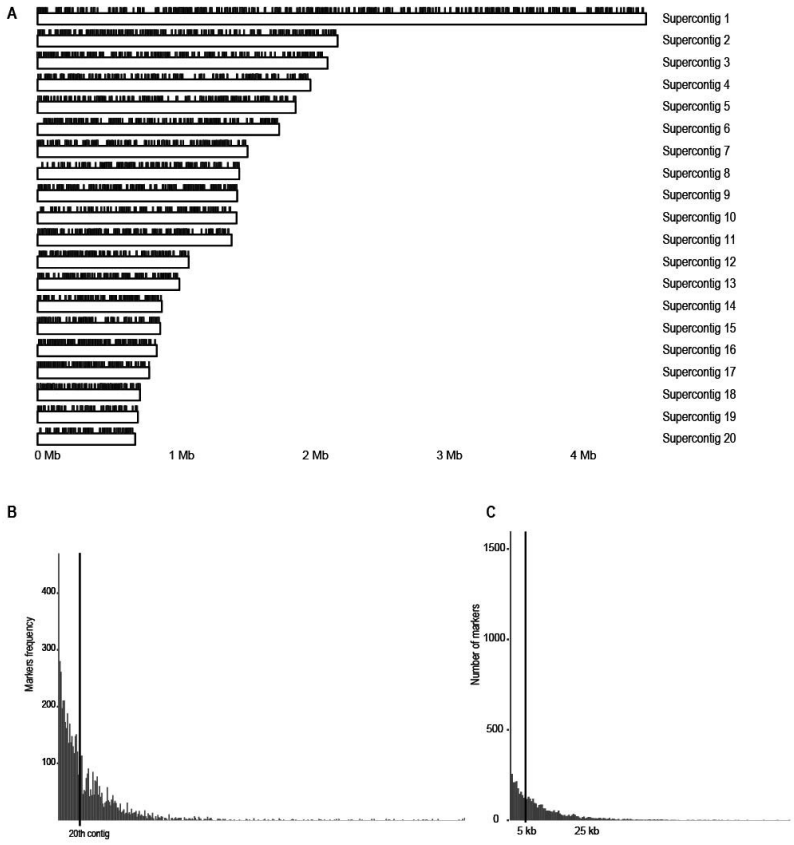

Fig. 2. Mapping of DArTSeq markers on the *Fusarium odoratissimum* II5 reference genome. **A.** Placement of DArTSeq markers on the longest 20 supercontigs. **B.** Number of markers per scaffold. **C.** Marker distances.

Identification of DArT Genotypic Group (DGG) across *Fusarium* species isolated from banana

To determine the genetic diversity among the isolates across species, we used all 13 150 polymorphic DArTseq markers to perform distance based hierarchical cluster analysis, which distributed the isolates over three of the five clades of the *Fusarium oxysporum* species complex (FOSC) (Fig. 3), hereafter denoted as Clades 1, 2, and 5 in concordance with the phylogeny of FOSC *sensu* O'Donnell *et al.* (1998, 2004) and Maryani *et al.* (2019). This encompasses 10 new phylogenetic species in the FOSC (Maryani *et al.* 2019; Table 1). All isolates were distributed over the three clades irrespective of their geographical origin and sampled host genotype (Fig. 3), demonstrating the high genetic diversity of Indonesian *Fusarium* spp. associated with Fusarium wilt in indigenous Indonesian bananas.

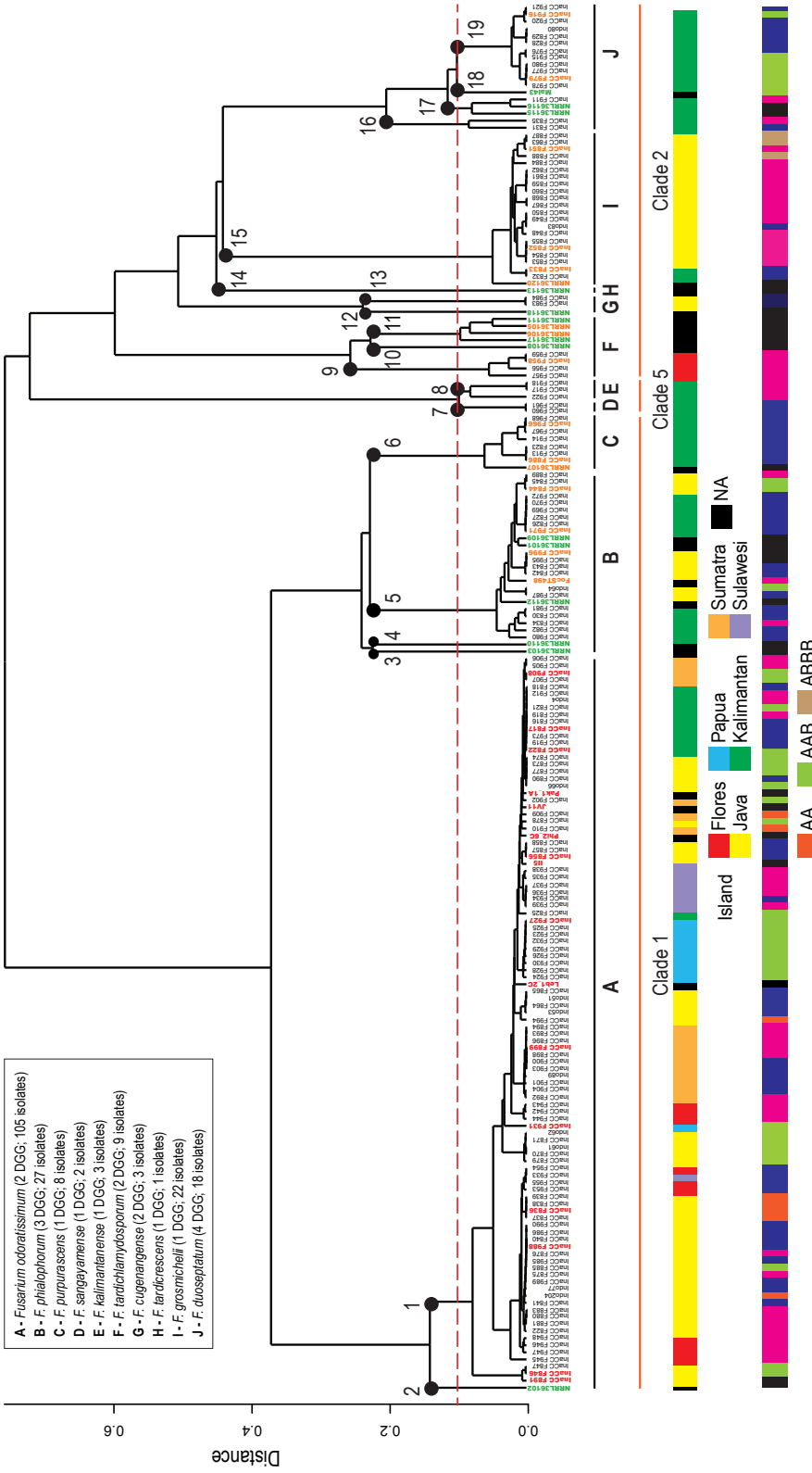

Fig. 3. Identified DArT Genotype Groups across 10 *Fusarium* spp. that were sampled from Fusarium wilt affected banana plants across Indonesia, with their geographical and host origins. Isolates in green have been phenotyped for vegetative compatibility. Isolates in red represent Tropical Race 4 strains and isolates in orange represent Race 1 strains. Indicated clades (1, 2 and 5) are according to the phylogeny of the *Fusarium oxysporum* species complex sensu O'Donnell et al. (1998, 2004) and Maryani *et al.* (2018a).

We identified 19 distinct DGGs along the tree (Fig. 3). The entire collection of Indonesian isolates is distributed over at least 10 of the 19 DGGs. Each *Fusarium* species contained a variable number of DGGs. The largest number of DGGs was observed in *Fusarium duoseptatum* with four DGGs, followed by *F. phialophorum* and *F. tardichlamidosporum* that have three DGGs and *F. odoratissimum* and *F. cugenangense,* which comprise two DGGs (Fig. 3). To assess the distribution of different DGGs in the known FOSC clades, we calculated the number of DGGs of each clade. Six DGGs were placed in Clade 1, including DGG1 which contained 55 % of the Indonesian isolates. Eleven DGGs were identified in Clade 2, with the Indonesian isolates belonging to six DGGs and two DGGs were positioned in Clade 5.

Indonesian isolates in DGG1, which contains all previously known/described TR4 isolates, are highly aggressive to Cavendish banana (Table 1; Fig. 3; Maryani *et al.* 2019) and originated from geographically very diverse environments (i.e. different Indonesian islands) mostly on Java and Sumatra (Fig. 1). This DGG was isolated from diverse hosts comprising 37 different local banana varieties, including diploid and triploid genotypes (Fig. 3; Table 1).

Unlike TR4 isolates, Race 1 isolates, which are highly aggressive to the banana variety Gros Michel (Table 1; Fig. 3) were placed in six different DGGs. These DGGs clustered in Clade 1 and Clade 2 of FOSC, suggesting that Race 1 evolved independently multiple times. Race 1 isolates of *F. duoseptatum*, contained DGG16, DGG17, DGG18 and DGG19 and were shown to be geographically isolated. Isolates belonging to these DGGs, as well as the reference strains NRRL 36116 and NRRL 36115, were obtained only from Kalimantan. Similarly, DGG9 contained only isolates from Flores, and DGG13 contained isolates from Java. Interestingly, the majority of the host genotypes from which these isolates were sampled are triploid banana species (Fig. 3).

To determine whether the VCG-typed isolates are circumscribing the genotype differences in the Indonesian population, we included reference strains in the DArTSeq analysis. This set of isolates is known to capture the worldwide diversity of *Fusarium* spp. affecting banana (Ordóñez *et al.* 2015). Cluster analyses of DArTseq markers demonstrated that previously identified VCGs are discordant with genotypic differences among isolates, as one DGG might include more than one VCG, e.g. DGG5 and DGG11 contain four VCGs and DGG17 comprises two VCGs. However, seven DGGs contained only one VCG. Therefore, the VCG-typed reference isolates do not necessarily represent different genotypes as defined by distinct DGGs, and thus DArTSeq markers were unable to resolve the VCG classification in *Fusarium* species causing wilt on banana.

DISCUSSION

Molecular techniques enable high-resolution diversity analyses amongst individuals of a given population (Milgroom 2015). An ideal molecular marker is independent, polymorphic, evenly distributed across the genome and is repeatable and unambiguous to score (Brown 1996, Schlötterer 2004). Here we demonstrate that DArTseq meets these criteria, and thus is a suitable tool to assess genetic variation amongst *Fusarium* spp. that are associated with banana plants affected by Fusarium wilt. Besides the number of generated markers (>10,000), DArTSeq has the advantage - contrary to other existing molecular technologies - that it allows parallel instead of serial analyses of marker data (Wittenberg 2007). With a genome size of ~46.5 MB (reference genome II5), our analysis of *Fusarium* spp. populations yielded a total of 13 150 polymorphic markers. This number is considerably higher than in other banana pathogens, such as *Pseudocercospora fijiensis*, the Black Sigatoka pathogen of banana, with a genome size of 74 Mb (Isaza *et al*. 2016), and from which a total 6 586 DArTseq polymorphic markers were obtained (Chong 2016). DArT markers were also reported to provide good genome coverage in wheat and barley (Wenzl *et al.* 2004), *Arabidopsis* (Wittenberg *et al.* 2005), and the wheat pathogen *Zymoseptoria tritici* (previously *Mycosphaerella graminicola*) (Wittenberg *et al.* 2007, Kema *et al.* 2018). Thus, the excellent genome coverage of DArTseq markers is an important advantage for our diversity analyses, as the genetic diversity assessment is expected to be more robust and reliable.

Hierarchical clustering of the 196 isolates using DArTseq markers as a proxy of genetic relatedness resulted in the identification of 19 DGGs. Moreover, these analyses provided a good resolution of genomic variation (i.e. genotype) within the 10 species that were recently described by Maryani *et al.* (2019). Thus, we conclude that our current data strongly support the phylogenetic species concept of *Fusarium* spp. affecting banana. The current 19 DGGs significantly extend diversity and also the most extensive genotypic diversity of *Fusarium* spp. affecting banana reported to date. Bentley *et al.* (1998) reported six genotype groups discerned in a worldwide collection, which was based on DNA fingerprinting. The same collection was subsequently studied by Groenewald *et al.* (2006) who identified seven AFLP genotypic groups. Recently Mostert *et al.* (2017) distinguished just six RFLP groups in an Asian collection. Therefore, technology seems to determine the resolution of biological complexity and hence, genotyping-by-sequencing is currently an outstanding technology for high-throughput diversity analyses of *Fusarium* spp. associated with banana, particularly in the centre of diversity where sympatric speciation is expected (Stukenbrock *et al.* 2007, Foote 2018).

The majority of the Indonesian isolates was identified as TR4 and clustered in a single group (DGG1) *F. odoratissimum*. These isolates were recovered from many local banana varieties across the Indonesian archipelago. The higher incidence of TR4 isolates could be explained by the ability to infect a wide range of banana varieties, thus raising the chance to

capture this specific race (Maryani *et al.* 2019). Therefore, TR4 strains are expected to dominate the Indonesian population, which corroborates with a previous report by Hermanto *et al.* (2009), who concluded that TR4 is well distributed across all the Indonesian islands, with the majority of isolates belonging to VCGs 01213/01216. However, this study comprised 100 isolates sampled from 14 banana accessions on 5 islands and was only based on VCGs. Here, DArTseq and cluster analyses clearly distinguish TR4 from the other sampled *Fusarium* spp. and assigns them unequivocally to *F. odoratissimum* (Maryani *et al.* 2019) showing that TR4 isolates share a similar genetic background. That observation that was also evident from phylogeographical analyses in the Greater Mekong area (Zheng *et al.* 2018) and recently developed diagnostics (Salacinas *et al.* 2018, Maryani *et al.* 2018). Additionally, our results confirm that Race 1 strains belong to different DGGs, recently recognized as several *Fusarium* species (Maryani *et al.* 2019). The diversity of Race 1 might be the result of its exposure to many different host varieties. A host population exerts selection pressure on pathogen populations, especially in different agroecosystems (Hansen 1987). Another factor which might contribute to Race 1 diversity is its soil dwelling nature that could facilitate horizontal gene transfer. It is well-known that *Fusarium oxysporum* undergoes horizontal gene transfer (Michielse & Rep 2009, Ma *et al.* 2010, Van Dam *et al.* 2016) and recent effector studies suggested a similar phenomenon in banana infecting *Fusarium* strains (Czislowski *et al*. 2018). Thus, in a plantation with different banana varieties, there is a higher chance for horizontal gene transfer between pathogenic strains once they occupy the same niche (Vlaardingerbroek *et al.* 2016).

We demonstrate that VCG classification is not reflecting genetic diversity of *Fusarium* isolates affecting banana. Of the 20 VCGs that we included in the analyses (Ordóñez *et al.* 2015), only seven VCGs were placed in single DGGs, while the others comprised three to six VCGs. This is in accordance with Fourie *et al.* (2009) who demonstrated that more than one VCG could cluster within a single lineage. Therefore, VCG determination among isolates might reflect genetic similarities but hide genetic differences (Kistler 1997, Fourie *et al.* 2011). Our results also suggest that the evolutionary origin of *Fusarium* spp. affecting banana is driven by two processes. Firstly, our data corroborate the generally accepted hypothesis that *Fusarium* co-evolves with its banana host in South-East Asia (Stover 1962, Ploetz & Pegg 1997, Buddenhagen 2007). Population studies of fungal pathogens have reported that co-evolution normally occurs in the centre origin of the hosts (Wyand & Brown 2003, Stukenbrock & McDonald 2008). For example, as *Magnaporthe grisea* displays higher diversity among Asian isolates where the host likely originated (Tharreau *et al.* 2009). Populations of the wheat pathogen *Z. tritici* were highly diverse in the fertile crescent where the origin and domestication of cereals occurred (Stukenbrock *et al.* 2007). Hence, our data representing the largest number of *Fusarium* spp. and genotypes to date, which were isolated from many local varieties in the centre origin of banana strongly suggest such a co-evolutionary process in the

Indonesian archipelago. Secondly, O'Donnell *et al.* (1998) suggested that this pathogen also evolves independently in areas where the pathogen was introduced and geographic isolation has occurred. This is consistent with distinct DGGs for isolates outside Indonesia, e.g. Australia, Malawi, Taiwan, Tanzania and USA. Interestingly, one of the Race 1 lineages identified in Brazil (Dita *et al.* 2010) and another regional collection in Latin America (Ordóñez *et al.* 2018), were not present in the Indonesian *Fusarium* spp. identified in our study. This strongly suggests ongoing secondary or allopatric speciation of banana infecting *Fusarium* spp. outside Indonesia.

Given the ongoing epidemic of Fusarium wilt in banana (Maryani *et al.* 2019, Ordóñez *et al.* 2015, Zheng *et al.* 2018), we plan future studies to decipher the pathogenicity of this extensive *Fusarium* collection on a suite of different (indigenous) banana varieties. Combining these datasets will provide more insight in the evolutionary process of host adaptation in the centre of origin of banana and is crucial for any breeding program aiming for new and resilient banana varieties (Pillay & Tenkouano 2011, Brown *et al.* 2017, http://www.promusa.org/INIBAP). Our study highlights the importance of analysing indigenous and international *Fusarium* populations to capture and unravel global diversity as a component towards sustainable banana production.

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Chapter 4

Pathogenic diversity of Indonesian Fusarium wilt pathogens in wild and cultivated bananas

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Abstract

Fusarium wilt of banana is caused by a group of *Fusarium* species in the *Fusarium oxysporum* species complex (FOSC). Recent diversity analyses on an extensive *Fusarium* collection from the Indonesian archipelago, which is the centre of diversity of banana and hence from co-evolving *Fusarium* species, revealed eleven new *Fusarium* species that were affecting banana. Here we assess the pathogenic variation of 78 isolates from these species on the four banana accessions Pisang Ambon Hijau (*Musa acuminata*, AAA, Cavendish subgroup 'Grand Naine'), Pisang Ambon Kuning (*Musa acuminata*, AAA, Gros Michel subgroup 'Gros Michel'), *Musa acuminata* ssp. *malaccensis* 'Pahang', and Pisang Rejang (*Musa acuminata*, AA, Sucrier subgroup 'Rose'). All isolates that are pathogenic on Grand Naine and Gros Michel belong to *Fusarium odoratissimum*, previously known as Tropical Race 4 (TR4), which also showed quantitative variation for aggressiveness. Isolates pathogenic on Gros Michel are more genetically diverse and belong to six *Fusarium* species. Taken together, these data showed significant isolate – banana accession interactions, indicating an underlying gene-for-gene (GFG) system. We also demonstrate the overall resistance of *Musa acuminata* ssp. *malaccensis* (Pahang) and Pisang Rejang to this suite of *Fusarium* species and even to the most aggressive TR4 isolates. Our study provides a first insight in pathogenic variation among indigenous *Fusarium* species to endemic banana accessions in Indonesia. The wide resistance in *M. acuminata* ssp. *malaccensis* and Pisang Rejang is promising for breeding programs aiming for new and resistant banana varieties.

Key words: Banana, *Fusarium odoratissimum*, phenotyping, Tropical Race 4, virulence

INTRODUCTION

Fungal plant pathogens are among the major constrains of global food production (Gurr *et al.* 2011). Particularly since the emergence of fungal strains that are resistant to fungicides, which threaten food security and urges for innovations and integrated management to control their spread (Fisher *et al.* 2018). This applies to the commonly known cereal staple foods, but also to crops such as banana. Banana is the number one fruit commodity in the world and also a staple food in many (sub)tropical countries (http://faostat.org/). Therefore, sustaining banana production is important to supply domestic and international markets to also support the livelihood of millions of smallholder farmers. However, banana is threatened by Fusarium wilt or Panama disease, one of the most devastating diseases (Simmonds 1962), that recently re-emerged in this crop (Ordóñez *et al.* 2015).

The disease is caused by a suite of soil-borne fungi belongs to the *Fusarium oxysporum* species complex (FOSC), which has been reported in all banana producing areas across the globe (Ploetz 1994, Ordóñez *et al.* 2015, Maryani *et al.* 2019). The decline of global banana production as a result of the disease significantly impacts food and employment security (http://www.fao.org/world-banana-forum/). The previous epidemic in the late 1960's was largely confined to Latin America where it destroyed hundreds of Gros Michel plantations, and is considered as one of the most destructive botanical epidemics in history (Ploetz 2015). The banana industry was eventually saved by planting Cavendish varieties that are resistant to the so-called Race 1, which effectively comprises a group of genetically diverse species with pathogenicity towards Gros Michel (Ordóñez *et al.* 2015, Maryani *et al.* 2019). However, already in the 1960s another genotype, colloquially named Tropical Race 4 (TR4), was found to kill Cavendish plants in Taiwan (Su *et al.* 1986). Since then, this virulent strain has disseminated regionally, internationally and even intercontinentally (Moore *et al.* 1991, Hermanto *et al.* 2009, Ordóñez *et al.* 2015, Zheng *et al.* 2018), and therefore there have been urgent calls for more attention and effort to study this pathogen and prevent further dissemination (Kema & Weise, 2013).

Studies on the diversity of fungi causing Fusarium wilt in the banana suggest that these co-evolved with the host in the centre of origin of banana in South-East Asia (Simmonds 1962, O'Donnell *et al.* 1998, Groenewald *et al.* 2006, Maryani *et al.* 2019). One of the earliest reports of Fusarium wilt on banana was in 1916, in a banana plantation in Java which from there disseminated to the African continent via Madagascar and to the American continent through Suriname (Simmonds 1962, Ploetz & Pegg 1997, Buddenhagen 2007). This was recently supported by Maryani *et al.* (2019) who showed that eight out of nine globally disseminated *Fusarium* species are indigenous in Indonesia. Therefore, Indonesia is a key area for the discovery of new sources of resistance and for pathogen population diversity analyses in order to better understand the banana – *Fusarium* pathosystem.

Many molecular studies report genetic diversity of *Fusarium* spp. affecting banana (Fourie *et al.* 2009, O'Donnell *et al.* 1998, Mostert *et al.* 2017). Maryani *et al.* (2019) recently identified eleven phylogenetic species in the FOSC isolated from local banana varieties suffered with Fusarium wilt in Indonesia. Using a genotyping-by-sequencing approach each of these species was shown to contain extensive genotypic diversity across Indonesia (Maryani *et al.* 2018). However, the relationship with pathogenicity towards different banana varieties is unknown. Traditionally three physiological races are recognized in the banana – *Fusarium* pathosystem. Race 1 is pathogenic on Gros Michel (AAA) and Silk (AAB) varieties and Race 2 is pathogenic on Bluggoe (ABB) (Stover & Waite 1960). Race 4 is pathogenic on Cavendish (AAA) varieties as well as on a wide range of local banana varieties in the tropics and was subdivided in the aggressive Tropical Race 4 (TR4) (Ploetz 1994, Gerlach *et al.* 2000) and subtropical Race 4 (ST4) that affects Cavendish merely under abiotic stresses (Ploetz 2005).

Traditionally, race identifications and nomenclature in fungal pathogens are based on large interaction data sets, which were later unravelled by the identification of resistance and (a)virulence genes that frequently comply with gene-for-gene (GFG) or inverse GFG systems (Person 1955, Friesen *et al.* 2008, Kema *et al.* 2018). However, the genetic basis of the race concept in the banana – *Fusarium* pathosystem is poorly understood. The first gene for resistance to Fusarium wilt in banana was only recently identified (Dale *et al.* 2017), and the understanding of pathogenicity is in its initial stages (Guo *et al.* 2015, Czislowski *et al.* 2018). Alternative systems based on the level of aggressiveness on a set of host cultivars which some consider likely for the banana – *Fusarium* pathosystem (Stover & Buddenhagen 1986, Larkin 1990, Correll 1991) are in our opinion largely due to the lack of (genetic) data and poor phenotyping protocols under variable conditions. Hence, the race concept of Fusarium wilt in banana is unclear and lacks accuracy. Here, we initiate a phenotyping program by screening many isolates of different *Fusarium* species causing Fusarium wilt on banana (Maryani *et al.* 2018) on a few Indonesian banana accessions. The data show both qualitative and quantitative variation for pathogenicity and provide a first step towards unveiling the genetic basis of the banana – *Fusarium* wilt pathosystem.

MATERIALS AND METHODS

Banana plants

Four banana accessions, comprising the wild diploid ancestor *Musa acuminata* ssp. *malaccensis* 'Pahang' and the cultivated Indonesian varieties Pisang Rejang (*Musa acuminata*, AA, Sucrier subgroup 'Rose'), Pisang Ambon Kuning (*Musa acuminata*, AAA, Gros Michel subgroup 'Gros Michel') and Pisang Ambon Hijau (*Musa acuminata*, AAA, Cavendish subgroup 'Grand Naine') were used in the phenotyping assays (Table 1; Fig. 1). All accessions were received as *in vitro* plantlets and were potted in Swedish sphagnum soil (peat 5 %, grinding clay granules 41 %, garden peat 5 %, beam structure 4 %, steamed compost 33 % and PG-Mix

15-10-20 12 %) for a two-weeks acclimatization under 100 % relative humidity at 25°C, Henceforward, plants were maintained for two to three months under greenhouse conditions (constant day temperature of 25°C, night temperature of 23°C, ambient light until max. 16 h, and relative humidity of ≥75 %) before they were used in phenotyping assays.

Isolates and pathogenicity assays

A collection of *Fusarium* species causing wilt on banana was obtained from symptomatic local banana varieties across Indonesia between 2014-2015 (Maryani *et al.* 2019). Seventy-eight isolates were selected that represent the widest genetic diversity of *Fusarium* species in banana and include isolates from all sampled locations and hosts (Table 2, Maryani *et al.* 2019). *Fusarium odoratissimum* strain II5 and CNPMF.R1 were used as positive controls for TR4 and Race 1, respectively. Recently developed inoculum production and phenotyping assay protocols Garcia-Bastidas *et al.*, (in prep.) were adopted with modifications. We added four infected maize kernels of each isolate to the potted banana plants in addition to the pouring inoculation method. After inoculation, plants were maintained in the same greenhouse with water/nutrients supplements twice per week during the eight weeks incubation period, after which the plants were scored for leaf chlorosis and then uprooted and dissected for internal corm evaluation.

Table 1. Banana varieties with details, synonym, genotype, origins, and source for pathogenicity test.

Variety	Synonym	Code	Genotype	Origin	Source
***Musa acuminata*, AAA, Cavendish subgroup 'Grand Naine'**	Pisang Ambon Hijau	GN	AAA (3x)	-	Rahan Meristem, Israel
***Musa acuminata*, AAA, Gros Michel subgroup 'Gros Michel'**	Pisang Ambon Kuning	GM	AAA(3x)	-	Corbana, Costa Rica
***Musa acuminata*, AA, Sucrier subgroup 'Rose'**	Pisang Rejang	Rejang	AA (2x)	Indonesia	ITC Leuven
***Musa acuminata* ssp. *malaccensis* 'Pahang'**	-	Pahang	AA (2x)	Malaysia	ITC Leuven

Experimental design and data analyses

Experiments were conducted between 2015 and 2016 following a partially balanced incomplete design. Four experiments were performed (Table 3), using a selection of strains based on their phylogenies in a previous study (Maryani *et al.* 2019). Sixty-three isolates were assayed on Grand Naine, 23 isolates on Grand Naine and Gros Michel and 15 isolates were assayed on these cultivars as well as on the resistant accessions Pahang and Rejang. Finally, we investigated whether the 25 *F. odoratissimum* TR4 isolates showed quantitative variation for aggressiveness towards Grand Naine (Table 3). In each experiment two disease parameters, corm discoloration (C) and foliar wilting (L), were scored following the aforementioned protocols with modification (Fig. 2 and Fig. 3). For data analyses we used the statistical package GENSTAT (VSN International 2015) for Restricted Maximum Likelihood

analysis (REML) to estimate fixed and random variances in a Linear Mixed Model (LMM) of both parameters for each experiment, which allows for unbalanced data in the estimation of variance components and fixed effects in a multi-stratum ANOVA with correlated error term. Genetic variation for virulence among isolates of different *Fusarium* species was determined with the fixed model (Best Linear Unbiased Estimates, BLUE's) and random model (Best Linear Unbiased Predictions, BLUP's). Given the unbalanced occurrence of the isolates across experiments which results in different precision estimates for the pairwise comparisons, we use isolate and therefore also the isolate.cultivar interaction as a random term to obtain mean values. Correlations between the corm and leaf data were performed using pairwise comparisons. Fisher's protected Least Significant Difference (LSD) test was used to determine groups based on their pathogenicity with *F. odoratissimum* II5 and Grand Naine as references for isolates and hosts, respectively. The means were subjected to hierarchical cluster based on group averages that endorse analyses pathogenicity group in a previous study (Maryani *et al.* 2019).

Table 2. Seventy-eight isolates comprising 11 *Fusarium* species used in this study with details of their accession number, host, origin and pathogenicity profile.

Species	Accession number	Host variety (Genotype)	Island	Location	Pathogenicity*	TR4 molecular detection	
						PCR	LAMP
Fusarium odoratissimum	InaCC F907	Pisang Tanduk (ABB)	Sumatra	Ogan Ilir	TR4	+	+
	InaCC F908	Pisang Tanduk (ABB)	Sumatra	Ogan Ilir	TR4	+	+
	InaCC F909	Pisang Mas (AA)	Sumatra	Ogan Ilir	TR4	+	+
	InaCC F825	Pisang Raja (AAB)	Kalimantan	Kutai Timur	TR4	+	+
	InaCC F924	Pisang Raja (AAB)	Papua	Jayapura	TR4	+	+
	InaCC F927	Pisang Raja (AAB)	Papua	Jayapura	TR4	+	+
	InaCC F929	Pisang Tanduk (ABB)	Papua	Jayapura	TR4	+	+
	InaCC F931	Pisang Tanduk (ABB)	Papua	Jayapura	TR4	+	+
	InaCC F933	Pisang Kepok (ABB)	Sulawesi	Barru	TR4	+	+
	InaCC F934	Pisang Kepok (ABB)	Sulawesi	Sidrap	TR4	+	+
	InaCC F935	Pisang Ambon (AAA)	Sulawesi	Maros	TR4	+	+
	InaCC F936	Pisang Ambon (AAA)	Sulawesi	Maros	TR4	+	+
	InaCC F942	Pisang Barangan (AAA)	Flores	Sikka	TR4	+	+
	InaCC F945	Pisang Barangan (AAA)	Flores	Sikka	TR4	+	+
	InaCC F946	Pisang Barangan (AAA)	Flores	Sikka	TR4	+	+
	InaCC F953	Pisang Kepok (ABB)	Flores	Sikka	TR4	+	+
	InaCC F817	Pisang Kepok (ABB)	Kalimantan	Kutai Timur	TR4	+	+
	InaCC F988	Pisang Kepok (ABB)	Java	Kendal	TR4	+	+
	InaCC F997	Cavendish (AAA)	Sumatra	Lampung	TR4	+	+
	InaCC F998	Cavendish (AAA)	Sumatra	Lampung	TR4	+	+
	InaCC F836	Pisang Mas Kirana (AA)	Java	Lumajang	TR4	+	+
	InaCC F840	Pisang Embuk (ABB)	Java	Lumajang	TR4	+	+
	InaCC F846	Pisang Susu (AAB)	Java	Purwodadi	TR4	+	+
	InaCC F856	Pisang Siem (ABB)	Java	Cianjur	TR4	+	+
	InaCC F864	Pisang Siem (ABB)	Java	Sukabumi	TR4	+	+

Table 2. (Continued).

	InaCC F870	Pisang Susu (AAB)	Java	Kendal	TR4	+	+
	InaCC F874	Pisang Susu (AAB)	Java	Kendal	TR4	+	+
	InaCC F875	Cavendish (AAA)	Java	Kendal	TR4	+	+
	InaCC F876	Cavendish (AAA)	Java	Semarang	TR4	+	+
	InaCC F878	Pisang Susu (AAB)	Java	Kendal	TR4	+	+
	InaCC F822	Pisang Raja (AAB)	Kalimantan	Kutai Timur	TR4	+	+
	InaCC F890	Pisang Kepok (ABB)	Java	Kendal	TR4	+	+
	InaCC F891	Pisang Glitung	Java	Purwodadi	TR4	+	+
	InaCC F899	Pisang Barangan (AAA)	Sumatra	Aceh	TR4	+	+
	InaCC F901	Pisang Kepok (ABB)	Sumatra	Aceh	TR4	+	+
	InaCC F903	Pisang Kepok (ABB)	Sumatra	Aceh	TR4	+	+
	II5	Pisang Manurung	Indonesia	NA	TR4	+	+
F. purpurascens	InaCC F886	Pisang Kepok (ABB)	Kalimantan	Kutai Timur	Race 1	-	-
	InaCC F966	Pisang Kepok (ABB)	Kalimantan	Tanah Bumbu	NT	-	-
	InaCC F913	Pisang Kepok (ABB)	Kalimantan	Kutai Timur	NT	-	-
F. phialophorum	InaCC F826	Pisang Awak (ABB)	Kalimantan	Tanah Bumbu	NT	-	-
	InaCC F827	Pisang Awak (ABB)	Kalimantan	Tanah Bumbu	NT	-	-
	InaCC F971	Pisang Awak (ABB)	Kalimantan	Tanah Bumbu	Race 1	-	-
	InaCC F834	Pisang Selendang (AAA)	Kalimantan	Pontianak	NT	-	-
	InaCC F843	Pisang Embuk (ABB)	Java	Lumajang	NT	-	-
	InaCC F844	Pisang Susu (AAB)	Java	Purwodadi	Race 1	-	-
	InaCC F869	Pisang Ambon Kuning (AAA)	Java	Sukabumi	NT	-	-
	InaCC F996	Pisang Kongkong	Java	Lumajang	Race 1	-	-
F. grosmichelii	InaCC F820	Pisang Ambon (AAA)	Kalimantan	Kutai Timur	Race 1	-	-
	InaCC F832	Pisang Awak (ABB)	Kalimantan	Pontianak	NT	-	-
	InaCC F833	Pisang Awak (ABB)	Kalimantan	Pontianak	Race 1	-	-
	InaCC F848	Pisang Ambon (AAA)	Java	Bogor	Race 1	-	-
	InaCC F849	Pisang Ambon (AAA)	Java	Bogor	NT	-	-
	InaCC F852	Pisang Ambon (AAA)	Java	Bogor	unknown	-	-

Table 2. (Continued).

	InaCC F853	Pisang Ambon Lumut (AAA)	Java	Bogor	Race 1	-	-
	InaCC F854	Pisang Ambon Lumut (AAA)	Java	Bogor	NT	-	-
	InaCC F855	Pisang Ambon Lumut (AAA)	Java	Bogor	NT	-	-
	InaCC F859	Cavendish (AAA)	Java	Cianjur	NT	-	-
	InaCC F861	Cavendish (AAA)	Java	Cianjur	NT	-	-
	InaCC F862	Cavendish (AAA)	Java	Cianjur	NT	-	-
	InaCC F887	Pisang Siem Jumbo (ABBB)	Java	Sukabumi	NT	-	-
	InaCC F888	Pisang Siem Jumbo (ABBB)	Java	Sukabumi	NT	-	-
F. duoseptatum	InaCC F916	Pisang Kepok (ABB)	Kalimantan	Kapuas	Race 1	-	-
	InaCC F920	Pisang Hawa (ABB)	Kalimantan	Katingan	Race 1	-	-
	InaCC F921	Pisang Hawa (ABB)	Kalimantan	Katingan	NT	-	-
	InaCC F829	Pisang Awak (ABB)	Kalimantan	Katingan	NT	-	-
	InaCC F979	Pisang Susu (AAB)	Kalimantan	Katingan	Race 1	-	-
	InaCC F980	Dwarf Cavendish (AAA)	Kalimantan	Pontianak	NT	-	-
	Indo80	Pisang Hawa (ABB)	Kalimantan	Katingan	NT	-	-
F. tardichlamydosporum	InaCC F956	Pisang Barangan (AAA)	Flores	Sikka	Race 1	-	-
	InaCC F958	Pisang Barangan (AAA)	Flores	Sikka	Race 1	-	-
F. cugenangense	InaCC F984	Pisang Kepok (ABB)	Java	Cianjur	unknown	-	-
F. hexaseptatum	InaCC F866	Pisang Ambon Kuning (AAA)	Java	Sukabumi	Race 1	-	-
F. kalimantensese	InaCC F917	Pisang Ambon (AAA)	Kalimantan	Katingan	unknown	-	-
	InaCC F918	Pisang Ambon (AAA)	Kalimantan	Katingan	unknown	-	-
	InaCC F922	Pisang Ambon (AAA)	Kalimantan	Katingan	unknown	-	-
F. sangayamense	InaCC F960	PisangKepok (ABB)	Kalimantan	Kota Baru	unknown	-	-
F. oxysporum	CNPMF.R1	Silk	Brazil	NA	Race 1	-	-

*Pathogenicity
NT= Non-pathogenic on GN, not tested yet on GM
Unknown = Non-pathogenic on both GN and GM

Fig. 1. Four banana varieties used for pathogenicity test. **A.** Gros Michel, **B.** Grand Naine, **C.** Pahang, **D.** Rejang.

Table 3. Four experimental set-ups of the phenotyping assays.

Experiment	Host variety	Σ isolates	Plants replicate	Σ experiment
1	Grand Naine	63	5	6
2	Grand Naine	25	5	5
3	Grand Naine, Gros Michel	23	5	1
4	Grand Naine, Gros Michel, Pahang, Rejang	15	3	1

DNA extraction and molecular detection of TR4

Monosporic cultures of each isolate were grown in Potato Dextrose Broth (PDB) and were incubated under continuous shaking (125 rpm) at room temperature. After seven days incubation, mycelia were collected by filtering the suspension through cheesecloth. Genomic DNA was extracted using the DNA Wizard Magnetic DNA Purification System for Food kit (Promega, USA) and was then quantified using the Invitrogen Quant-iT™ PicoGreen™ dsDNA Reagent on a TECAN 2000 analyser (Thermo Fisher Scientific, Switzerland) and finally quality was checked by agarose gel electrophoresis. We used the PCR-TR4 diagnostic primers described by Dita *et al.* (2010) and a newly loop-mediated isothermal amplification (LAMP) assay as described by Salacinas *et al.* (2018).

Fig. 2. Phenotyping Fusarium wilt in banana. Foliar diseases severities which were used to assess the pathogenicity of *Fusarium* spp. eight weeks after inoculation. **A.** Score 1 (healthy plant), **B.** Score 2 (initial chlorosis), **C.** Score 3 (advanced chlorosis ≤ 10 %), **D.** Score 4 (extensive chlorosis 10 % – 50 %), **E.** Score 5 (extensive chlorosis and necrosis ≤50 % – 90 %), **F.** Score 6 (extensive necrosis >90 % or dead plant).

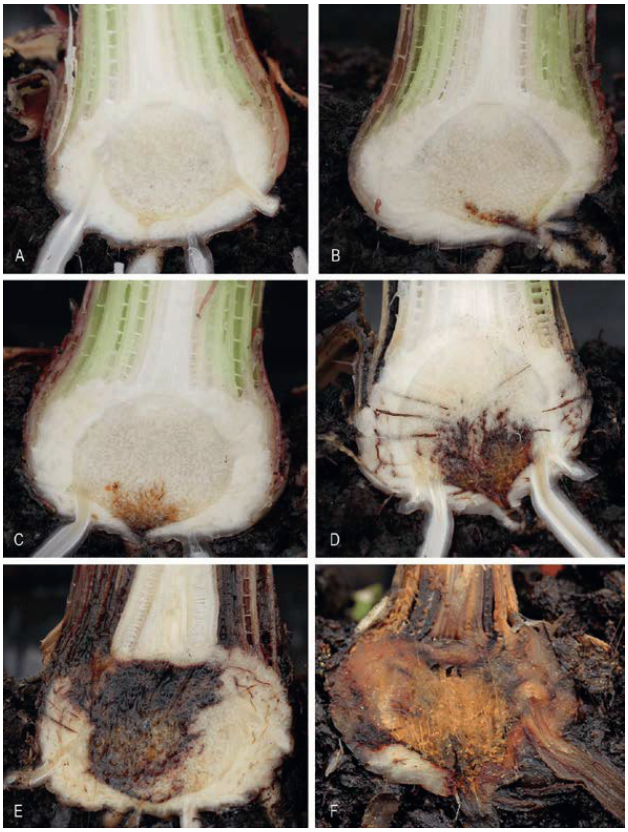

Fig. 3. Phenotyping Fusarium wilt in banana. Internal diseases severities in the corm which were used to assess the pathogenicity of *Fusarium* spp. eight weeks after inoculation. **A.** Score 1 (No discoloration), **B.** Score 2 (≤5 % discoloration), **C.** Score 3 (6 % – 10 % discoloration), **D.** Score 4 (21 % – 50 % discoloration), **E.** Score 5 (50 % – 90 % discoloration), **F.** Score 6 (>90 % discoloration).

RESULTS

Symptom development

The characteristic symptoms of Fusarium wilt on banana, such as leaf chlorosis/ necrosis and internal vascular discoloration were observed on all susceptible plants. Highly pathogenic isolates resulted in plant death within eight weeks after inoculation. The first foliar chlorosis appeared in the third week after inoculation and progressed from the older to the younger leaves, eventually resulting in petiole collapse and desiccation (Fig. 4). The natural anthocyanin blotches on Cavendish and Gros Michel foliage turned yellowish-red on wilting leaves (Fig. 4), but the young furled cigar leaves remained erect and green. Highly pathogenic isolates caused rapid and progressive wilting as well as pseudostem splitting, which eventually resulted in severely stunted plants with constricted pseudostem and curly leaves (Fig. 5).

Vascular symptoms started from the outer part of the corm, went up into the pseudostem and discoloured the first and second strands of the pseudostem leaf sheaths from

white (see controls) to a myriad of discolorations and patterns. They frequently varied from yellow - reddish-brown and eventually dark brown-black, depending on the severity of infection. Initial colonization by the pathogen was sometimes already observed in the hairy and lateral roots and varied from bright to dark-yellow, from light to dark-brown, or from reddish to brown or almost black (Fig. 6). We also observed discontinued flecking or spotting, discoloured patches, but most frequently a dense discoloration of the bottom of the corm (Fig. 6). These colour and pattern variations were regardless of the isolates or varieties used across this study and severities of foliar symptoms generally correlated well with those in the corm.

Fig. 4. Phenotyping Fusarium wilt in banana. Overview of symptoms development of Fusarium wilt on banana. Left panels showing plants before inoculation and right panels show plants eight weeks after inoculation.

Fig. 5. Phenotyping Fusarium wilt in banana. Secondary external symptoms caused by very aggressive *Fusarium odoratissimum* isolates. **A.** Stunting, **B.** Splitting of pseudostem, **C.** Wilting

Fig. 6. Phenotyping Fusarium wilt in banana. Overview of corm disease severities at eight weeks after inoculation of Grand Naine with isolates InaCC F836 (A), InaCC F988 (G), InaCC F909 (H), InaCC F988 (M) and InaCC F856 (N) and Gros Michel with isolates InaCC F998 (B), InaCC F997 (C), InaCC F909 (D), InaCC F998 (E), InaCC F936 (F), InaCC F908 (I), InaCC F916 (J), InaCC F998 (K), InaCC F958 (L) and InaCC F978 (O).

Banana – *Fusarium* interactions

We performed four rounds of phenotyping to determine the pathogenicity of a significant subsample of the newly described *Fusarium* species (Maryani *et al.* 2019) that are associated with Fusarium wilt in banana. The screen with 63 isolates (experiment 1) on Grand Naine showed significant differences in pathogenicity for both the C and L parameters (Table

4; Supplementing Table 1). An LSD test was used to group the isolates according to their means (Supplementing Fig.1 and Fig.2) and hierarchical cluster analysis resulted in two categories (Fig.7; Supplementing Fig.3). Thirty-four avirulent isolates and 27 virulent isolates (Fig. 7), which essentially matched with the reference TR4 (II5) and Race 1 (CNPMF.R1) strains that are virulent and avirulent on Cavendish banana varieties, respectively. We also observed substantial quantitative variation for aggressiveness among the *F. odoratissimum* TR4 isolates (Fig. 8; Table 4; Supplementing Table 2). An additional statistical analysis of 25 of these isolates showed that the majority of this panel of TR4 isolates is more aggressive than the reference strain II5 (Fig. 8; Table 4; Supplementing Table 2).

Table 4. REML analyses from the fixed model of corm and foliar responses on each experimental set-up.

Fixed term	Corm (C)				Foliar (L)				Pairwise correlation (C – L)
	[1]n.d.f.	F statistic	[2]d.d.f.	F probability	n.d.f.	F statistic	d.d.f.	F probability	
Experiment 1: GN									
experiments	6	20.95	110.9	**<0.001**	6	8.62	108.3	**<0.001**	
isolates	63	8.53	106.6	**<0.001**	63	4.27	105.7	**<0.001**	0.73
Experiment 2: TR4 on GN									
biological replicates	4	25.97	36.9	**<0.001**	4	12.07	37.4	**<0.001**	
isolates	24	1.48	37.0	**0.137**	24	1.93	37.5	**0.034**	0.68
Experiment 3: GN.GM									
varieties	1	144.88	185.0	**<0.001**	1	62.71	184.0	**<0.001**	
isolates	23	13.16	185.0	**<0.001**	23	8.45	184.0	**<0.001**	
varieties. isolates	23	7.25	185.0	**<0.001**	23	2.21	184.0	**<0.001**	0.76
Experiment 4: GN.GM. Rejang. Pahang									
varieties	3	51.57	6.6	**<0.001**	3	12.27	6.6	**0.004**	
isolates	13	10.32	91.7	**<0.001**	13	2.88	92.4	**0.002**	
varieties. isolates	33	5.78	91.7	**<0.001**	33	2.36	92.4	**<0.001**	0.73

[1]n.d.f.= number degrees of freedom
[2]d.d.f.= denominator degrees of freedom

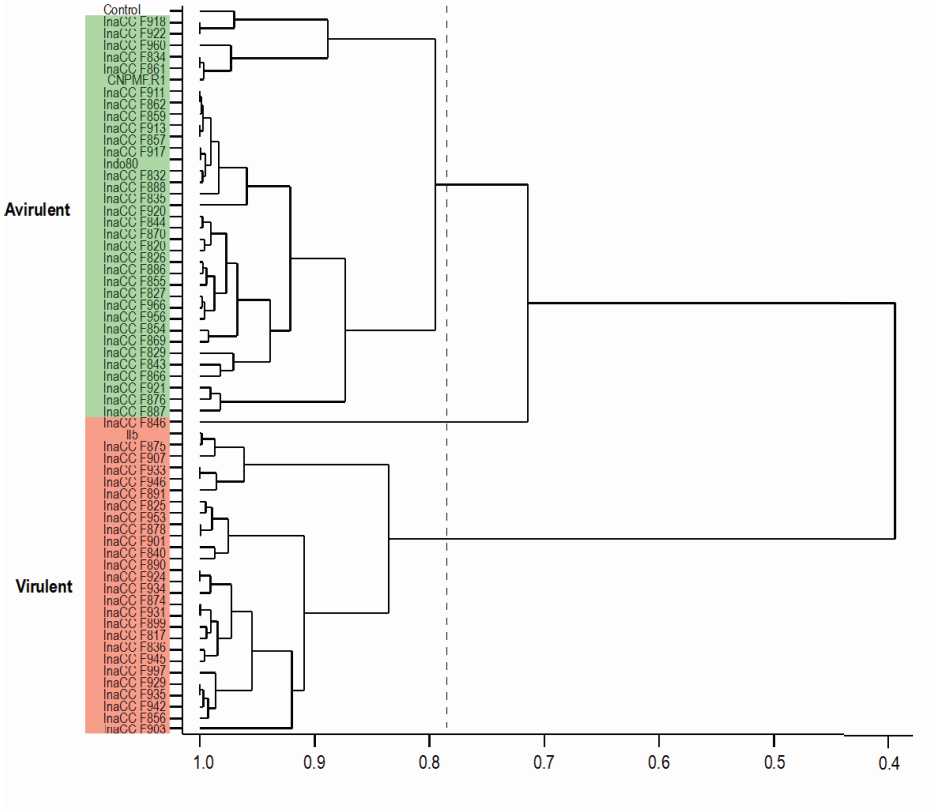

Fig. 7. Hierarchical cluster analysis of 63 *Fusarium* spp. (experiment 1) that were phenotyped (eight weeks after inoculation) on Grand Naine banana plants using averages of Fusarium wilt disease severities in the corms. Mean values were obtained from the Best Linear Unbiased Predictions (BLUP's, see materials and methods). X-axis values indicate the degree of similarity based on pairwise difference, the vertical dashed line is an arbitrary threshold between significantly different virulence levels of the phenotyped isolates indicated by different colours.

To validate the two observed groups with either virulence or non-virulence for Grand Naine we tested 23 isolates across the collection of new species described by Maryani *et al.* (2019) on Grand Naine and Gros Michel (experiment 3). This resulted in a highly significant host x pathogen interaction component for the C and L disease parameters (Table 4; Fig. 9), indicating specificity between banana accessions and *Fusarium* species. In this experiment, we confirmed the abovementioned quantitative variation for aggressiveness on Grand Naine, which was absent on Gros Michel (Supplementing Table 3). In total, 11 isolates were virulent on both banana accessions, whereas 10 isolates were avirulent and virulent on Grand Naine and Gros Michel, respectively. Two isolates, InaCC F984 and InaCC F851, were avirulent on both varieties. Finally, we selected 15 isolates that were virulent on Grand Naine and Gros Michel to investigate whether these strains would cause any disease on wild Pahang and seedless Rejang diploids (experiment 4), but none of the isolates caused any significant

disease symptoms on these diploids. The fungus was unable to invade the inner parts of the corm, but rather remained outside the cortex without causing any further disease symptoms (Fig. 10; Supplementing Table. 4). Statistical analyses also indicated significant host – pathogen terms for both disease parameters in this experiment (Table 4).

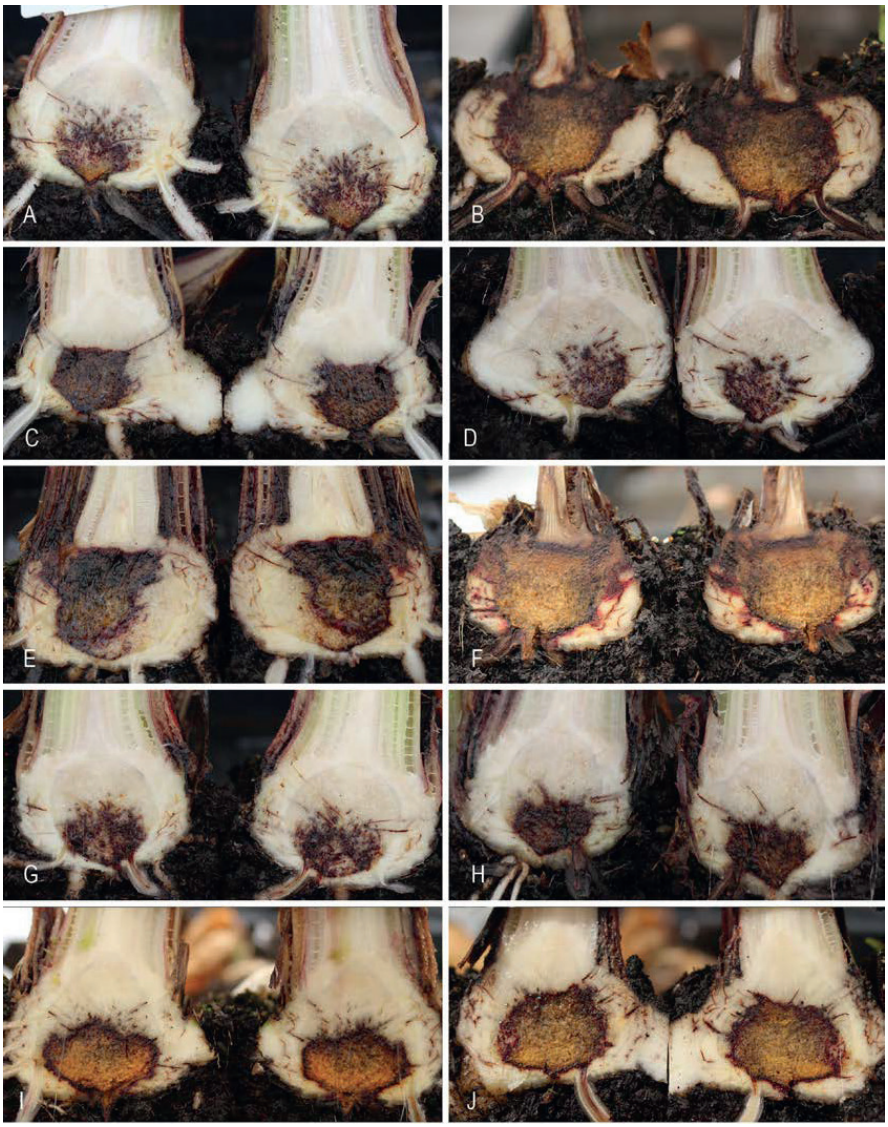

Fig. 8. Phenotyping Fusarium wilt in banana (experiment 2). Overview of corm disease severities at eight weeks after inoculation of Grand Naine with different isolates of *Fusarium odoratisimum.* **A.** InaCC F846. **B.** InaCC F899. **C.** InaCC F909. **D.** InaCC F988. **E.** InaCC 817. **F.** InaCC F997. **G.** InaCC F908. **H.** II5. J. InaCC F874.

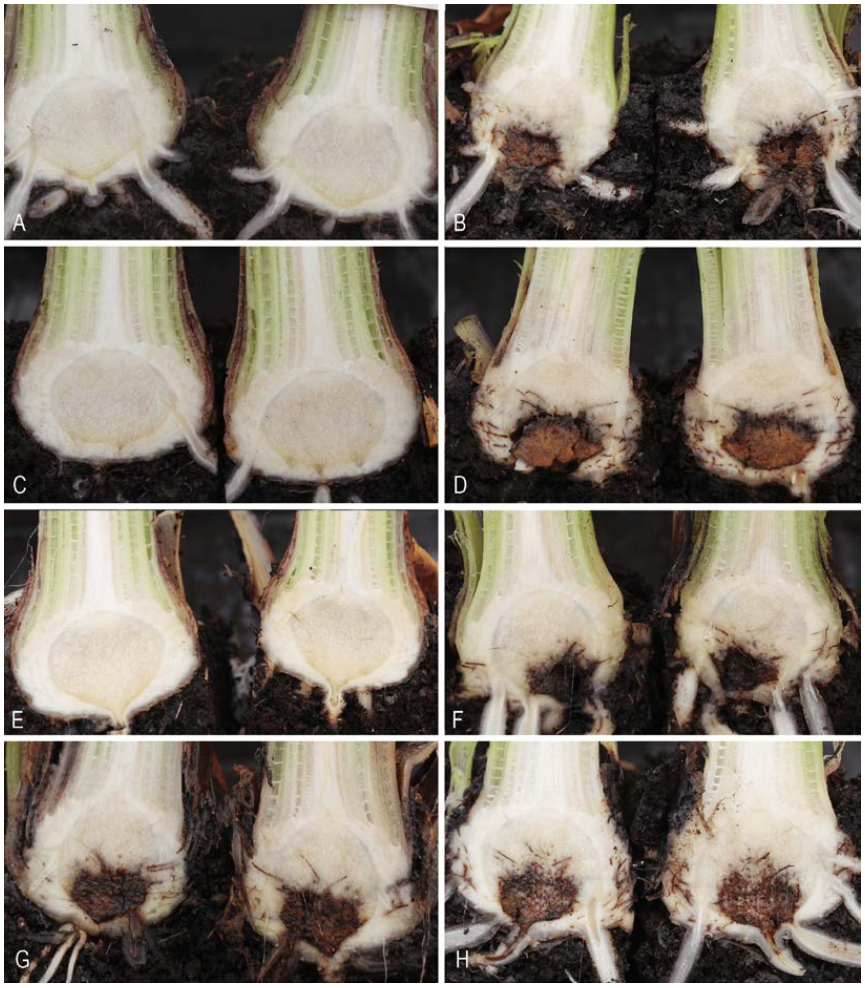

Fig. 9. Phenotyping Fusarium wilt in banana (experiment 3). Overview of corm disease severities at eight weeks after inoculation of Grand Naine (left panel) and Gros Michel (right panel) with various *Fusarium* spp.. **A-B.** *Fusarium duoseptatum* InaCC F916, **C-D.** *F. tardichlamydosporum* InaCC F958, **E-F.** *F. grosmichelii* InaCC F852, **G-H.** *F. odoratissimum* II5.

Molecular diagnosis of *Fusarium odoratissimum*, TR4 isolates

All isolates that were assayed in the abovementioned phenotyping assays were tested with PCR and LAMP diagnostics. Isolates classified as virulent on Grand Naine were without exception positive for both assays (Table 2).

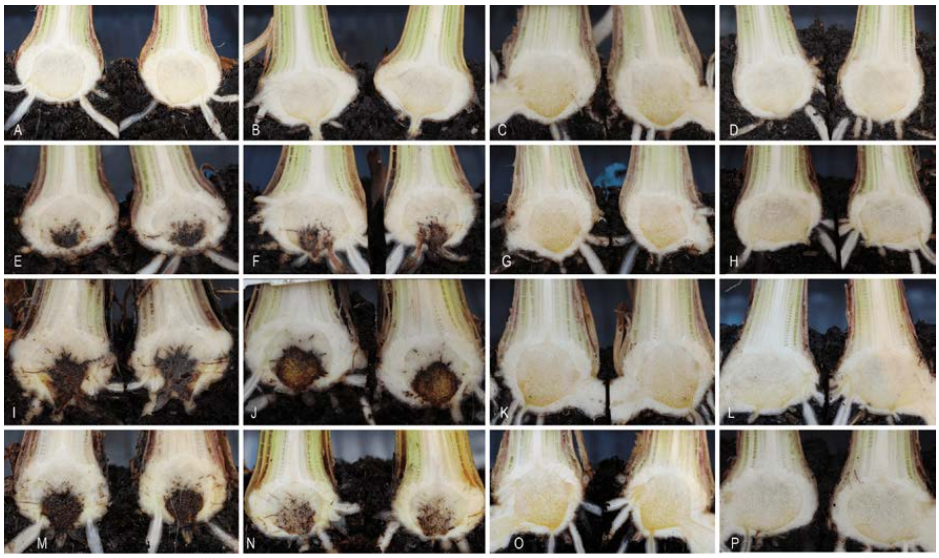

Fig. 10. Phenotyping Fusarium wilt in banana (experiment 4). Overview of corm disease severities at eight weeks after inoculation of Grand Naine (first column), Gros Michel (second column), Pahang (third column) and Rejang (fourth columns) with *Fusarium odoratissimum.* First row control (**A-D**), second row reference strain II5 (**E-H**), third row InaCC F822 (**I-L**), and fourth row InaCC F856 (**M-P**).

DISCUSSION

Host – pathogen interaction data sets are crucial as a foundation for gene discovery (Roelfs & Martens 1987, Kema 1996, 2000, Figueroa *et al.* 2013). Early attempts to conceptualize such data were published by Person (1959). Clearly, such data sets are to be complemented by genetic data of both the host and the pathogen whenever possible to identify resistance genes or effectors, respectively. Recent gene discoveries in the *Zymoseptoria tritici* pathogen of wheat demonstrate the value of isolate collections and complete host – pathogen data sets (Zhang *et al.* 2017, Kema *et al.* 2018). Studies in Fusarium wilt have greatly advanced in genomics and genetics such as in tomato and cotton (Ma *et al.* 2013), but not in banana. Since the epic epidemic in the previous century (Stover 1962), little progress has been made over the past 50 years since TR4 emerged on Cavendish bananas (Su *et al.* 1986). It is therefore not surprising that the focal occurrence has developed in a current pandemic (Ordóñez *et al.* 2015, Zheng *et al.* 2018). Moreover, the first resistance gene to TR4 was only identified recently (Dale *et al.* 2017). Thus, despite the fact that Cavendish saved the industry due to its resistance to Fusarium wilt, the underlying genetics remains elusive.

Some vascular wilt pathogens such as *Fusarium oxysporum* exclusively colonize xylem vessels which are essential for water and minerals transport in plants. As a result, systemic wilting symptoms and chlorosis are the first exterior symptoms of disease development. However, wilting is a nonspecific symptom that also can be due to other pathogens, or abiotic factors such as drought. The development of diagnostics (Dita *et al.* 2010, Lievens 2008, Salacinas *et al.* 2018) is therefore crucial for rapid identification of causal organisms and

intervention strategies. Here, we combined pathogenicity of *Fusarium* species belonging to the FOSC on various banana hosts with molecular diagnostics, which confirmed in all cases that the *Fusarium* strains identified as TR4 unified in one species of *F. odoratissimum* (Maryani *et al.* 2019). The PCR assay for pathogenic strains on Grand Naine (TR4), has proven to be indispensable for rapid alerts and diagnosis of this particular pathotype in suspicious plants (Dita *et al.* 2010) as well as the newly developed LAMP assay (Salacinas *et al.* 2018). Both methods are congruent and unequivocally identified which strains are TR4, as validated by all phenotyping assays. Nevertheless, molecular identification of pathogenic strains should ideally be based on DNA sequences directly related to genes for host-specificity or pathogenicity rather than conserved genes or anonymous molecular marker (Recorbet 2003).

Similar to field evaluations (Stover & Waite 1960, Sutanto *et al.* 2013), we used foliage wilting and corm discoloration to assess the pathogenicity of the Indonesian-wide *Fusarium* panel causing wilt on wild and cultivated banana varieties under greenhouse conditions. However, as pointed out above, corm symptoms are generally more reliable in assessing disease severity (Garcia-Bastidas *et al.*, in prep.), which was also observed in our trials as leaf symptoms were highly variable.

The majority of species in the FOSC is non-pathogenic to any particular host (O'Donnell *et al.* 2009). Thus, pathogenicity assays can be used to explore genetic diversity as a start to elucidate the underlying genetic basis. Many studies reported genetic diversity in Fusarium wilt pathogens on banana at the molecular level (Groenewald *et al.* 2006, Fourie *et al.* 2009, Mostert *et al.* 2017), but testing pathogenic diversity is still largely void of extended experimental data. All 35 isolates of *F. odoratissimum* pathogenic on Grand Naine, were sampled across the Indonesian archipelago and belong to the physiological group of TR4 strains. None of the other identified species is able to cause disease in Grand Naine. The identification of TR4 in Indonesia was to be expected as the reference strain II5 is of Indonesian origin and was identified in 1992 on Sumatra Island (Budenhagen 2007). It is also believed to be the origin of the first encounter with Cavendish monoculture in Taiwan in 1976 (Hwang & Ko 2004). Still, the distribution of 35 TR4 isolates of a single species across Indonesia is surprising and seems to be due to demographic factors and the distribution of infected plant materials, similar to recent findings in the Greater Mekong area (Zheng *et al.* 2018).

Thirteen strains that were not pathogenic on Cavendish, caused disease on Gros Michel, hence represent the genetically diverse Race 1 strains that belong to six different *Fusarium* species in the FOSC (Maryani *et al.* 2019). In addition to this broad division of pathogenicity towards Gros Michel and Grand Naine, we observed substantial quantitative variation among the TR4 strains, with many being more aggressive than the II5 reference strain. In general, TR4 isolates are isolated from Cavendish monoculture plantations (Ordóñez *et al.* 2015), but the Indonesian TR4 isolates in our study were isolated from at least 40 local banana varieties grown in different ecosystem at 34 geographically different locations on six

Indonesian islands (Maryani *et al.* 2019). This could contribute to small evolutionary changes, which is reflected by their variable aggressiveness towards Cavendish bananas. It is known that pathogens show extensive genetic diversity in their centres of origin (Islam *et al.* 2016, Kema *et al.* 1996, Stukenbrock & McDonald 2008). We observed a similar situation for *Fusarium* species with pathogenicity to banana in Indonesia (Maryani *et al.* 2019), which revealed eleven phylogenetically diverse species as well as genomic variation within the species by genotyping-by-sequencing across these *Fusarium* species (Maryani *et al.* 2018). We do not know how such diversity is generated, but presume it results from extended co-evolution between the wide range of genetic diversity in banana germplasm in Indonesia (Perrier *et al.* 2011, Nasution 1990). Until now, sexual reproduction in this pathogen has not been discovered and population analyses (Fourie *et al.* 2006) do not hint towards such a niche. Taylor *et al.* (1999) demonstrate that the phylogenetic species concept of asexual and clonal fungi does not preclude recombination and used *Fusarium* causing Panama disease as an example that asexual and sexual reproductive biology not necessarily align with clonal or recombining populations, respectively. This is in accord with Buxton (1962), who demonstrated parasexuality in *Fusarium oxysporum* strains causing wilting in Gros Michel, which evidently could result in any form of diversification that might manifest itself through subtle morphological changes, including pathogenicity. Other *F. oxysporum* ff.spp. such as f. sp. *niveum* on watermelon (Larkin *et al.* 1990) and f. sp. *ciceris* on chickpea (Jiménez-Gasco *et al.* 2004) showed also a wide range of aggressiveness which was attributable to particular races.

Gene-for-gene interactions have been described for various ff.spp. of *F. oxysporum* (Michelse & Rep 2009). However, the race concept in Fusarium wilt pathogens on banana is crude and merely depends on pathogenicity of a limited number of strains on a few banana accessions, usually evaluated under field conditions. Stover and Waite (1960) in their first experiments used three isolates and found Race 1 and Race 2 to be highly pathogenic on Gros Michel and Bluggoe, respectively. The latter accession and Cavendish were highly resistant to Race 1, but Race 4 affects Cavendish bananas (Su *et al.* 1986). In these experiments, susceptible varieties succumbed to pathogenic isolates, while resistant accessions did not develop any symptom. However, expanding the number of *Fusarium* species and banana accessions will almost certainly extend and complicate this rudimentary system. Here, we tested 23 isolates across seven *Fusarium* species that are pathogenic on Grand Naine and Gros Michel and found a strong interaction between isolates and banana genotypes, indicative for GFG, which is in accord with previous reports (Stover & Waite 1960, Su *et al.* 1986) as well as the most recent reports of Garcia-Bastidas *et al.* (in prep.), who phenotyped 242 banana accessions with TR4 strain II5 and Race 1 strain CNPMF.R1. In our study, six *Fusarium* spp. are pathogenic on Gros Michel, clearly demonstrating that pathogenicity is not species specific unlike the pathogenicity toward Grand Naine. We also validated the resistance to the diverse

Indonesian TR4 isolates as well as other *Fusarium* species in *M. acuminata* var. *malaccensis*, a highly dispersed species originating from Sumatra and the Malaysian peninsula (Simmonds & Shepherd 1995). The genome of this species was sequenced and it is the source of the first identified and cloned resistance gene to TR4 (D'Hont *et al.* 2012, Dale *et al.* 2017). In our surveys across Indonesia we never observed any external Fusarium wilt symptoms in the forests of Java and Sumatra (Maryani *et al.* 2019, Ahmad & Maryani 2014, personal observation). Likewise, Rejang was found to be resistant to this wide *Fusarium* diversity in our greenhouse assays, which confirmed our observations from banana backyard home plantations in Java and Sumatra (Poerba & Pangesti 2017, pers. comm.). Also, recent reports on the screening of local Indonesian banana varieties against Fusarium wilt showed that both diploid and tetraploid Pisang Rejang genotypes were highly resistant to TR4 (Handayani *et al.* 2017). Hence, the deciphering of the genetic basis of resistance to Fusarium wilt in cultivated and wild bananas is relevant, possible and urgent. Our study provides the well genotyped and phenotyped pathogen isolates that should be used for such analyses.

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Supplementary data

Supplementing table 1. Disease severities in the corm and foliar symptoms of experiment 1. Mean values were obtained from the random model (see materials and methods).

Species	Accession number	Mean		∑ Experiment	∑Plant
		C	L		
Fusarium duoseptatum	InaCC F829	1.92	2.72	1	3
	InaCC F920	2.11	2.25	3	15
	Indo80	2.41	2.72	1	3
	InaCC F835	2.45	2.21	3	15
	InaCC F921	2.69	2.50	1	5
	InaCC F911	2.38	3.30	1	2
F. grosmichelii	InaCC F861	1.80	2.19	3	15
	InaCC F820	2.13	2.24	4	20
	InaCC F855	2.17	2.51	2	7
	InaCC F854	2.30	2.30	3	15
	InaCC F862	2.38	2.77	1	2
	InaCC F859	2.38	2.18	2	10
	InaCC F888	2.42	2.47	3	15
	InaCC F832	2.43	2.40	3	15
	InaCC F887	2.65	2.72	1	3
F. hexaseptatum	InaCC F866	2.05	2.24	4	17
F. kalimantanense	InaCC F918	1.43	2.72	1	3
	InaCC F922	1.43	2.74	1	3
	InaCC F917	2.41	2.55	5	25
F. oxysporum	CNPMF.R1	1.79	1.63	4	20
F. phialophorum	InaCC F834	1.80	2.65	3	15
	InaCC F843	1.99	2.36	3	15
	InaCC F844	2.10	2.43	4	20
	InaCC F826	2.19	2.17	4	20
	InaCC F827	2.22	2.33	2	10
	InaCC F869	2.27	3.42	2	7
F. purpurascens	InaCC F886	2.18	2.38	4	20
	InaCC F966	2.22	2.31	3	15
	InaCC F913	2.39	2.50	1	5
F. sangayamense	InaCC F960	1.70	2.01	4	20
F. tardichlamydosporum	InaCC F956	2.23	2.02	3	15
F. odoratissimum	InaCC F870	2.15	2.03	1	5
	InaCC F857	2.39	3.34	1	5
	InaCC F876	2.73	2.77	1	3
	InaCC F846	3.14	2.91	1	5
	InaCC F891	3.72	3.05	3	15
	InaCC F933	3.77	3.87	3	15
	InaCC F946	3.77	3.23	2	10
	InaCC F875	3.86	3.68	3	15
	II5	3.87	3.83	7	35
	InaCC F907	3.91	3.49	3	15
	InaCC F890	4.08	3.64	2	10

	InaCC F840	4.12	3.11	1	5
	InaCC F825	4.16	3.48	1	5
	InaCC F953	4.17	3.96	3	15
	InaCC F878	4.20	4.07	3	15
	InaCC F901	4.20	3.67	3	15
	InaCC F874	4.31	3.63	3	15
	InaCC F924	4.34	3.73	3	15
	InaCC F934	4.34	3.59	3	15
	InaCC F931	4.39	3.93	2	10
	InaCC F899	4.39	4.30	3	15
	InaCC F817	4.41	3.66	2	10
	InaCC F836	4.43	3.59	1	5
	InaCC F997	4.45	4.04	3	15
	InaCC F945	4.46	3.79	3	15
	InaCC F935	4.52	3.68	3	15
	InaCC F942	4.52	3.62	3	15
	InaCC F822	4.53	4.08	5	25
	InaCC F856	4.55	3.57	4	20
	InaCC F929	4.58	3.87	3	15
	InaCC F903	4.71	4.19	3	15

Supplementing table 2. Disease severities in the corm and foliar symptoms of experiment 2. Mean values were obtained from the fixed model (see materials and methods).

Accession number	Mean		Σ Experiment	Σ Plant
	C	L		
InaCC F933	3.3	4.1	2	10
InaCC F870	3.4	3.3	1	5
InaCC F891	3.4	3.1	2	10
InaCC F907	3.8	3.8	1	5
InaCC F946	3.8	3.7	1	5
InaCC F878	3.8	4.3	2	10
InaCC F953	3.8	4.1	2	10
InaCC 924	4.1	3.8	2	10
InaCC F934	4.2	3.7	2	10
InaCC F942	4.3	3.7	2	10
InaCC F945	4.3	3.9	2	10
InaCC F817	4.4	4.7	1	5
II5	4.5	4.0	5	25
InaCC F935	4.5	3.8	3	15
InaCC F875	4.5	4.3	2	10
InaCC F856	4.6	4.0	2	10
InaCC F890	4.6	4.2	2	10
InaCC F929	4.7	4.2	2	10
InaCC F822	4.7	4.3	3	15
InaCC F901	4.7	4.2	3	15
InaCC F931	4.8	4.5	1	5
InaCC F997	4.8	4.4	2	10
InaCC F874	4.9	3.5	2	10
InaCC F899	4.9	4.9	3	15

InaCC F903	5.3	4.8	3	15

Supplementing table 3. Disease severities in the corm and foliar symptoms of experiment 3. Mean values were obtained from the fixed model (see materials and methods).

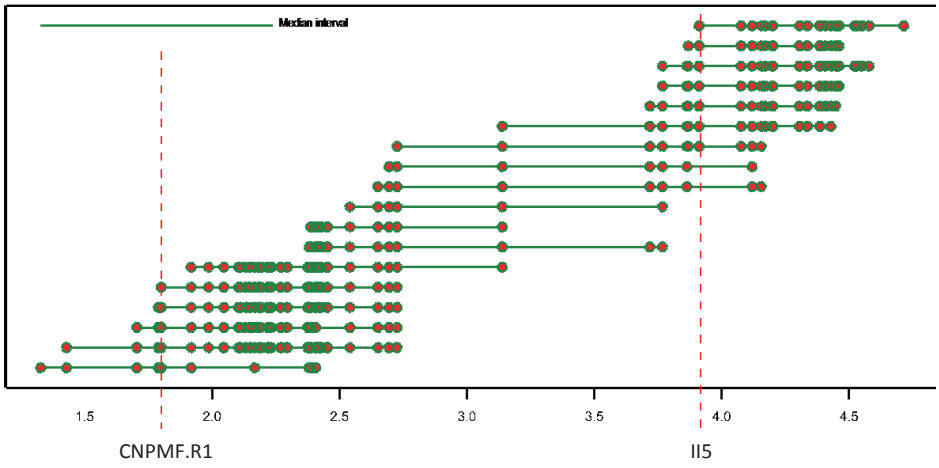
Species	Accession number	Mean				Pathogenicity
		Grand Naine		Gros Michel		
		C	L	C	L	
Fusarium cugenangense	InaCC F984	1.4	1.2	1.4	1.2	NA
F. duoseptatum	InaCC F971	1.4	1.2	4.2	2.4	Race 1
	InaCC F979	1.4	1.2	5.0	4.4	Race 1
	InaCC F916	2.2	1.0	4.8	4.4	Race 1
F. grosmichelii	InaCC F848	1.6	1.4	4.2	2.8	Race 1
	InaCC F851	1.6	1.4	2.6	1.6	NA
	InaCC F853	1.6	1.6	4.6	3.6	Race 1
	InaCC F833	2.0	1.8	4.6	4.2	Race 1
F. oxysporum	CNPMF.R1	1.2	1.0	4.4	2.8	Race 1
F. phialophorum	InaCC F996	2.4	2.2	4.0	2.6	Race 1
F. tardichlamydosporum	InaCC F958	1.0	1.6	4.4	3.8	Race 1
F. odoratissimum	InaCC F927	3.2	2.6	4.4	3.8	TR4
	InaCC F936	3.6	2.2	4.2	3.8	TR4
	InaCC F908	4.0	3.2	5.2	5.0	TR4
	II5	4.2	2.6	4.2	2.5	TR4
	InaCC F909	4.2	4.2	5.6	5.4	TR4
	InaCC F817	4.3	3.7	3.8	4.3	TR4
	InaCC F988	4.4	3.6	4.6	4.0	TR4
	InaCC F836	4.4	3.6	4.2	3.6	TR4
	InaCC F846	4.6	4.3	4.8	5.2	TR4
	InaCC F998	4.8	4.0	4.2	4.2	TR4
	InaCC F997	5.3	4.8	4.0	4.0	TR4

Supplementing table 4. Disease severities in the corm and foliar symptoms of experiment 4. Mean values were obtained from the fixed model (see materials and methods).

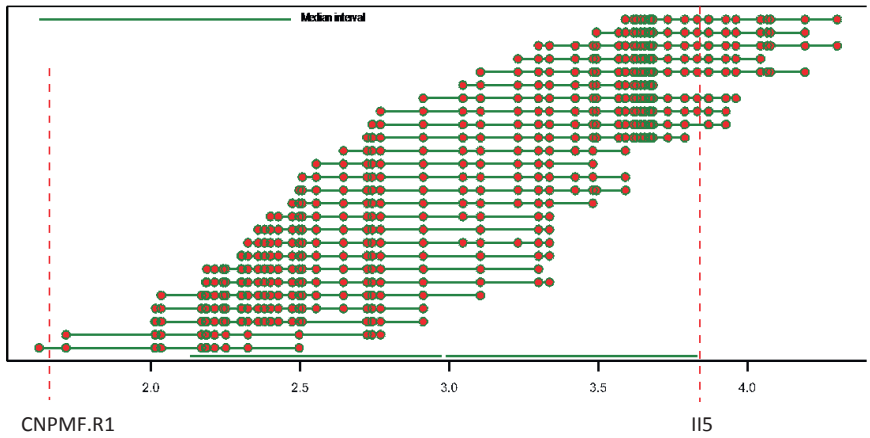
Species	Accession number	Mean							
		Pahang		Rejang		Grand Naine		Gros Michel	
		C	L	C	L	C	L	C	L
Fusarium sangayamense	InaCC F960	1.0	1.7	NA	NA	1.0	1.3	1.3	2.3
F. kalimantanense	InaCC F917	1.0	1.7	1.0	2.0	1.3	1.7	1.0	2.3
F. hexaseptatum	InaCC F866	1.0	1.7	NA	NA	1.3	1.7	4.7	4.7
F. purpurascens	InaCC F886	1.0	2.0	NA	NA	1.3	2.0	3.7	3.3
F. phialophorum	InaCC F844	1.0	2.0	NA	NA	1.7	2.0	3.3	2.3
F. grosmichelii	InaCC F820	2.3	2.3	NA	NA	1.0	1.3	4.0	3.3
F. odoratissium	InaCC F891	1.0	2.0	1.3	2.0	2.7	3.0	4.0	5.0
	InaCC F817	1.0	2.0	NA	NA	3.6	3.3	4.5	4.5
	InaCC F997	1.0	2.3	NA	NA	4.8	4.7	4.0	5.0
	INACC F899	1.3	1.7	1.3	2.0	3.0	2.7	2.7	2.3
	II5	1.3	1.3	1.3	2.0	3.3	2.7	3.7	3.0
	InaCC F931	1.3	1.3	1.0	2.0	3.7	3.3	3.3	4.3
	InaCC F856	1.3	1.7	1.3	1.3	4.0	3.0	4.3	3.7
	InaCC F822	1.3	1.7	1.0	1.3	4.3	3.7	4.3	3.3
F. oxysporum	CNPMF.R1	1.7	1.7	NA	NA	1.3	2.0	3.7	3.3

Supplementing table 5. Estimated variance components from the full random model on each experimental set-up.

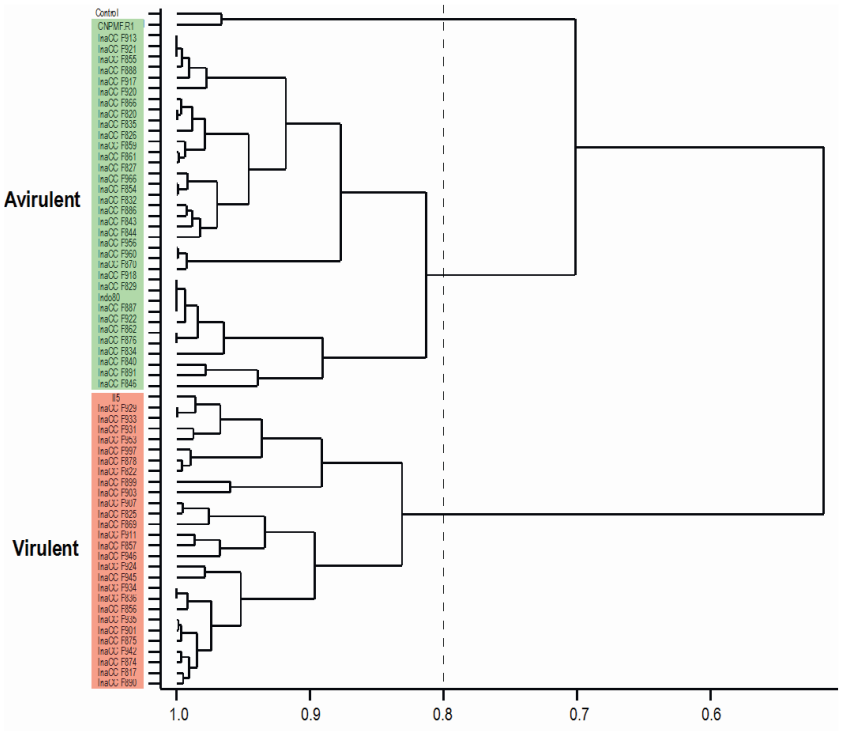
Random term	Corm (C)		Foliar (L)	
	Component	s.e.	Component	s.e.
Experiment 1: GN				
experiment (exper.)	0.3184	0.2043	0.1332	0.0977
isolates (isol.)	1.2999	0.2739	0.6206	0.1547
replicates (rep.)	0.0086	0.0097	0.0008	0.0043
exper.isol	0.3185	0.0643	0.4003	0.0708
exper.rep	0.0025	0.0083	0.0075	0.0085
isol.rep	0.0106	0.0258	0.0068	0.0200
exper.isol.rep	0.6120	0.0437	0.4710	0.0339
Experiment 2: TR4 on GN				
experiment (exper.)	0.4679	0.3508	0.2786	0.2279
isolates (isol.)	0.0300	0.0391	0.0289	0.0681
replicates (rep.)	-0.0080	0.0088	-0.0132	0.0060
exper.isol	0.0657	0.0582	0.2342	0.0940
exper.rep	0.0249	0.0306	0.0231	0.0251
isol.rep	-0.0797	0.0457	-0.0336	0.0473
exper.isol.rep	0.8320	0.0925	0.7380	0.0849
Experiment 3: GN.GM				
varieties (var.)	0.8365	1.2526	0.674	0.973
isolates (isol.)	0.4226	0.3454	0.888	0.347
replicates (rep.)	-0.0103	0.0161	0.013	0.012
cult.isol	1.0088	0.3363	0.259	0.175
cult.rep	0.0087	0.0252	-0.050	0.015
isol.rep	0.1440	0.0840	-0.200	0.142
var.isol.rep	0.6070	0.0929	1.517	0.229
Experiment 4: GN.GM. Rejang. Pahang				
varieties (var.)	0.8586	0.7588	0.3913	0.3676
isolates (isol.)	0.1656	0.1533	0.0446	0.0736
replicates (rep.)	0.0044	0.0186	0.0046	0.0340
cult.isol	0.6116	0.1838	0.2610	0.1216
cult.rep	0.0194	0.0294	0.0580	0.0623
isol.rep	-0.0090	0.0335	-0.0316	0.0514
var.isol.rep	0.3950	0.0683	0.6400	0.1110



Supplementing Fig. 1. Homogenous subsets of pairwise comparisons of mean corm symptom severities, indicated by connected dots. Each distinct group of means is displayed above each other, on the corm score, based on Fischer's protected LSD. Median LSD size holds for most pairwise comparisons of isolates. II5 strain is used as reference pathogenic group and CNPMF.R1 as non-pathogenic group.



Supplementing Fig. 2. Homogenous subsets of pairwise comparisons of mean foliar symptom severities, indicated by connected dots. Each distinct group of means is displayed above each other, on the corm score, based on Fischer's protected LSD. Median LSD size holds for most pairwise comparisons of isolates. II5 strain is used as reference pathogenic group and CNPMF.R1 as non-pathogenic group.



Supplementing Fig. 3. Hierarchical cluster analysis of 63 *Fusarium* spp. (experiment 1) that were phenotyped (eight weeks after inoculation) on Grand Naine banana plants using averages of Fusarium wilt disease severities in the foliar symptom. Mean values were obtained from the Best Linear Unbiased Predictions (BLUP's, see materials and methods). X-axis values indicate the degree of similarity based on pairwise difference. The vertical dashed line is an arbitrary threshold between significantly different virulence levels of the phenotyped isolates indicated by different colours.

Chapter 5

New endemic Fusarium species hitchhiking with pathogenic Fusarium strains causing Panama disease in small-holder banana plots in Indonesia

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Abstract

Fusarium species are well known for their abundance, diversity and cosmopolitan life style. Many members of the genus *Fusarium* are associated with plant hosts, either as plant pathogens, secondary invaders, saprotrophs, and/or endophytes. We previously studied the diversity of *Fusarium* species in the *Fusarium oxysporum* species complex (FOSC) associated with Fusarium wilt of banana in Indonesia. In that study, several *Fusarium* species not belonging to the FOSC were found to be associated with Fusarium wilt of banana. These *Fusarium* isolates belonged to three *Fusarium* species complexes, which included the *Fusarium fujikuroi* species complex (FFSC), *Fusarium incarnatum-equiseti* species complex (FIESC) and the *Fusarium sambucinum* species complex (FSSC). Using a multi-gene phylogeny that included partial fragments of the beta-tubulin (*tub),* calmodulin (*cmdA*)*,* translation elongation factor 1-alpha (*tef1*)*,* the internal transcribed spacer region of the rDNA (ITS)*,* the large subunit of the rDNA (LSU)*,* plus the RNA polymerase II large subunit (*rpb1*) and second largest subunit (*rpb2*) genes, we were able to identify and characterize several of these as new *Fusarium* species in the respective species complexes identified in this study.

Key words: Indonesia, new species, non-pathogenic, phylogeny, species complex

INTRODUCTION

Fusarium is one of the most diverse fungal genera that has been given much attention by mycologists and plant pathologists (Snyder & Hansen 1940, Nelson *et al.* 1983, Geiser *et al.* 2013, Aoki *et al.* 2014). Its global distribution, ability to adapt to manifold climatic conditions, and colonisation of a wide number of ecological niches and hosts, makes the diversity and abundance of *Fusarium* species unparalleled (Booth 1971, Gerlach & Nirenberg 1982, Geiser *et al.* 2013, Aoki *et al.* 2014). The genus *Fusarium* includes some of the most devastating plant pathogens, affecting many agronomical crops. Two of its species*, Fusarium graminearum* and *F. oxysporum,* were included in the top 10 list of fungal plant pathogens regarded as important in terms of scientific and economic impact (Dean *et al.* 2012, Geiser *et al*. 2013, Aoki *et al*. 2014).

Besides their role as plant pathogens, *Fusarium* species are also known as endophytes or saprophytic colonisers (Leslie *et al.* 1990, Bacon & Yates 2000). Many different *Fusarium* species are associated with symptomatic and asymptomatic plants (Leslie *et al.* 1990, Wang *et al.* 2004, Pinaria *et al.* 2010). Their role as pathogens can easily be identified through pathogenicity tests. However, many *Fusarium* species have not been associated with any disease symptoms on plants (Wang *et al.* 2004, Pinaria *et al.* 2010). Therefore, they are considered as endophytes and their association with their known host plants is difficult to discern (Kuldau & Yates 2000).

A complex of *Fusarium* spp. in the *Fusarium oxysporum* species complex (FOSC) is causing Fusarium wilt on banana (Maryani *et al.* 2019), also known as Panama disease (Stover 1962). The ability of these notorious fungi to infect a wide range of banana varieties has resulted in substantial economic strive in several banana producing regions (Ploetz *et al.* 2015, http://fusariumwilt.org/). Several studies acknowledged the diversity of *Fusarium* spp. pathogenic on banana and their worldwide distribution, thus recognising the threat to global banana cultivation (Ploetz 2006a, Ordonez *et al.* 2015, Maryani *et al.* 2019). However, to our knowledge, no study has been done to assess which other *Fusarium* species might be associated with Fusarium wilt on bananas.

In this study, we report *Fusarium* species hitchhiking with pathogenic *Fusarium* spp. causing Panama disease, isolated from local banana varieties in Indonesia. Therefore, we aim to characterise these non-*Fusarium oxysporum* isolates, based on multi-gene phylogenetic inference, supported by morphological observations.

MATERIALS AND METHODS

Isolates

Isolates were obtained from the pseudostems of local banana plants clearly displaying symptoms of Fusarium wilt, which were sampled in small-holder backyard plantations across Indonesia in 2014–2015 (Maryani *et al.* 2019). The dried pseudostem samples were cut into pieces of 2 x 3 cm and plated on Komada medium (Komada 1975). Single-spore isolates were derived from resulting fungal colonies, and transferred to potato dextrose agar (PDA), on which they were maintained as working cultures, or stored in 20 % (v/v) glycerol at –80 °C for long term preservation. All isolates were deposited in the Indonesian Culture Collection (InaCC) Cibinong, Indonesia.

Morphological characterisation

Morphological characterisations of the *Fusarium* species were performed on PDA for colony growth rates, pigmentation and production of aerial conidia; carnation leaf agar (CLA; Fisher *et al.* 1982) for formation of sporodochia and sporodochial conidia, and synthetic low-nutrient agar (SNA; Nirenberg 1981) for chlamydospores. To induce sporulation, cultures were incubated under continuous white light (Osram L18W/840 Cool White) for 7 d at 25 °C. Growth rates of all isolates were determined on PDA after 7 d incubation at 25 °C in the dark. Colony colour notation followed the mycological colour charts of Rayner (1970). Morphological characters were examined after mounting fungal structures in sterile water and observed using light microscopy (Nikon Eclipse 80i microscope) with Differential Interference Contrast (DIC) optics and a Nikon AZ100 stereomicroscope, both equipped with Nikon DS-Ri2 high definition colour digital cameras. Photographs and measurements were taken using the Nikon software NIS-elements D software v. 4.50. The length and width of at least 30 conidiogenous cells and 50 conidia were measured, and the mean values, standard deviation (SD) with maximum-minimum values were calculated. All descriptions, illustrations and nomenclatural data were deposited in MycoBank (Crous *et al.* 2004).

DNA isolation, amplification and analyses

Genomic DNA was isolated using the DNA Wizard Magnetic DNA Purification System for Food kit (Promega, USA). Partial gene sequences were determined for the RNA polymerase largest subunit gene (*rpb1*) using primers RPB1-Fa & RPB1-G2R (O'Donnell *et al.* 2010), RNA polymerase second largest subunit gene (*rpb2*) using primers RPB2-5f2 & RPB2-7cr (O'Donnell *et al.* 2010), the translation elongation factor 1-alpha gene (*tef1*) using primers EF1 & EF2 (O'Donnell *et al*. 1998a), calmodulin (*cmdA*) CAL-228F & CAL-2RD (Carbone & Kohn 1999, Quaedvlieg *et al.* 2011), beta-tubulin (*tub*) using primers TUB-T1 & TUB-4RD (O'Donnell & Cigelnik 1997, Woudenberg *et al.* 2009), the internal transcribed spacer region (ITS) using primers ITS4 & ITS5 (White *et al.* 1990) and the large subunit of the ribosomal DNA (LSU) using

primers LR0R & LR5 (Rehner & Samuels 1994, Vilgalys & Hester 1990). PCR conditions followed those described by Lombard *et al.* (2015). Amplicons were sequenced in both directions using the same primer pairs as were used for amplification to ensure integrity of the sequences. Consensus sequences were analysed and assembled using MEGA v. 7 (Kumar *et al.* 2016). Subsequent alignments for each individual locus were generated using MAFFT v. 7.110 (Katoh *et al.* 2017) and manually corrected if necessary. The individual sequences generated in this study were compared with those maintained in the *Fusarium*-MLST database (http://www.westerdijkinstitute.nl/fusarium/) and GenBank, and relevant sequences were included in the subsequent phylogenetic inferences.

Phylogenetic analyses were based on Maximum Likelihood (ML) and Bayesian Inference (BI). The ML analysis was performed using RAxML v. 8 (randomised accelerated (sic) maximum likelihood for high performance computing) (Stamatakis 2014) through RAxML BlackBox (http://embnet.vital-it.ch/raxml-bb/index.php) or the CIPRES science gateway portal (Miller *et al.* 2012). To assess the robustness of the analyses, the Bootstrap support (BS) was determined automatically by the software using default parameters. The BI analysis was performed using MrBayes v. 3.2.6 (Ronquist *et al.* 2012) on the CIPRES science gateway portal (Miller *et al*. 2012), using four Markov chain Monte Carlo (MCMC) chains starting from a random tree topology. The MCMC analyses lasted until the average standard deviations of split frequencies were below 0.01 with phylogenies saved every 1 000 generations. The first 25 % of saved trees were discarded as the "burn-in" phase and the 50 % consensus trees and posterior probabilities (PP) were determined from the remaining trees. All the sequences generated in this study were deposited in GenBank and the European Nucleotide Archive (ENA) and the alignments in TreeBASE.

Pathogenicity

Representative isolates from the different *Fusarium* species were selected for pathogenicity assays. *Fusarium odoratissimum*, Tropical Race 4 (TR4) isolate InaCC F856, was used as a positive control, and negative controls were treated with sterile water only. Two to three-month-old banana plants of the Cavendish variety Grand Naine were used in greenhouse-controlled conditions (constant day temperature of 25 °C, night temperature of 23 °C, ambient light until max. 16 h, and a relative humidity of ≥75 %). Preparation of the fungal inoculum, pathogenicity tests and severity scoring followed the protocol of Maryani *et al.* (2019). Five plant replicates were included for each isolate tested and 7 wk after inoculation disease severity was evaluated by scoring external foliage and internal corm symptoms.

RESULTS

In total, 20 isolates were identified that did not belong to the *Fusarium oxysporum* species complex (FOSC). These isolates were recovered from 13 banana varieties from the islands of Flores, Java, Kalimantan and Sulawesi (Table 1). An initial preliminary phylogenetic inference based on *rpb2* sequence data, demonstrated that most isolates belonged to the *Fusarium incarnatum-equiseti* species complex (FIESC, 11 isolates), followed by the *F. fujikuroi* species complex (FFSC, eight isolates) and the *F. sambucinum* species complex (FSSC, one isolate) (Fig. 1). Nine isolates in FIESC originated from Kalimantan, isolated from *Musa* sp. variety Pisang Awak (ABB), Pisang Kepok (ABB), and Pisang Talas (AA) and two isolates from Sulawesi, isolated from *Musa acuminata* var. Pisang Cere (AAA). The majority of the isolates in FFSC were isolated from bananas varieties in Java. The only isolate in the FSSC was isolated from the variety Pisang Awak (ABB) in Central Kalimantan. *Fusarium* isolates belonging to different species complexes were in some cases recovered from the same sample: isolate InaCC F962 in the FFSC and isolate Indo175 in the FIESC were isolated from the same sample of *Musa acuminata* var. Pisang Talas (AA) from South Kalimantan. In the FFSC, isolate InaCC F993 and Indo 213 were also isolated from a sample of *Musa acuminata* var. Pisang Mas Kirana (AA) from East Java. Additionally, different banana varieties were found to be associated with the same *Fusarium* species (Table 1).

***Fusarium fujikuroi* species complex (FFSC) phylogeny – Fig. 2.**

The eight isolates belonging to the FFSC were further analysed using a multi-gene phylogeny based on *cmdA, rpb1, rpb2, tef* and *tub.* The final alignment included 4 791 characters (*cmdA* 544, *rpb1* 1533, *rpb2* 1550, *tef* 676 and *tub* 488) including alignment gaps, and encompassed 52 isolates, with two outgroup taxa (*F. oxysporum* CBS 716.74 and CBS 744.97) (Table 2). The analysis was consistently able to distinguish the three biogeographical clades known as the African, American and Asian clades sensu O'Donnell *et al.* (1998). All of the Indonesian isolates clustered within the Asian clade of FFSC except for isolate InaCC F991, identified as *F. verticilloides*, and clustered within the African clade (Fig. 2). According to the multi-gene analysis, two isolates (InaCC F962 and InaCC F992) were identified as *F. proliferatum*, while two new phylogenetic species were recognised among the Indonesian isolates. Isolates InaCC F872 and InaCC F993, from central and East Java, respectively, clustered in a distinct, highly supported clade (96 bs/0.99 pp) closely related to *F. mangiferae*. Isolates InaCC F950–152, formed a distinct group (100 bs/1.0 pp), closely related to, but genetically distinct from *F. sacchari*.

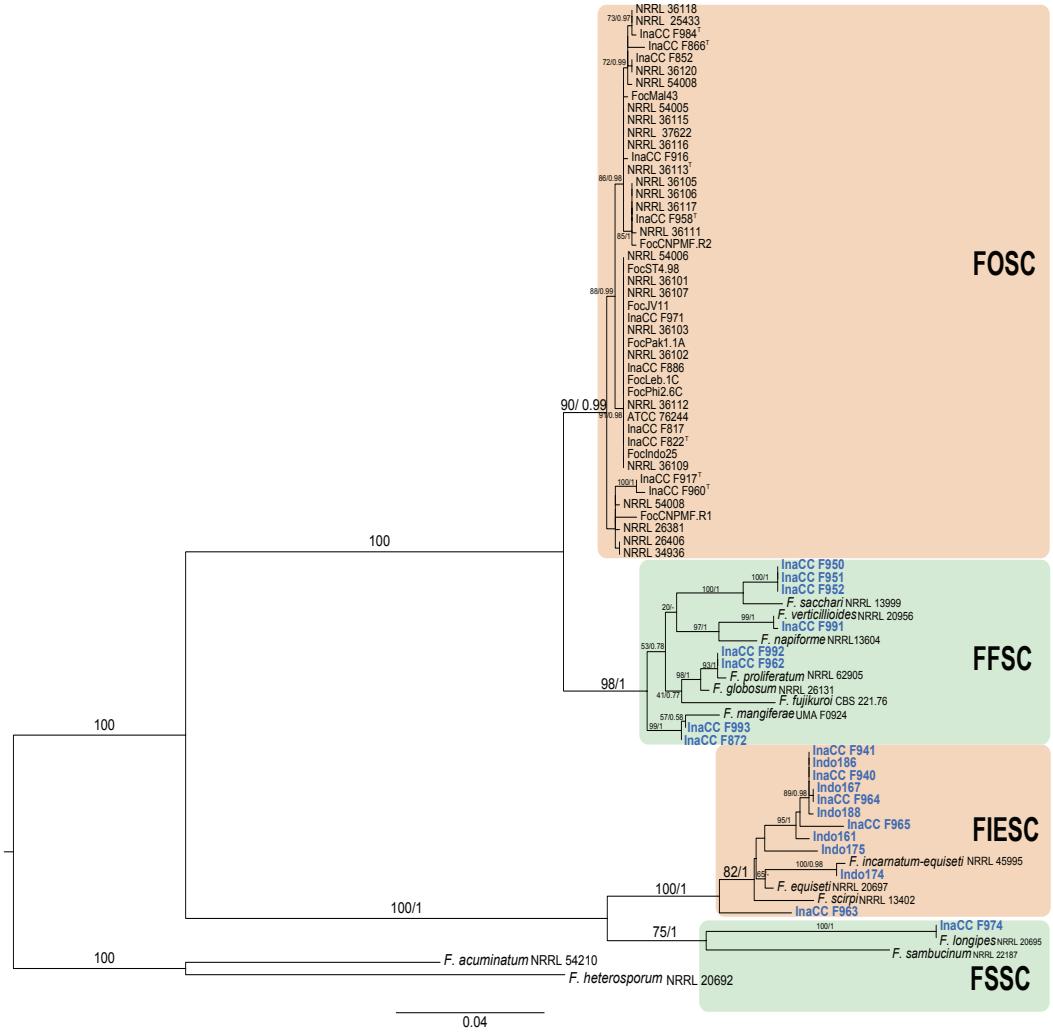

Fig. 1. Maximum Likelihood tree inferred using the *rpb2* gene region of the Indonesian isolates in the *Fusarium fujikuroi* species complex (FFSC), *Fusarium incarnatum-equiseti* species complex (FIESC), *Fusarium sambucinum* species complex (FSSC) and *Fusarium oxysporum* species complex (FOSC) isolates from a previous study (Maryani *et al.* 2019). Bootstrap support values and Bayesian posterior probabilities are given at each node. The tree is rooted to *Fusarium acuminatum* (NRRL 54210) and *Fusarium heterosporum* (NRRL 20692).

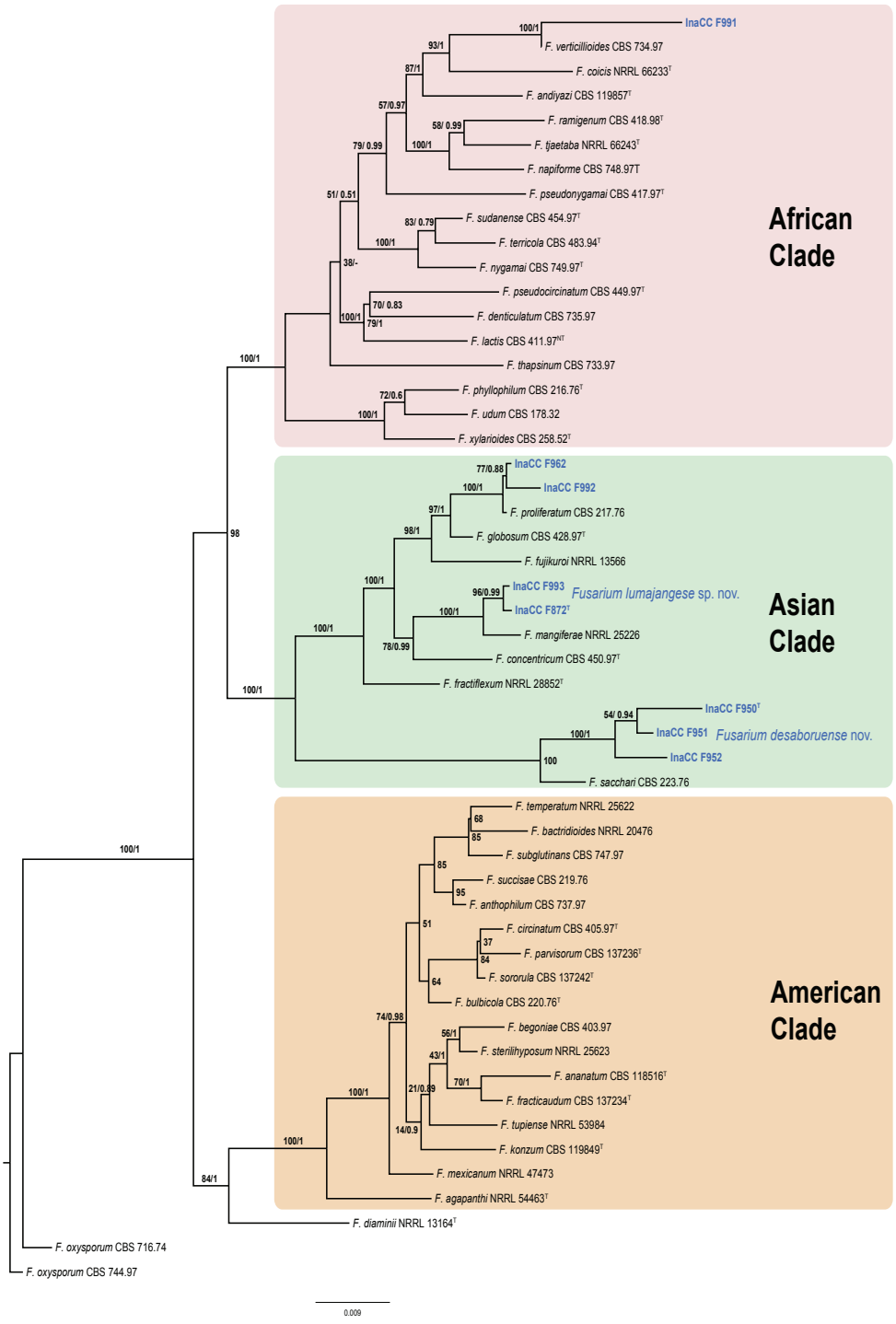

Fig. 2. Maximum likelihood tree inferred from the combined *cmdA*, *tef1*, *tub*, *rpb1* and *rpb2* sequence datasets of the *Fusarium fujikuroi* species complex (FFSC) including eight Indonesian isolates (Indicated in blue). Bootstrap support values and Bayesian posterior probabilities are given at each node. The tree is rooted to *Fusarium oxysporum* (CBS 716.74 and CBS 744.97).

Table 1. *Fusarium* species recovered from pseudostems of banana with Fusarium wilt symptoms in Indonesia, with details information on origin, year of collection and GenBank/ ENA accession numbers.

Species name	Strain number[1]	Location	Host[2]	Host genotype[2]	GenBank/ENA accession number[3]						
					cal	ITS	LSU	*rpb1*	*rpb2*	*tef*	*tub*
Fusarium desaboruense	InaCC F950^T	Sikka, Flores	*Musa* sp. var. Pisang Kepok	ABB				LS479870	LS479852		**LS479435**
	InaCC F951	Sikka, Flores	*Musa* sp. var. Pisang Kepok	ABB				LS479871	LS479853		**LS479436**
	InaCC F952	Sikka, Flores	*Musa* sp. var. Pisang Kepok	ABB				LS479872	LS479854		**LS479437**
F. kotabaruense	InaCC F963^T	Kota Baru, South Kalimantan	*Musa* sp. var. Pisang Awak	ABB	**LS479429**	**LS479417**	**LS479890**	LS479875	LS479859	LS479445	
F. longipes	InaCC F974	Katingan, Central Kalimantan	*Musa* sp. var. Pisang Awak	ABB				LS479880	LS479866	LS479451	
F. lumajangense	InaCC F872^T	Kendal, Central Java	*Musa* sp. var. Pisang Raja Nangka	AAB					LS479850	LS479441	**LS479433**
	InaCC F993	Lumajang, East Java	*Musa acuminata* var. Pisang Mas Kirana	AA					LS479851	LS479442	**LS479434**
F. verticilloides	InaCC F991	Bondowoso, East Java	*Musa* sp. var. Pisang Kepok	ABB	**LS479421**			LS479881	LS479867	LS479452	**LS479438**
F. proliferatum	InaCC F962	Kota Baru, South Kalimantan	*Musa acuminata* var. Pisang Talas	AA					LS479868	LS479453	**LS479439**
	InaCC F992	Lumajang, East Java	*Musa acuminata* var. Pisang Mas Kirana	AA				LS479882	LS479869	LS479454	**LS479440**
F. sulawense	InaCC F940^T	Bone, South Sulawesi	*Musa acuminata* var. Pisang Cere	AAA	**LS479422**	**LS479410**	**LS479883**		LS479855	LS479443	
	InaCC F941	Bone, South Sulawesi	*Musa acuminata* var. Pisang Cere	AAA	**LS479423**	**LS479411**	**LS479884**		LS479856	LS479444	
	Indo167	Kota Baru, South Kalimantan	*Musa* sp. var. Pisang Kepok	ABB	**LS479424**	**LS479412**	**LS479885**	LS479874	LS479858		
	InaCC F964	Kota Baru, South Kalimantan	*Musa* sp. var. Pisang Awak	ABB	**LS479425**	**LS479413**	**LS479886**	LS479876	LS479860	LS479446	
	Indo186	Banjar, South Kalimantan	*Musa* sp. var. Pisang Kepok	ABB	**LS479426**	**LS479414**	**LS479887**	LS479878	LS479864	LS479449	
	Indo188	Benajam, East Kalimantan	*Musa* sp. var. Pisang Awak	ABB	**LS479427**	**LS479415**	**LS479888**	LS479879	LS479865	LS479450	
F. tanahbumbuense	InaCC F965^T	Kota Baru, South Kalimantan	*Musa acuminata* var. Pisang Talas	AA	**LS479432**	**LS479420**	**LS479893**	LS479877	LS479863	LS479448	
Fusarium sp. FIESC 33	Indo161	Kota Baru, South Kalimantan	*Musa acuminata* var. Pisang Talas	AA	**LS479428**	**LS479416**	**LS479889**	LS479873	LS479857		
Fusarium sp. FIESC 29	Indo174	Kota Baru, South Kalimantan	*Musa* sp. var. Pisang Awak	ABB	**LS479430**	**LS479418**	**LS479891**		LS479861		
Fusarium sp. FIESC 30	Indo175	Kota Baru, South Kalimantan	*Musa acuminata* var. Pisang Talas	AA	**LS479431**	**LS479419**	**LS479892**		LS479862	LS479447	

[1]InaCC: Indonesian Culture Collection, Research Center for Biology, Indonesian Institute of Sciences (LIPI) Cibinong, Indonesia; Indo: Collection of N. Maryani; ^{T}: ex-type strain.
[2] According to https://www.crop-diversity.org/mgis/taxonomy.
[3] *cal*: calmodulin; ITS: internal transcribed spacer region of the rDNA. LSU: large subunit of the rDNA; rpb1: RNA polymerase largest subunit gene; rpb2: RNA polymerase second largest subunit gene; *tef*: translation elongation factor 1-alpha gene; *tub*: beta-tubulin. Sequences newly generated in this study are shown in **bold**.

Table 2. *Fusarium* species included in this study.

Species name	Strain number[1]	Identification	Country	Host	GenBank/ENA accession number[2]						
					cal	ITS	LSU	*rpb1*	*rpb2*	*tef*	*tub*
Fusarium acuminatum	NRRL 54210								GQ505484		
F. agapanthi	NRRL 54463^T		Australia	*Agapanthus* sp.	KU900611			KU900620	KU900625	KU900630	KU900635
F. ananatum	CBS 118516^T		South Africa	*Ananas comosus* fruit	LT996175			LT996188	LT996137	LT996091	LT996112
F. andiyazi	CBS 119857^T = NRRL 21727		South Africa	*Sorghum bicolor* soil debris	LT996176			LT996189	LT996138	LT996092	LT996113
F. anthophilum	CBS 737.97 = NRRL 13602		Germany	*Hippeastrum* sp.	LT996177			LT996190	LT996139	LT996093	LT996114
F. armeniacum	NRRL 6227		USA	Fescue hay				JX171446	JX171560		
F. asiaticum	CBS 110257 = NRRL 13818		Japan	Barley				JX171459	JX171573		
F. bactridioides	NRRL 20476		USA	*Cronartium conigenum*	AF158343					AF160290	U34434
F. begoniae	CBS 403.97^T = NRRL 25300		Germany	*Begonia* elatior hybrid	AF158346			LT996191	LT996140	AF160293	U61543
F. bulbicola	CBS 220.76^T = NRRL 13618		Germany	*Nerine bowdenii*	KF466327			KF466394	KF466404	KF466415	KF466437
F. cf. *compactum*	NRRL 13829		Japan	River sediments				JX171460	JX171574		
F. circinatum	CBS 405.97^T = NRRL 25331		USA	*Pinus radiata*	KM231393			JX171510	HM068354	KM231943	KM232080
F. coicis	NRRL 66233^T		Australia	*Coix gasteenii*	LT996178			KP083269	KP083274	KP083251	LT996115
F. concentricum	CBS 450.97^T = NRRL 25181		Costa Rica	*Musa sapientum* fruit	AF158335			LT996192	JF741086	AF160282	U61548
F. cugenangense	InaCC F984^T	f. sp. *cubense*	Indonesia	*Musa* sp. var. Pisang Kepok					LS479308		
	NRRL 36118	f. sp. *cubense*	Thailand	*Musa* sp. var. Kluai nam wa					LS479221		
	NRRL 25433	f. sp. *vasinvectum*	China	*Gosypium* sp.					LS479202		
F. culmorum	CBS 417.86 = NRRL 25475		Denmark	Moldy barley kernel				JX171515	JX171628		
F. denticulatum	CBS 735.97 = NRRL 25302		USA	*Ipomoea batatas*	AF158322			LT996195	LT996143	AF160269	U61550
F. dlaminii	CBS 119860^T = NRRL 13164		South Africa	Soil debris in cornfield	AF158330			KU171681	KU171701	AF160277	U34430
F. duoseptatum	InaCC F916	f. sp. *cubense*	Indonesia	*Musa* sp. var. Pisang Kepok					LS479239		
	FocMal43	f. sp. *cubense*	Malaysia	*Musa* sp. var. Pisang Rastali					LS479207		
	NRRL 36115	f. sp. *cubense*	Malaysia	*Musa acuminata* var. Pisang Ambon					LS479218		
	NRRL 36116	f. sp. *cubense*	Malaysia	*Musa* sp. var. Pisang Keling					LS479219		
F. equiseti	CBS 107.07 = NRRL 36136	FIESC 14a			GQ505556	GQ505733	GQ505733		GQ505822	GQ505644	

Table 2. (Continued).

	CBS 185.34 = NRRL 36321	FIESC 14a	Nehterlands	Soil	GQ505559	GQ505736	GQ505736		GQ505825	GQ505647	
	CBS 307.94^{NT} = NRRL 26419	FIESC 14a	Germany	Soil	GQ505511	GQ505688	GQ505688		GQ505777	GQ505599	
	CBS 414.86 = NRRL 36466	FIESC 14a	Denmark	Potato peel	GQ505565	GQ505742	GQ505742		GQ505831	GQ505653	
F. fracticaudum	CBS 137234^{PT}		Colombia	*Pinus maximonoii* stem	LT996179			LT996196	LT996144	KJ541059	KJ541051
F. fractiflexum	NRRL 28852^{T}		Japan	*Cymbidium* sp.	AF158341			LT575064	LT575064	AF160288	AF160315
F. fujikuroi	NRRL 13566		China	*Oryza sativa*	AF158332	U34557	U34528	JX171456	JX171570	AF160279	U34415
	CBS 221.76								KU604255		
F. globosum	CBS 428.97^{T} = NRRL 26131		South Africa	*Zea mays*	KF466329			KF466396	KF466406	KF466417	KF466439
F. goolgardi	NRRL 66250^{T} = RBG 5411		Australia	*Xanthorrhoea glauca*				KP083270	KP083280		
F. graminearum	CBS 123657 = NRRL 31084		USA	Corn				JX171531	JX171644		
F. grosmichelii	InaCC F852	f. sp. *cubense*	Indonesia	*Musa acuminata* var. Pisang Ambon Lumut					LS479342		
	NRRL 36120	f. sp. *cubense*	Thailand	*Musa sapientum*					LS479222		
F. heterosporum	NRRL 20692		Ethiopia	*Cynodon dactylon*					JX171593		
F. hexaseptatum	InaCC F866^{T}	f. sp. *cubense*	Indonesia	*Musa acuminata* var. Pisang Ambon Kuning					LS479359		
F. kalimantanense	InaCC F917^{T}		Indonesia	*Musa acuminata* var. Pisang Ambon					LS479241		
F. konzum	CBS 119849^{T}		USA	*Sorghastrum nuttans*	LT996182			LT996200	LT996148	LT996098	LT996118
F. kyushuense	NRRL 25349		Japan	*Triticum aestivum*					GQ915492		
F. lacertarum	CBS 102300 = NRRL 36123	FIESC 4b			GQ505555	GQ505732	GQ505732		JX171581	GQ505593	
	CBS 130185^{T} = NRRL 20423	FIESC 4a	India	Lizard skin	GQ505505	GQ505682	GQ505682		GQ505821	GQ505643	
F. lactis	CBS 411.97^{NT} = NRRL 25200		USA	*Ficus carica*	AF158325			LT996201	LT996149	AF160272	U61551
F. langsethiae	NRRL 54940		Norway	Oats				JX171550	JX171662		
F. longipes	NRRL 13368		Australia	Soil				JX171448	JX171562		
	NRRL 20695								GQ915493		
F. mangiferae	NRRL 25226		Israel	*Mangifera indica*	AF158334			JX171509	HM068353	AF160281	U61561
	UMA F0924			*Mangifera indica*					KP753442		
F. mexicanum	NRRL 47473		Mexico	*Mangifera indica* inflorescence	GU737389			Not public	Not public	GU737416	GU737308
F. napiforme	CBS 748.97^{T} = NRRL 13604		Namibia	*Pennisetum typhoides*	AF158319			HM347136	EF470117	AF160266	U34428

Table 2. (Continued).

F. nygamai	CBS 749.97^{T} = NRRL 13448		Australia	*Sorghum bicolor* necrotic root	AF158326	LT996202	EF470114	AF160273	U34426
F. odoratissimum	InaCC F817	f. sp. *cubense*	Indonesia	*Musa* sp. var. Pisang Kepok			LS479304		
	InaCC F822^{T}	f. sp. *cubense*	Indonesia	*Musa* sp. var. Pisang Raja			LS479386		
	NRRL 54006	f. sp. *cubense*	Indonesia	*Musa acuminata* var. Pisang Manurung			LS479198		
	FocJV11	f. sp. *cubense*	Jordan	*Musa acuminata* var. Cavendish			LS479205		
	FocLeb1.2C	f. sp. *cubense*	Lebanon	*Musa acuminata* var. Cavendish			LS479206		
	NRRL 36102	f. sp. *cubense*	China	*Musa acuminata* var. Cavendish			LS479209		
	FocPak1.1A	f. sp. *cubense*	Pakistan	*Musa acuminata* var. Cavendish			LS479223		
	FocPhi2.6C	f. sp. *cubense*	The Philippines	*Musa acuminata* var. Cavendish			LS479224		
F. oxysporum	CBS 716.74		Germany	*Vicia faba*	AF158366	JX171469	JX171583	AF008479	U34435
	CBS 744.97		USA	*Pseudotsuga menziesii*	AF158365	LT996203	LT575065	AF160312	U34424
	NRRL 26381	f. sp. *lycopersici*	USA	*Solanum lycopersicum*			LS479195		
	NRRL 54002			Soil			LS479194		
	FocCNPMF.R1	f. sp. *cubense*	Brazil	*Musa* sp. var. Silk			LS479196		
	NRRL 34936	f. sp. *lycopersici*	Spain	*Solanum lycopersicum*			LS479200		
	NRRL 26406	f. sp. *melonis*		*Cucumis melo*			LS479201		
F. palustre	NRRL 54056^{T}		USA	*Spartina alterniflora*		KT597718	KT597731		
F. parvisorum	CBS 137236^{T}		Colombia	*Pinus patula* roots	LT996183		LT996150	KJ541060	KJ541055
F. phialoporum	InaCC F971	f. sp. *cubense*	Indonesia	*Musa* sp. var. Pisang Awak			LS479292		
	FocST4.98	f. sp. *cubense*	Spain	*Musa acuminata* var. Dwarf Cavendish			LS479227		
	FocIndo25	f. sp. *cubense*	Indonesia	*Musa acuminata* var. Pisang Ambon			LS479204		
	NRRL 36101	f. sp. *cubense*	Australia	*Musa* sp. var. Mons Mari			LS479208		
	NRRL 36103	f. sp. *cubense*	The Philippines	*Musa acuminata* var. Cavendish			LS479210		
	NRRL 36109	f. sp. *cubense*	Australia	*Musa acuminata* var. SH3142			LS479214		
	NRRL 36112	f. sp. *cubense*	South Africa	*Musa acuminata* var. Cavendish			LS479216		

Table 2. (Continued).

F. phyllophilum	CBS 216.76^{T} = NRRL 13617		Italy	*Dracaena deremensis* leaf	KF466333			KF466399	KF466410	KF466421	KF466443
F. poae	NRRL 13714							JX171458	JX171572		
F. proliferatum	CBS 217.76 = NRRL 22944		Germany	*Cattleya* pseudobulb, hybrid	AF158333			JX171504	HM068352	AF160280	U34416
	NRRL 62905								KU171707		
F. pseudocircinatum	CBS 449.97^{T} = NRRL 22946		Ghana	*Solanum* sp.	AF158324			LT996204	LT996151	AF160271	U34427
F. pseudograminearum	CBS 109956^{T} = NRRL 28062		Australia	*Hordeum vulgare* crowns				JX171524	JX171637		
F. pseudonygamai	CBS 417.97^{T} = NRRL 13592		Nigeria	*Pennisetum typhoides*	AF158316			LT996205	LT996152	AF160263	U34421
F. purpurascens	InaCC F886	f. sp. *cubense*	Indonesia	*Musa* sp. var. Pisang Kepok					LS479385		
	ATCC 76244	f. sp. *cubense*	USA	*Musa acuminata* var. Apple					LS479199		
	NRRL 36107	f. sp. *cubense*	Honduras	*Musa* sp. var. Maqueno					LS479213		
F. ramigenum	CBS 418.98^{T} = NRRL 25208		USA	*Ficus carica*	KF466335			KF466401	KF466412	KF466423	KF466445
F. sacchari	CBS 223.76 = NRRL 13999		India	*Saccharum officinarum*	AF158331			JX171466	JX171580	AF160278	U34414
F. sambucinum	NRRL 22187 = NRRL 20727		England	*Solanum* sp.				JX171493	JX171606		
F. sangayamense	InaCC F960^{T}		Indonesia	*Musa* sp. var. Pisang Kepok					LS479283		
F. scirpi	CBS 447.84 = NRRL 36478	FIESC 9a	Australia	Pasture soil	GQ505566	GQ505743	GQ505743		GQ505832	GQ505654	
	CBS 448.84 = NRRL 29134	FIESC 9a	Australia	Pasture soil	GQ505517	GQ505694	GQ505694		GQ505783	GQ505605	
	CBS 610.95 = NRRL 26922	FIESC 9c	France	Soil	GQ505513	GQ505690	GQ505690		GQ505779	GQ505601	
	NRRL 13402	FIESC 9b	Australia	Pine nursery soil	GQ505504	GQ505681	GQ505681		JX171566	GQ505592	
F. sibiricum	NRRL 53430^{T}		Russia	*Avena sativa*					HQ154472		
F. sororula	CBS 137242^{T}		Colombia	*Pinus patula* stems	LT996184			LT996206	LT996153	KJ541067	KJ541057
F. tardichlamydosporum	InaCC F958^{T}	f. sp. *cubense*	Indonesia	*Musa acuminata* var. Pisang Barangan					LS479280		
	FocCNPMF.R2	f. sp. *cubense*	Brazil	*Musa* sp. var. Monthan					LS479197		
	NRRL 36105	f. sp. *cubense*	Honduras	*Musa* sp. var. Bluggoe					LS479211		
	NRRL 36106	f. sp. *cubense*	Australia	*Musa acuminata* var. Lady Finger					LS479212		
	NRRL 36111	f. sp. *cubense*	Australia	*Musa* sp. var. Bluggoe					LS479215		
	NRRL 36117	f. sp. *cubense*	Malaysia	*Musa* sp. var. Pisang Awak Legor					LS479220		

Table 2. (Continued).

F. tardicrescens	NRRL 36113^{T}	f. sp. *cubense*	Malawi	*Musa* sp. var. Harare					LS479217	
	NRRL 37622	f. sp. *pisi*		*Cicer* sp.					LS479203	
	NRRL 54008	f. sp. *conglutinans*	Brazil	Silk					LS479225	
	NRRL 54005	f. sp. *raphani*		*Raphanus* sp.					LS479226	
Fusarium sp.	NRRL 3020	FIESC 10a			GQ505498	GQ505675	GQ505675		GQ505764	GQ505586
	NRRL 3214	FIESC 10a			GQ505499	GQ505676	GQ505676		GQ505765	GQ505587
	NRRL 5537	FIESC 8a	USA	Fescue hay	GQ505500	GQ505677	GQ505677		GQ505766	GQ505588
	NRRL 6548	FIESC 12a	Germany	*Hordeum vulgare* seedling	GQ505501	GQ505678	GQ505678		GQ505767	GQ505589
	NRRL 13335	FIESC 21a			GQ505502	GQ505679	GQ505679		GQ505768	GQ505590
	NRRL 20722	FIESC 27a	Kenya	*Pyrethrum* sp.	GQ505507	GQ505684	GQ505684		GQ505773	GQ505595
	NRRL 22244	FIESC 25a	China	Rice	GQ505508	GQ505685	GQ505685		GQ505774	GQ505596
	NRRL 25795	FIESC 5c	Germany	*Disphyma crassifolium* seed	GQ505509	GQ505686	GQ505686		GQ505775	GQ505597
	NRRL 26417	FIESC 26a	Cuba	Plant leaf litter	GQ505510	GQ505687	GQ505687		GQ505776	GQ505598
	NRRL 26921	FIESC 12a	Germany	Culm base of *Triticum aestivum*	GQ505512	GQ505689	GQ505689		GQ505778	GQ505600
	NRRL 28029	FIESC 3b	USA		GQ505514	GQ505691	GQ505691		GQ505780	GQ505602
	NRRL 28577	FIESC 28a	Romania	Grave stone	GQ505515	GQ505692	GQ505692		GQ505781	GQ505603
	NRRL 28714	FIESC 26b			GQ505516	GQ505693	GQ505693		GQ505782	GQ505604
	NRRL 31008		Australia	Soil				JX171529	JX171642	
	NRRL 31011	FIESC 12a	Germany	*Thuja* sp.	GQ505518	GQ505695	GQ505695		GQ505784	GQ505606
	NRRL 31160	FIESC 15c	USA	Human lung	GQ505519	GQ505696	GQ505696		GQ505785	GQ505607
	NRRL 31167	FIESC 18a	USA	Human sputum	GQ505520	GQ505697	GQ505697		GQ505786	GQ505608
	NRRL 32175	FIESC 15a	USA	Human sputum	GQ505521	GQ505698	GQ505698		GQ505787	GQ505609
	NRRL 32181	FIESC 15c	USA	Human blood	GQ505522	GQ505699	GQ505699		GQ505788	GQ505610
	NRRL 32182	FIESC 15b	USA	Human blood	GQ505523	GQ505700	GQ505700		GQ505789	GQ505611
	NRRL 32522	FIESC 18b	USA	Human diabetic cellulitis	GQ505524	GQ505701	GQ505701		GQ505790	GQ505612
	NRRL 32864	FIESC 17a	USA	Human	GQ505525	GQ505702	GQ505702		GQ505791	GQ505613
	NRRL 32865	FIESC 21b	Brazil	Human endocarditis	GQ505526	GQ505703	GQ505703		GQ505792	GQ505614

Table 2. (Continued).

NRRL 32866	FIESC 23a	USA	Human cancer patient	GQ505527	GQ505704	GQ505704	GQ505793	GQ505615
NRRL 32867	FIESC 23a	USA	Human	GQ505528	GQ505705	GQ505705	GQ505794	GQ505616
NRRL 32868	FIESC 25c	USA	Human blood	GQ505529	GQ505706	GQ505706	GQ505795	GQ505617
NRRL 32869	FIESC 15c	USA	Human cancer patient	GQ505530	GQ505707	GQ505707	GQ505796	GQ505618
NRRL 32871	FIESC 5a	USA	Human abscess	GQ505531	GQ505708	GQ505708	GQ505797	GQ505619
NRRL 32994	FIESC 15c	USA	Human ethmoid sinus	GQ505533	GQ505710	GQ505710	GQ505799	GQ505621
NRRL 32995	FIESC 15c	USA	Human sinus	GQ505534	GQ505711	GQ505711	GQ505800	GQ505622
NRRL 32996	FIESC 15c	USA	Human leg wound	GQ505535	GQ505712	GQ505712	GQ505801	GQ505623
NRRL 32997	FIESC 7a	USA	Human toenail	GQ505536	GQ505713	GQ505713	GQ505802	GQ505624
NRRL 34001	FIESC 15e	USA	Human foot wound	GQ505537	GQ505714	GQ505714	GQ505803	GQ505625
NRRL 34002	FIESC 22a	USA	Human ethmoid sinus	GQ505538	GQ505715	GQ505715	GQ505804	GQ505626
NRRL 34003	FIESC 20a	USA	Human sputum	GQ505539	GQ505716	GQ505716	GQ505805	GQ505627
NRRL 34004	FIESC 16a	USA	Human BAL	GQ505540	GQ505717	GQ505717	GQ505806	GQ505628
NRRL 34005	FIESC 24a	USA	Human intravitreal fluid	GQ505541	GQ505718	GQ505718	GQ505807	GQ505629
NRRL 34006	FIESC 15a	USA	Human eye	GQ505542	GQ505719	GQ505719	GQ505808	GQ505630
NRRL 34007	FIESC 15a	USA	Human sputum	GQ505543	GQ505720	GQ505720	GQ505809	GQ505631
NRRL 34008	FIESC 15d	USA	Human lung	GQ505544	GQ505721	GQ505721	GQ505810	GQ505632
NRRL 34010	FIESC 15c	USA	Human maxullary sinus	GQ505545	GQ505722	GQ505722	GQ505811	GQ505633
NRRL 34011	FIESC 15a	USA	Human sputum	GQ505546	GQ505723	GQ505723	GQ505812	GQ505634
NRRL 34032	FIESC 5a	USA	Human abscess	GQ505547	GQ505724	GQ505724	GQ505813	GQ505635
NRRL 34034	FIESC 1c	USA	Human leg	GQ505548	GQ505725	GQ505725	GQ505814	GQ505636
NRRL 34035	FIESC 5d	USA	Human sinus	GQ505549	GQ505726	GQ505726	GQ505815	GQ505637
NRRL 34037	FIESC 5b	USA	Human abscess	GQ505550	GQ505727	GQ505727	GQ505816	GQ505638
NRRL 34039	FIESC 1b	USA	Human	GQ505551	GQ505728	GQ505728	GQ505817	GQ505639
NRRL 34056	FIESC 16b	USA	Human bronchial wash	GQ505552	GQ505729	GQ505729	GQ505818	GQ505640
NRRL 34059	FIESC 16c	USA	Human blood	GQ505553	GQ505730	GQ505730	GQ505819	GQ505641
NRRL 34070	FIESC 17c	USA	Tortoise	GQ505554	GQ505731	GQ505731	GQ505820	GQ505642
NRRL 36269	FIESC 12b	Croatia	*Pinus nigra* seesling	GQ505557	GQ505734	GQ505734	GQ505823	GQ505645

Table 2. (Continued).

	NRRL 36318	FIESC 3a			GQ505558	GQ505735	GQ505735		GQ505824	GQ505646	
	NRRL 36323	FIESC 3a	England	Cotton yarn	GQ505560	GQ505737	GQ505737		GQ505826	GQ505648	
	NRRL 36351								GQ915484		
	NRRL 36372	FIESC 11a	Netherlands Antilles	Air	GQ505561	GQ505738	GQ505738		GQ505827	GQ505649	
	NRRL 36392	FIESC 12c	Germany	Seedling	GQ505562	GQ505739	GQ505739		GQ505828	GQ505650	
	NRRL 36401	FIESC 2a	Mozambique	Cotton	GQ505563	GQ505740	GQ505740		GQ505829	GQ505651	
	NRRL 36448	FIESC 2b	Sudan	*Phaseolus vulgaris* seed	GQ505564	GQ505741	GQ505741		GQ505830	GQ505652	
	NRRL 36548	FIESC 17b	Congo	Banana	GQ505567	GQ505744	GQ505744		GQ505833	GQ505655	
	NRRL 36575	FIESC 20b	USA	*Juniperus chinensis* leaf	GQ505568	GQ505745	GQ505745		GQ505834	GQ505656	
	NRRL 43297	FIESC 24b	USA	*Spartina* rhizomes	GQ505569	GQ505746	GQ505746		GQ505835	GQ505657	
	NRRL 43619	FIESC 15a	USA	Human finger	GQ505570	GQ505748	GQ505748		GQ505837	GQ505659	
	NRRL 43622	FIESC 15c	USA	Human lung	GQ505571	GQ505749	GQ505749		GQ505838	GQ505660	
	NRRL 43635	FIESC 13a	USA	Horse	GQ505573	GQ505751	GQ505751		GQ505840	GQ505662	
	NRRL 43638	FIESC 6a	USA	Manatee	GQ505576	GQ505754	GQ505754		GQ505843	GQ505665	
	NRRL 43639	FIESC 19a	USA	Manatee	GQ505577	GQ505755	GQ505755		GQ505844	GQ505666	
	NRRL 43640	FIESC 1a	USA	Dog nose	GQ505578	GQ505756	GQ505756		GQ505845	GQ505667	
	NRRL 43694	FIESC 6a	USA	Human eye	GQ505579	GQ505757	GQ505757		GQ505846	GQ505668	
	NRRL 43730	FIESC 16c	USA	Contact lens	GQ505580	GQ505758	GQ505758		GQ505847	GQ505669	
	NRRL 45995	FIESC 5b	USA	Human abscess	GQ505581	GQ505759	GQ505759		GQ505848	GQ505670	
	NRRL 45997	FIESC 5f	USA	Human sinus	GQ505583	GQ505761	GQ505761		GQ505850	GQ505672	
	NRRL 45998	FIESC 6b	USA	Human toe	GQ505584	GQ505762	GQ505762		GQ505851	GQ505673	
F. sporotrichioides	NRRL 3299		USA	Corn				JX171444	HQ154454		
F. sterilihyposum	NRRL 25623		South Africa	Mango	AF158353			Not public	Not public	AF160300	AF160316
F. subglutinans	CBS 747.97 = NRRL 22016		USA	Corn	AF158342			JX171486	JX171599	AF160289	U34417
F. succisae	CBS 219.76 = NRRL 13613		Germany	*Succisa pratensis* flower	AF158344			LT996207	LT996154	AF160291	U34419
F. sudanense	CBS 454.97^{T} = NRRL 25451		Sudan	*Striga hermonthica*	LT996185			LT996208	LT996155	KU711697	KU603909
F. temperatum	NRRL 25622 = NRRL 26616		South Africa	*Zea mays*	AF158354			Not public	Not public	AF16030	AF160317

Table 2. (Continued).

F. terricola	CBS 483.94^{T}	Australia	Soil	KU603951	LT996209	LT996156	KU711698	KU603908
F. thapsinum	CBS 733.97 = NRRL 22045	South Africa	*Sorghum bicolor*	LT996186	JX171487	JX171600	AF160270	U34418
F. tjaetaba	NRRL 66243^{T}	Australia	*Sorghum interjectum*	LT996187	KP083267	KP083275	KP083263	GU737296
F. tupiense	NRRL 53984	Brazil	*Mangifera indica*	GU737377	Not public	Not public	GU737404	GU737296
F. udum	CBS 178.32 = NRRL 22949	Germany	*Lactarius pubescens*	AF158328	LT996220	LT996172	AF160275	U34433
F. venenatum	CBS 458.93^{T}	Austria	Winter wheat halm base			KM232382		
F. verticillioides	CBS 734.97 = NRRL 22172	Germany	*Zea mays*	AF158315	LT996221	EF470122	AF160262	U34413
	NRRL 20956		*Zea mays*			JX171598		
F. xylarioides	CBS 258.52 = NRRL 25486	Ivory Coast	*Coffea* trunk		JX171517	HM068355	AY707136	AY707118

[1]CBS: collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; Foc: collection of Wageningen Plant Research, Wageningen University, The Netherlands; InaCC: Indonesian Culture Collection, Research Center for Biology, Indonesian Institute of Sciences (LIPI) Cibinong, Indonesia; NRRL: Agricultural Research Service Culture Collection, USA; ATCC: American Type Culture Collection, USA; UMAF: Microbiology and Plant Pathology Laboratory Collection, University of Malaga, Spain; Indo: Collection of N. Maryani, Wageningen Plant Research, Wageningen University, The Netherlands; UMAF: Microbiology and Plant Pathology Laboratory Collection, University of Malaga, Spain; PT: ex-paratype culture; T: ex-type culture; NT: neotype.

[2] *cal*: calmodulin; ITS: internal transcribed spacer region of the rDNA; LSU: large subunit of the rDNA; *rpb1*: RNA polymerase largest subunit gene; *rpb2*: RNA polymerase second largest subunit gene; *tef*: translation elongation factor 1-alpha gene; *tub*: beta-tubulin; Sequences marked as "Not public" were obtained from Kerry O'Donnell's alignment datasets.

***Fusarium incarnatum-equiseti* species complex (FIESC) phylogeny – Fig. 3.**

The 11 isolates belonging to the FIESC were assessed using a more inclusive analysis based on five loci (*cmdA,* ITS, LSU, *rpb2 and tef1*). The alignment consisted of a total 2 746 characters (*cmdA* 653, ITS 510, LSU 562, *rpb2* 597 and *tef1* 424), from 93 isolates, including all the phylogenetic clades known in this species complex plus two outgroup taxa (*Fusarium circinatum* NRRL 25331 and *F. fujikuroi* NRRL 13566). Multi-gene phylogenetic inference was able to recognise six new phylogenetic species in the FIESC. The number of new phylogenetic species recognised is equally distributed in the incarnatum clade and the equiseti clade (three new phylospecies each) *sensu* O'Donnell *et al*. (2009). In the *incarnatum* clade, isolates InaCC F940, InaCC F941, Indo167, InaCC F964, Indo186, and Indo188 clustered in a distinct clade (55 bp/0.99 pp) closely related to the phylogenetic species FIESC-16 which is introduced here as phylogenetic species FIESC-32. These isolates were obtained from five different banana variety hosts in Sulawesi and Kalimantan. The other two new species in the *incarnatum* clade are monotypic lineages represented by isolate Indo161 (99 bp/1 pp) closely related to FIESC-26 and isolate InaCC F965 (50bp/ 1 pp) closely related to FIESC-24, introduced as phylogenetic species FIESC-33 and FIESC-34 respectively. In the *equiseti* clade, three isolates: Indo174 (99 bp/1 pp) closely related to FIESC-1; Indo175 (-/ 1 pp) and InaCC F963 (55 bp/1 pp), both isolates closely related to FIESC-13, formed monotypic lineages which are introduced here as FIESC-29, FIESC-30, and FIESC-31 respectively. These phylogenetic species were isolated from two banana varieties in relatively close proximity in South Kalimantan.

***Fusarium sambucinum* species complex (FSSC) phylogeny – Fig. 4.**

The single Indonesian isolate in the FSSC was further analysed using a two-gene phylogeny based on *rpb1* and *rpb2* sequences. The analysis included a total of 2 461 characters (*rpb1* 854 and *rpb2* 1607) from a total of 21 isolates representing the FSSC and two outgroup taxa (*F. circinatum* NRRL 25331 and *F. fujikuroi* NRRL 13566). Isolate InaCC F974 was identified as *Fusarium longipes* (Fig. 4) based on phylogenetic inference.

Pathogenicity

Representative isolates from each species complex were tested for their pathogenicity against banana variety Cavendish (Fig. 5). Selected isolates included InaCC F872, InaCC F950, and InaCC F992 (FFSC), InaCC F962 (FIESC), InaCC F974 and (FSSC). None of the isolates was able to cause any disease symptoms in the inoculated plants. All of the isolates tested caused only slight discoloration in the corm without any further disease development.

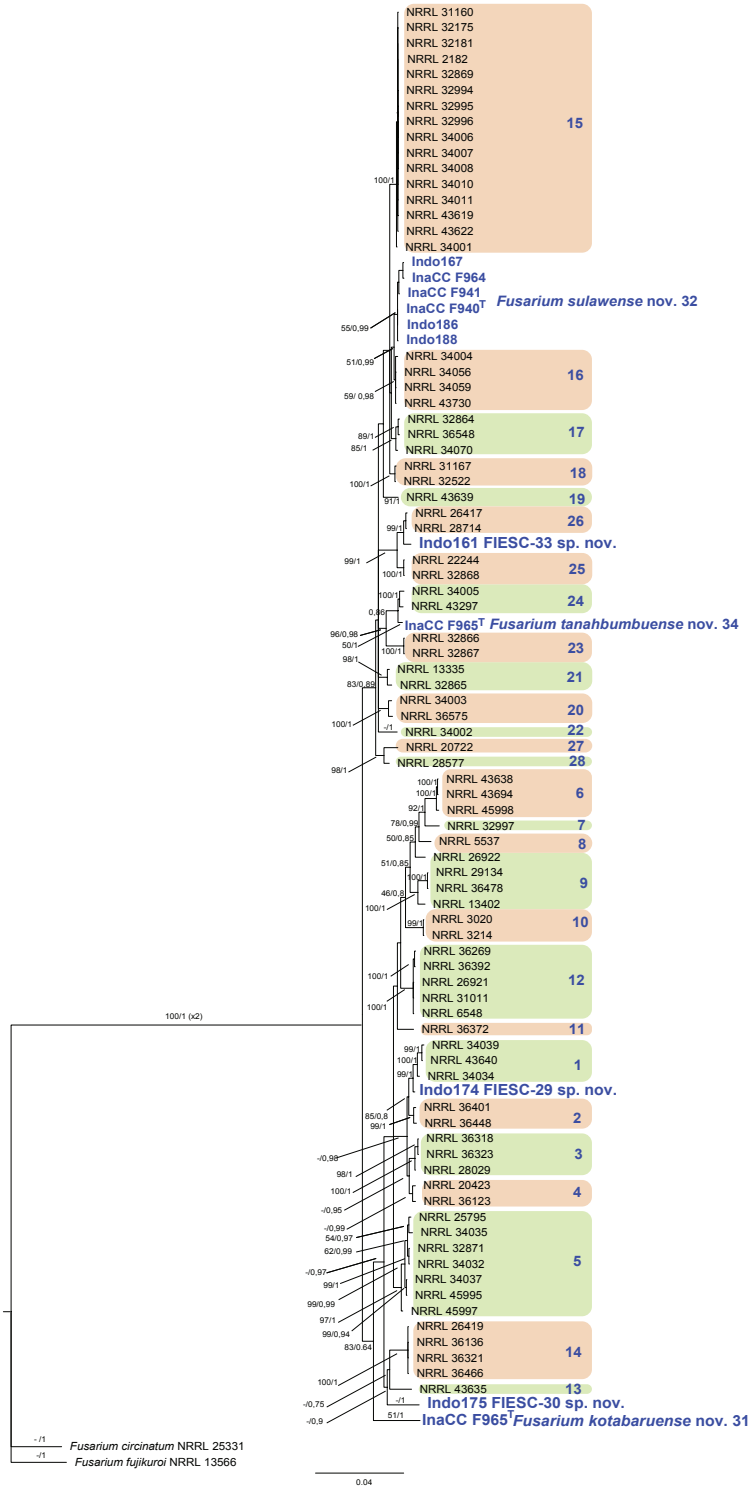

Fig. 3. Maximum likelihood tree inferred from the combined *cmdA, ITS, rpb2, tef1* and *LSU* sequence datasets of the *Fusarium incarnatum-equiseti* species complex (FIESC) including 11 Indonesian isolates (Indicated in blue). Bootstrap support values and Bayesian posterior probabilities are given at each node. The tree is rooted to *Fusarium circinatum* (NRRL 25331) and *Fusarium fujikuroi* (NRRL 13566).

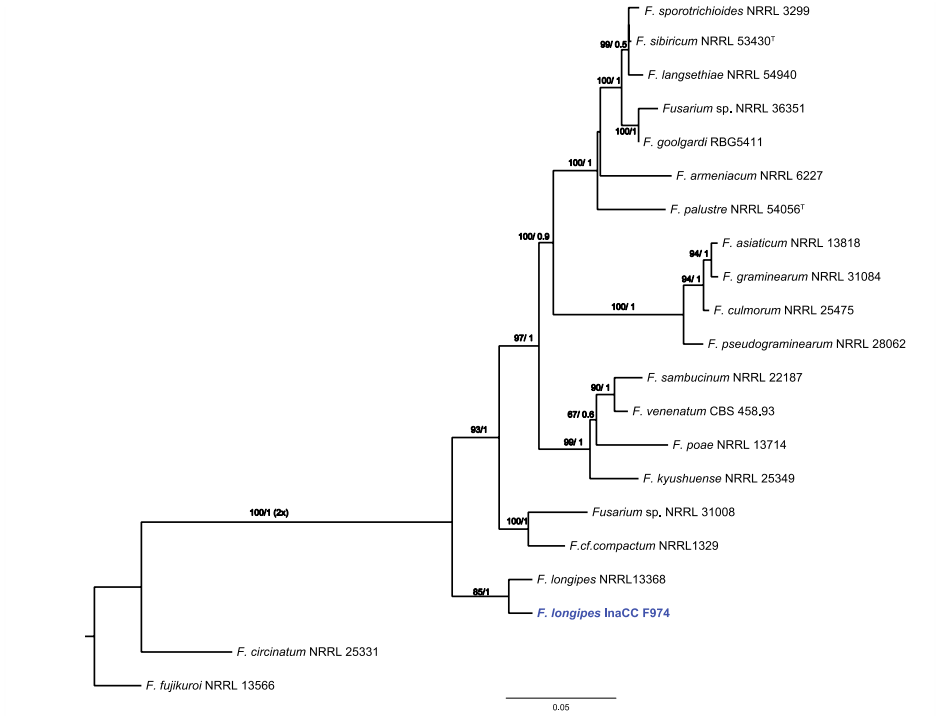

Fig. 4. Maximum likelihood tree inferred from the combined *rpb1* and *rpb2* sequence datasets of the *Fusarium sambucinum* species complex (FSSC) including one Indonesian isolate InaCC F974 (indicated in blue). Bootstrap support values and Bayesian posterior probabilities are given at each node. The tree is rooted to *Fusarium circinatum* (NRRL 25331) and *Fusarium fujikuroi* (NRRL 13566).

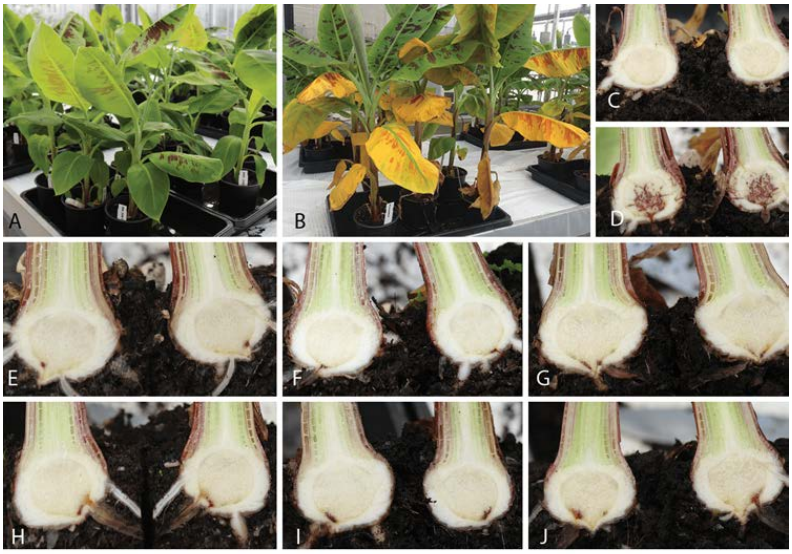

Fig. 5. Pathogenicity test of *Fusarium* spp. that belong to other species complexes. **A.** Plants before inoculation, **B.** Wilting symptom caused by *Fusarium odoratissimum* InaCC F856, seven weeks after inoculation, **C.** Control, **D.** Positive control *Fusarium odoratissimum* InaCC F856, **E.** *Fusarium proliferatum* (InaCC F992), **F.** *Fusarium desaboruense* (InaCC F950), **G.** *Fusarium lumajangense* (InaCC F872^{T}), **H.** *Fusarium longipes* (InaCC F974), **I.** FIESC (Indo161), **J.** *Fusarium lumajangense* (InaCC F993).

Taxonomy

The *Fusarium* species in each complex and novel species identified in this study are described below.

Fusarium lumajangense N. Maryani, M. Sandoval, L. Lombard, Kema & Crous, **sp. nov.**
MycoBank MBXXXXXXX. Fig.6

Etymology: Name refers to Lumajang, the region from where this species was collected in Indonesia.

Sporulation abundant from conidiophores carried on aerial mycelium and from sporodochia. *Conidiophores* on aerial mycelium, septate, branching profusely, irregularly or sympodially or reduced to solitary conidiogenous cells formed laterally on aerial hyphae; *conidiogenous cells* mono- or polyphialidic, acute, subulate or subcylindrical, smooth- and thin-walled (6–)10–22.5(–31.5) × 2–3(–4) µm, formed terminally and singly on conidiophores or intercalary, often proliferating percurrently; periclinal thickening inconspicuous or absent; *conidia* of two types: a) (microconidia) ovoid to ellipsoid, smooth- and thin-walled, (6–)9–18(–23) × (2–)3(–5) µm (av. 13 × 4 µm), 0–1-septate, arranged in false heads on monophialides; and b) (macroconidia) falcate and multiseptate, apical cells papillate, basal cells indistinct or foot-shaped, (1–2–)3-septate, formed on polyphialides; 1-septate conidia 18.5 × 3.5 µm; 2-septate conidia 40 × 4 µm; 3-septate conidia (26–)29–39.5(–44.5) × (3–)3.5–4.5(–5.5) µm; av. (18.5–)28–39.5(–44.5) × (3–)3.5–4.5(–5.5) µm. *Sporodochia* formed abundantly on surface of carnation leaves after 7 d, pale orange to orange. *Conidiophores* on sporodochia, septate, mostly unbranched or rarely sparsely and irregularly branched, bearing terminal monophialides, carried singly or grouped in verticillately branched; *conidiogenous cells* monophialidic, ampulliform, doliiform to subcylindrical, smooth- and thin-walled, (11.5–)12.5–18.5(–23.5) × (2–)3–4(–4.5) µm, proliferating percurrently several times, with short collarets and inconspicuous periclinal thickening; *sporodochial conidia* falcate, apical cells gently curved, papillate, basal cells slightly curved, foot-shaped, 3–5 septate: 3-septate conidia, (30–)34.5–46.5(–54) × 3.5–4.5 µm; 4-septate conidia, 41–48(–52.5) × (3–)3.5–4.5 µm; 5-septate conidia, (42.5–)45–53(–56) × 3.5–4.5 µm; av. (30–)40–50.5(–56) × (3–)3.5–4(–4.5) µm. *Chlamydospores* not observed.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 3.5–4.6 mm/d. Colony reverse, liliac to violet becoming white towards the margin, later becoming dark purple with time. Colony surface dry, white becoming livid purple towards the margin, turning completely purple with age. Aerial mycelium abundant, cottony, with moderate sporulation and lacking exudates.

Geography and host: Lumajang, East Java, *Musa acuminata.* var. Pisang Mas Kirana (AA).

Pathogenicity: Non-pathogenic on Cavendish (AAA).

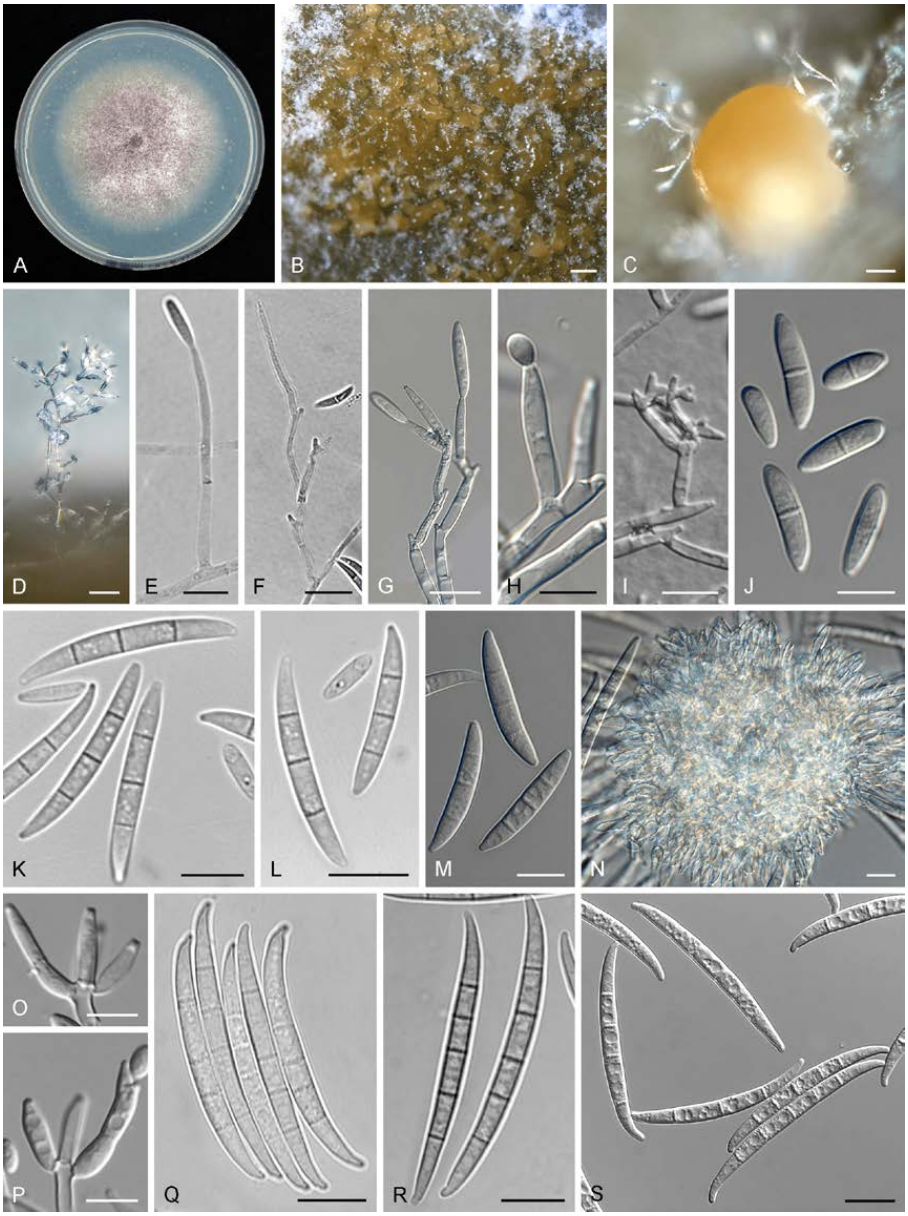

Fig. 6. *Fusarium lumajangense* (ex-type InaCC F993). **A.** Culture grown on PDA. **B–C.** Sporodochia on carnation leaves. **D–I.** Aerial conidiophores and phialides. **J–M.** Aerial conidia. **N–P.** Sporodochial conidiophores and phialides. **Q–S.** Sporodochial conidia. Scale bars: **B–D=** 50 μm, E= 5 μm, F–S= 10 μm.

Material examined: **Indonesia**, Desa Kandang Kepus, Kecamatan Senduro, Lumajang, East Java (113°4'157"E, 8°4'46"S), in infected pseudostem of *Musa acuminata* var. Pisang Mas Kirana (AA), 17 Jul. 2014, N. Maryani (**holotype** InaCC F872, ex-type culture InaCC F993; Desa Kandang Kepus, Kecamatan Senduro, Lumajang, East Java (113°4'157"E, 8°4'46"S), in infected pseudostem of *Musa acuminata* var. Pisang Mas Kirana (AA), 17 Jul. 2014, N. Maryani (InaCC F993).

Notes: *Fusarium lumajangense* exhibits similar morphological features to *F. mangiferae* (Britz *et al*. 2002), also clustering in a sister relationship with the latter species. However, besides its clear phylogenetic delimitation, the polyphialides found in *F. lumajangense* commonly present two conidiogenous loci. Similar to *F. subglutinans* described by Nelson *et al.* (1983), except that the microconidia in *F. lumajangense* were formed both on mono- and polyphialides.

Fusarium desaboruense N. Maryani, M. Sandoval, L. Lombard, Kema & Crous, **sp. nov.** MycoBank MBXXXXXXX. Fig. 7

Etymology: Name refers to Desa Boru, the village from where this species was collected in Indonesia.

Sporulation abundant from conidiophores carried on aerial mycelium and from sporodochia. *Conidiophores* on aerial mycelium abundant on PDA and SNA, less frequent on CLA, septate, sparingly or profusely branching irregularly or sympodially, rarely reduced to solitary conidiogenous cells, formed laterally on aerial hyphae; *conidiogenous cells* mono- or polyphialidic, acute, subulate or subcylindrical, smooth- and thin-walled (6–)15–33(–44) × (2–)2.5–4(–7) µm (av. 21.5 × 3 µm), formed terminally, singly or in whorls on conidiophores or intercalary, proliferating percurrently, periclinal thickening inconspicuous or absent; *conidia* of two types: a) (microconidia) ovoid to ellipsoid, smooth- and thin-walled, (10–)11–16(–18) × (4–)6(–7) µm (av. 13 × 5 µm), 0–1-septate, arranged in false heads on monophialides; and b) (macroconidia) falcate and multiseptate, apical cells papillate, basal cells indistinct or foot-shaped, 1–3-septate, formed on polyphialides: 1-septate conidia 22.5–26(–27) × 3.4–4 µm; 2-septate conidia (21.5–)22–26 × 3–4.5 µm; 3-septate conidia (23–)24.5–34(–37) × 3–4.5 µm; av. (21.5–)22–30.5(–37) × 3–4.5 µm. *Sporodochia* formed abundantly on CLA after 7 d, pale orange to orange. *Conidiophores* in sporodochia unbranched, rarely laterally branched up to two times; *conidiogenous cells* monophialidic, smooth- and thin-walled (15.5–)16.5–24(–29) × (2.5–)3–4 µm (av. 20 × 3.5 µm), solitary, terminal or lateral, or in terminal groups of up to three conidiogenous cells, with minute collarettes and periclinal thickening; *sporodochial conidia* falcate, apical cells gently curved, papillate, basal cells gently curved, foot-shaped, 1–3(–4)-septate. 1-septate conidia (14.5–)15–20.5(–22) × 3.5–4.5 µm; 2-septate conidia (20.5–)21.5–24 × 3.5–4.5(–5) µm; 3-septate conidia (21–)24–29(–31.5) × (3.5–)4–5(–5.5) µm; 4-

septate conidia 34 × 5.5 µm; av. (14.5–)20–28(–34.5)×(3.5–)4–5(–5.5) µm. *Chlamydospores* not observed.

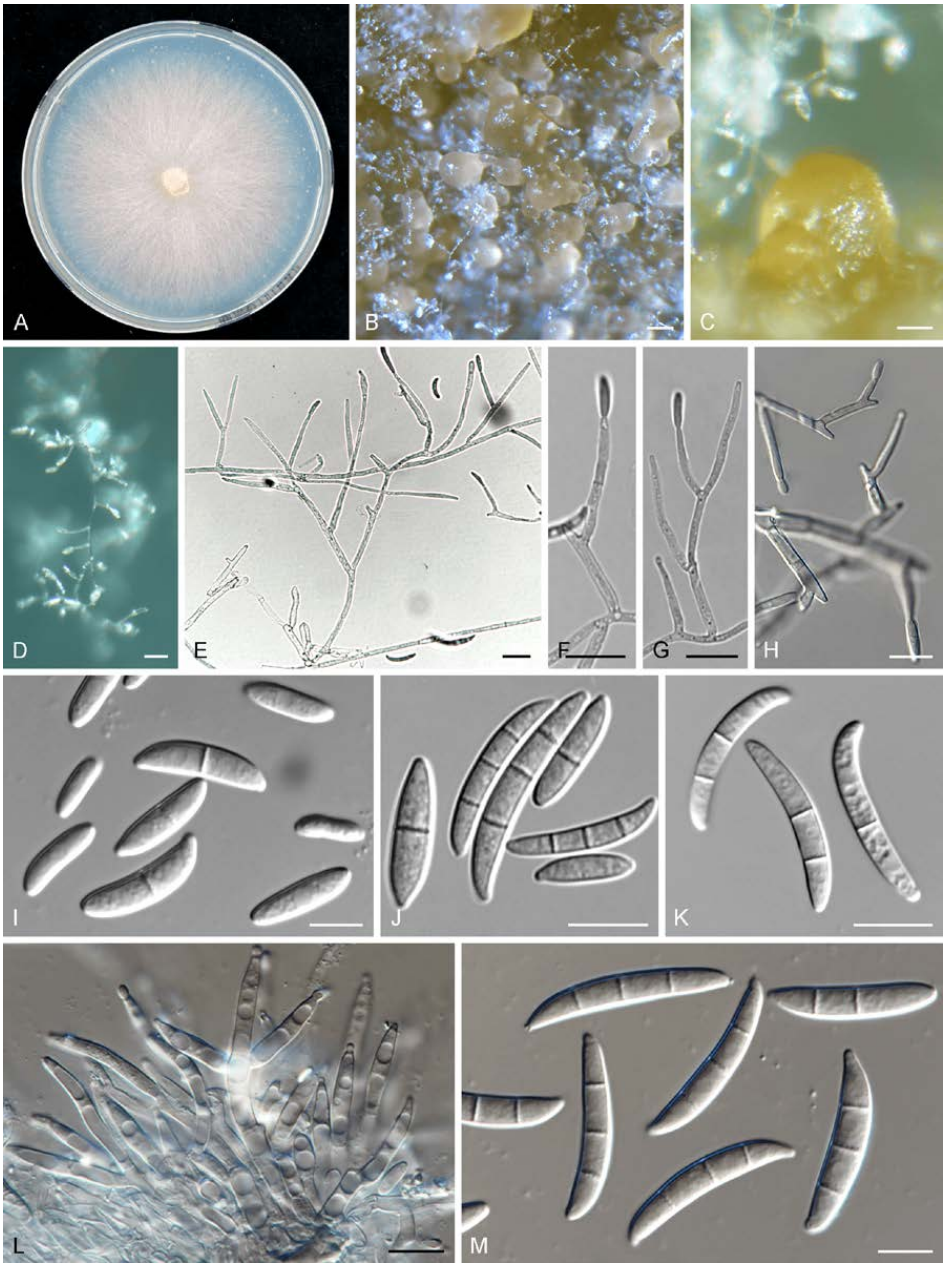

Fig. 7. *Fusarium desaboruense* (ex-type InaCC F950). **A.** Culture grown on PDA. **B–C.** Sporodochia on carnation leaves. **D–H.** Aerial conidiophores and conidiogenous cells. **I–K.** Aerial conidia. **L.** Sporodochial conidiophores and phialides. **M**. Sporodochial conidia. Scale bars: **B–E**= 20 µm, D**–M**= 10 µm.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 4.9–5.2 mm/d. Colony reverse, pale violet becoming white towards the margins, turning violet with age and pigmented. Colony surface cottony, pale violet, becoming white with age, immersed mycelium becoming purple and lacking exudates. Aerial mycelium abundant, cottony, with abundant sporulation.

Geography and host: Sikka Flores, East Nusa Tenggara, *Musa* sp. var. Pisang Kepok (ABB).

Pathogenicity: Not pathogenic on Cavendish (AAA).

Material examined: **Indonesia**, Desa Boru, Kecamatan Waigate, Sikka Flores, East Nusa Tenggara (122°22'7"E and 8°36'49"S), on infected pseudostem of *Musa* sp. var. Pisang Kepok (ABB), 17 Aug. 2015, N. Maryani (**holotype** InaCC F950, ex-type culture InaCC F950); Desa Boru, Kecamatan Waigate, Sikka Flores, East Nusa Tenggara (122°22'7"E and 8°36'49"S), on infected pseudostem of *Musa* sp. var. Pisang Kepok (ABB), 17 Aug. 2015, N. Maryani (InaCC F 951, InaCC F 952).

Notes: Morphologically very similar to *F. sacchari* ((J. Gams) Leslie & Summerell (2006)) and *F. subglutinans* (Nelson *et al.* 1983), except that this species produces sporodochia abundantly under regular culturing conditions. *F. desaboruense* can be distinguished by the septation of its macroconidia (1–4-septate) and microconidia (1–3-septate), not observed in *F. saccari* (Leslie & Summerell 2006). Phylogenetic analyses of partial *rpb2* gene sequences recognized this species as distinct from *F. sacchari* with strong support of BP 99 %.

***Fusarium tanahbumbuense* (FIESC-34)** N. Maryani, M. Sandoval, L. Lombard, Kema & Crous, **sp. nov.** MycoBank MBXXXXXXX. Fig.8

Etymology: Name refers to Tanah Bumbu, the region from where this species was collected in Indonesia.

Sporulation abundant from conidiophores borne on aerial mycelium and from sporodochia. *Conidiophores* on aerial mycelium abundant on PDA, SNA, and CLA, septate, irregularly of verticillately branched; conidiogenous cells monophialidic or polyphialidic, subulate or subcylindrical, smooth- and thin-walled, (11–)13–24(–38) × (4–)5–6(–7) µm (av. 19 × 6 µm), formed terminally, singly or in groups of up to three cells on a stipe, or carried singly and laterally on aerial mycelium, collarettes and periclinal thickening inconspicuous or absent; *conidia* of one type (macroconidia) falcate and multiseptate, apical cells conical to papillate, basal cells indistinct or foot-shaped, 3–5-septate, formed on both mono- and polyphialides, 3-septate conidia, 31–36(–38.5) × 3.5–5(–5.5) µm; 4-septate conidia, (31–)33.5–43.5(–48) × 3.5–5(–5.5) µm; 5-septate conidia, (30–)37–45(–47) × 4–5.5(–6) µm; av. (30–)34.5–44(–48) × (3.5–

)4–5.5(–6) µm. *Sporodochia* formed abundantly on CLA after 7 d, pale orange; *conidiophores* in sporodochia irregularly and laterally branched; *conidiogenous cells* monophialidic, doliiform to ampulliform, smooth- and thin-walled, (9.5–)10–13(–15) × (2.5–)3–4 µm (av. 11.5 × 3.5 µm), collarettes or periclinal thickening inconspicuous or absent; *sporodochial conidia* falcate, apical cells gently curved, papillate; basal cells slightly curved, foot-shaped; (2–)3–5-septate; 2-septate conidia, 40.5 × 4.5 µm; 3-septate conidia, (25.5–)29–36.5(–41) × 3.5–4.5 µm; 4-septate conidia, (32.5–)34–40(–46) × 3.5–4.5(–5) µm; 5-septate conidia, (36–)37–43.5(–49) × 3.5–4.5(–5) µm; av. (25.5–)32–41.5(49) × 3.5–5 µm. *Chlamydospores* not observed.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 1.3–2.2 mm/d. Colony reverse, rosy buff becoming white towards the margins, turning cinnamon to fawn with age and pigmented. Colony surface cottony, rosy buff becoming white towards the margin, turning hazel with age. Aerial mycelium abundant, cottony, with high sporulation and lacking exudates.

Geography and host: Tanah Bumbu, South Kalimantan, *Musa* sp. var. Pisang Hawa (ABB).

Pathogenicity: NA.

Material examined: **Indonesia**, Desa Betung, Kecamatan Kusan Hilir, Tanah Bumbu, Kalimantan Selatan (115°37’477”E, 3°50’77”S), on infected pseudostem of *Musa* sp. var. Pisang Hawa (ABB), 20 Jun. 2014, N. Maryani (**holotype** InaCC F965).

Notes: Fusarium tanahbumbuense can be distinguished from *F. semitectum* (Leslie & Summerell 2006, Nelson *et al.* 1983) by the absence of microconidia and chlamydospores. The polyphialides observed for this species also greatly differed from those that have been observed for *F. semitectum* which have 3–5 openings (Nelson *et al.* 1983).

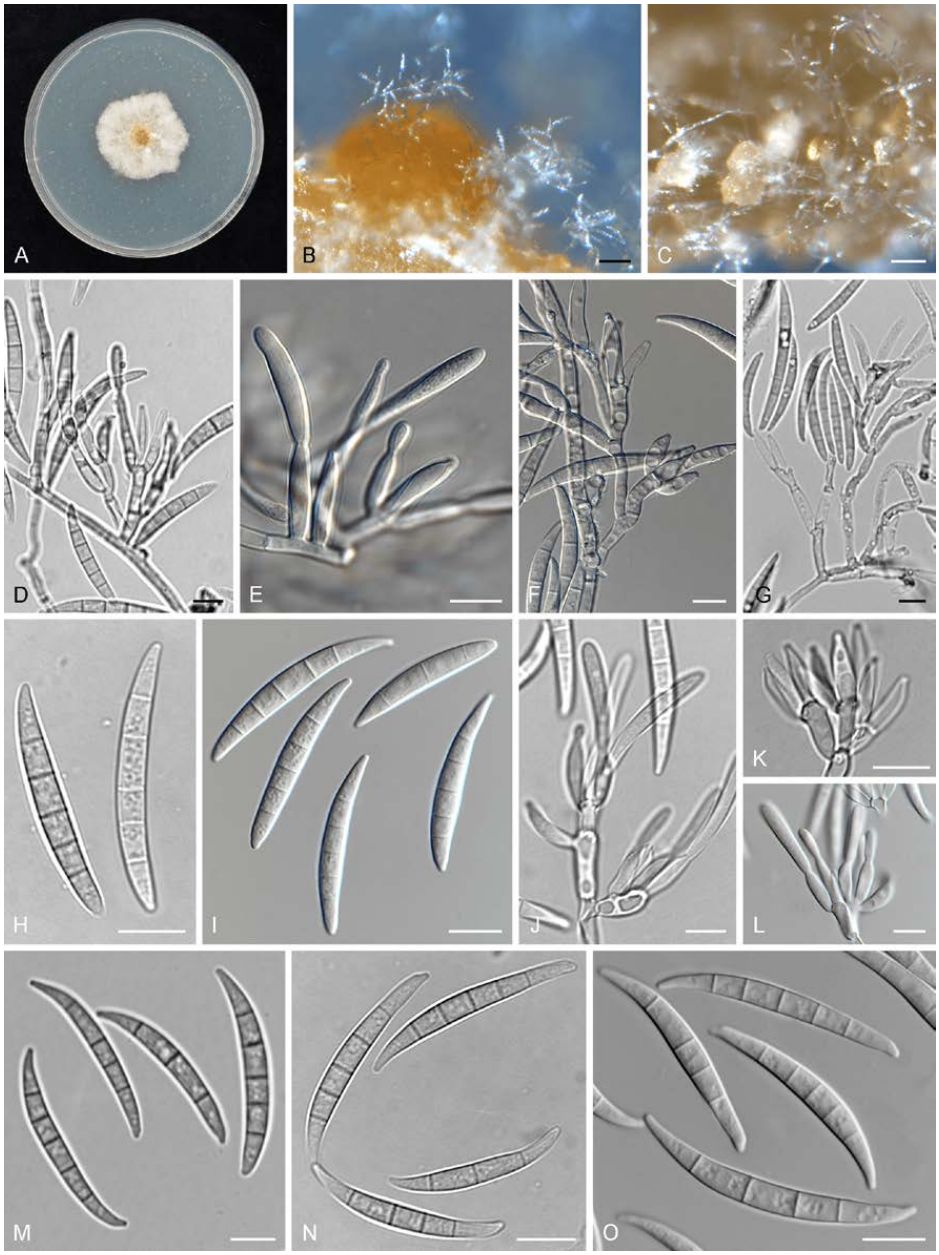

Fig. 8. *Fusarium tanahbumbuense*, FIESC-34 (ex-type InaCC F965). **A.** Culture grown on PDA. **B–C.** Sporodochia on carnation leaves. **D–G.** Aerial conidiophores and conidiogenous cells. **H–I.** Aerial conidia. **J–L.** Sporodochial conidiophores and conidiogenous cells. **M–O.** Sporodochial conidia. Scale bars: B–C= 50 μm, D–O= 10 μm.

Fusarium sulawense (FIESC-32) N. Maryani, M. Sandoval, L. Lombard, Kema & Crous, **sp. nov.** MycoBank MBXXXXXXX. Fig.9

Etymology: Name refers to Sulawesi, the island from where this species was collected in Indonesia.

Sporulation abundant from conidiophores carried on aerial mycelium and from sporodochia. *Conidiophores* on aerial mycelium abundant on PDA and SNA, less frequent on CLA, septate, irregularly or verticillately branched; *conidiogenous cells* mono- or polyphialidic, subulate to subcylindrical, smooth- and thin-walled, (8.5–)14–22.5(–27) × (2–)2.5–4(–4.5) µm (av. 18 × 3 µm), formed singly, laterally or terminally, or more often in groups of 2–3 cells, sometimes proliferating percurrently, collarettes and periclinal thickening inconspicuous or absent; *conidia* of one type (macroconidia), falcate and multiseptate, apical cells papillate, basal cells indistinct or foot-shaped, 3–5(–9)-septate, formed on both mono- and polyphialides, 3-septate conidia, 20.5–47.5(–55) × 3.5–5 µm; 5-septate conidia, (33.5–)39.5–48(–50.5) × (4–)4.5–5.5 µm; 6-septate conidia 51.5 × 6 µm; 9-septate conidia, 67 × 5.5 µm; av. (20.5–)36–51(–67.5) × (3.5–)4–5.5(–6) µm.

Sporodochia formed rarely on CLA after 7 d, pale orange; *conidiophores* in sporodochia unbranched or irregularly branched, densely packed, bearing terminal clusters of 2–5 conidiogenous cells; *conidiogenous cells* monophialidic, short ampulliform, smooth- and thin-walled, (8.5–)9–11.5(–13) × (3–)3.5–5(–5.5) µm (av. 10.5 × 4.5 µm) with a minute collarette and inconspicuous periclinal thickening; *sporodochial conidia* falcate, apical cells gently curved, papillate; basal cells slightly curved, foot-shaped, (3–)5(–6)-septate; 3-septate conidia, (29.5–)30–44 × 4–4.5 µm; 4-septate conidia, 30 × 5.5 µm; 5-septate conidia, (30–)36–41.5(–43.5) × (3.5–)4–5(–5.5) µm; 6-septate conidia 43.5 × 5 µm; av. (30–)36–41.5(–44) × (3.5–)4–5(–5.5) µm. *Chlamydospores* not observed.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 5.2–6.0 mm/d. Colony reverse rosy buff becoming white towards the margins. Colony surface dry, cottony, saffron. Aerial mycelium abundant, cottony, with high sporulation and lacking exudates.

Geography and host: Bone, South Sulawesi, *Musa acuminata* var. Pisang Cere (AAA).

Pathogenicity: Non-pathogenic on Cavendish (AAA).

Material examined: **Indonesia**, Desa Seli, Kecamatan Bengo, Bone, Sulawesi Selatan (120°1'12.8"E, 4°37'26"S), on infected pseudostem of *Musa acuminata* var. Pisang Cere (AAA), 12 Aug. 2015, N. Maryani (**holotype** InaCC F940), ex-type culture InaCC F940); Desa Sungai Birah, kecamatan Pamukan Barat, Kota Baru, Kalimantan Selatan (115°59'982"E, 2°22'883"S), on infected pseudostem of *Musa* sp. var. Pisang Hawa (ABB), 19 Jun. 2014, N. Maryani (InaCC F964).

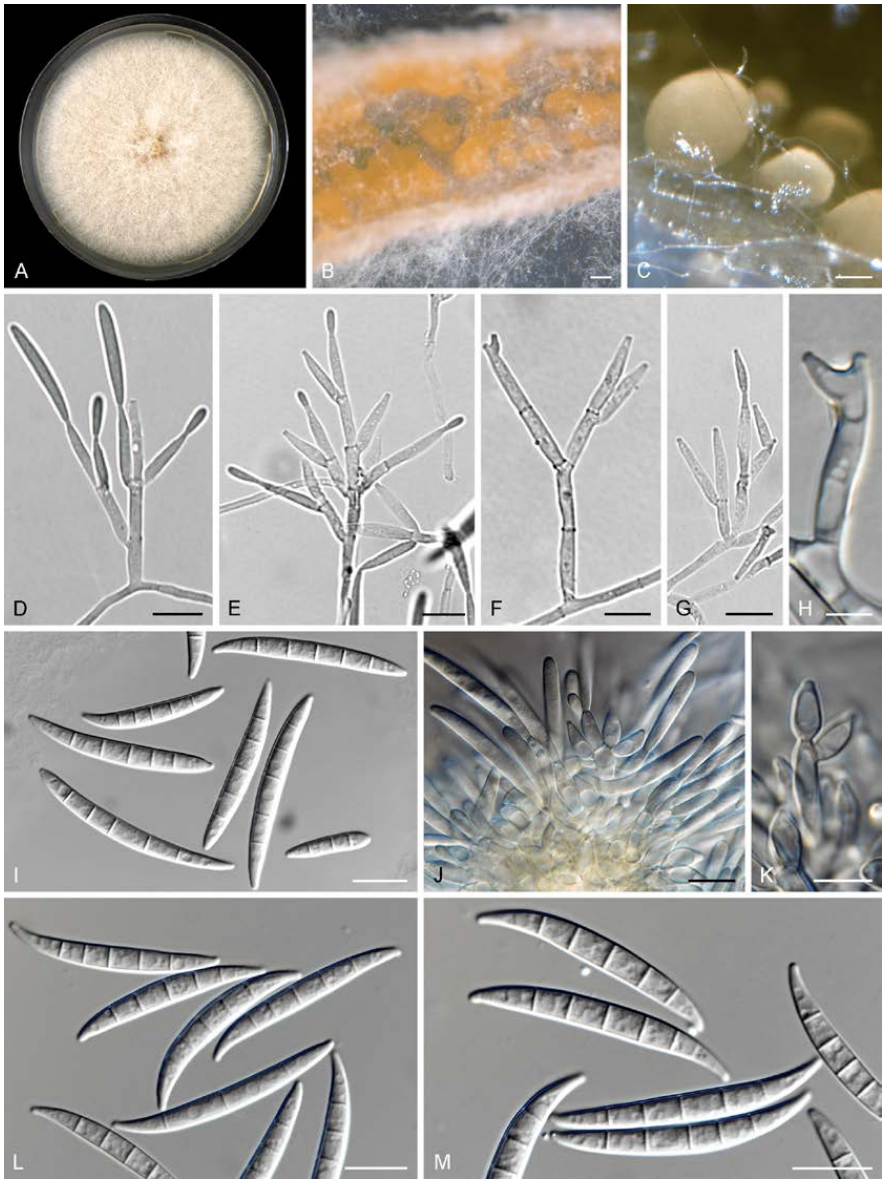

Fig. 9. *Fusarium sulawense* FIESC-32 (ex-type InaCC F964). **A.** Culture grown on PDA. **B–C.** Sporodochia on carnation leaves. **D–H.** Aerial conidiophores and conidiogenous cells. **I.** Aerial conidia. **J–K.** Sporodochial conidiophores and conidiogenous cells. **L–M.** Sporodochial conidia. Scale bars: B–C= 50 μm, H= 5 μm, D–G, I–M= 10 μm.

Notes: *Fusarium sulawense* is relatively fast growing (av. 5.2–6.0 mm/d) compared to its sister species in the Incarnatum clade *F.* FIESC-34 (av. 1.3–2.2 mm/d). Members of this species were recovered from different banana varieties in the Kalimantan and Sulawesi islands of Indonesia.

Fusarium kotabaruense (FIESC-31) N. Maryani, M. Sandoval, L. Lombard, Kema & Crous, **sp. nov.** MycoBank MBXXXXXXX. Fig.10

Etymology: Name refers to Kota Baru one of the nine regencies in the Indonesian province of South Kalimantan.

Sporulation abundant from conidiophores carried on aerial mycelium. *Conidiophores* on aerial mycelium abundant on PDA and SNA, less frequent on CLA, septate, irregularly branching; *conidiogenous cells* mono- or polyphialidic, subulate to subcylindrical, smooth- and thin-walled, (15–)19–33(–40) × 4–7 µm (av. 26 × 5 µm), forming terminally, singly or in verticillately branched conidiophores, less commonly laterally or intercalary, proliferating percurrently, periclinal thickening inconspicuous or absent; falcate and multiseptate, apical cells papillate, basal cells indistinct or foot-shaped, (2–)3–5(–7)-septate, formed on both mono- and polyphialides, 2-septate conidia, (21–)21.5–25(–26) × 5–6 µm; 3-septate conidia, (24.5–)28–35(–36.5) × 5.5–6.5(–7) µm; 4-septate conidia, (32–)34–39.5(–41.5) × 5.5–6.5(–7) µm; 5-septate conidia, (34.5–)36–42.5(–45) × (5–)5.5–6.5(–7.5) µm; 6-septate conidia, 39–40.5 × 5.5–7 µm; 7-septate conidia, (38.5–)39.5–44(–45) × 6–7 µm; av. (21–)31.5–41.5(–45) × (5–)5.5–6.5(–7.5) µm. Sporodochia and chlamydospores not observed.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 5.0–6.85 mm/d. Colony reverse rosy buff. Colony surface cottony rosy buff. Aerial mycelium abundant, cottony, with high sporulation and lacking exudates.

Geography and host: Kota Baru, South Kalimantan, *Musa* sp. var. Pisang Hawa (ABB).

Pathogenicity: Non-pathogenic on Cavendish (AAA).

Material examined: **Indonesia**, Desa Sungai Birah, Kecamatan Pamukan Barat, Kota Baru, Kalimantan Selatan (115°59’982”E, 2°22’883”S), on infected pseudostem of *Musa* sp. var. Pisang Hawa (ABB), 19 Jun. 2014, N. Maryani (**holotype** InaCC F963).

Notes: *Fusarium kotabaruense* represents a species in the Equiseti clade of the FIESC and relatively fast growing (5.0–6.85 mm/d). Most distinguishing characteristic of this species is

the absence of sporodochia on CLA culture. However, aerial conidiophores are abundant with conidia produced with high variability on its septation, (0–)3–5(–7)-septate.

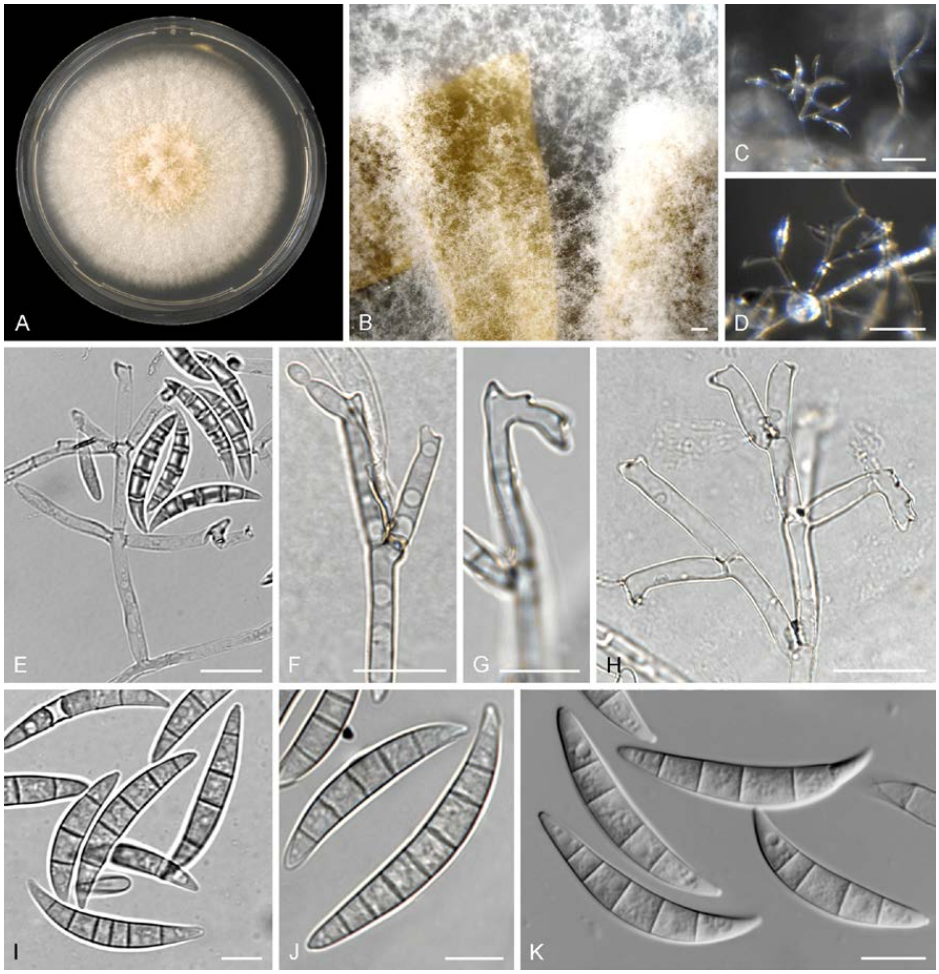

Fig. 10. *Fusarium kotabaruense*, FIESC-31 (ex-type InaCC F963). **A.** Culture grown on PDA. **B.** Mycelium on carnation leaves. **C–H.** Conidiophores and conidiogenous cells. **I–K.** Conidia. Scale bars: B= 200 µm**,** C–D= 50 µm, G= 5 µm, E–F, H–K= 10 µm.

Fusarium longipes InaCC F974 Fig.11

Sporulation abundant from conidiophores carried on aerial mycelium and from sporodochia. *Conidiophores* on aerial mycelium abundant on PDA and SNA, rare on CLA, septate, branching irregularly, mostly reduced to solitary conidiogenous cells formed singly and laterally on aerial hyphae; *conidiogenous cells* monophialidic, doliiform to ampulliform, smooth- and thin-walled, (7–)10–13(–15) × 3–4(–5) µm (av. 12 × 6 µm), formed laterally on aerial hyphae or clustering terminally on conidiophores, with a minute collarette; *conidia* (microconidia)

obovoid to ellipsoid, rough- and thin-walled, (7–)10–19(–23) × (3–)4(–5) µm (av. 15 × 4 µm), 0–2-septate, arranged in false heads on monophialides. *Sporodochia* formed abundantly on CLA after 7 d, bright orange, later turning red to purple; *conidiophores* in sporodochia highly irregularly or verticillately branched, sympodially to solitary conidiogenous cells; *conidiogenous cells* monophialidic, doliiform, ampulliform to subcylindrical, 7–11(–14) × (2–)2.5–3.5(–4) µm (av. 9.5 × 3 µm), with inconspicuous collarets; *sporodochial conidia* falcate, apical cell strongly curved, tapering and whip-like with rounded apex, basal cell foot-shaped and elongated, (3–)4–5-septate: 3-septate conidia, 28.5 × 3.5 µm; 4-septate conidia, (37)–38–43 (–43.5) × 4.5–5.5 µm; 5-septate conidia, (37–)42–49.5(–53.5) × (3.5–)4.5–5(–6) µm; av. (28.5–)40.5–49.5(–53.5) × (3–)4–5(–6) µm. *Chlamydospores* ellipsoid, sub-globose to globose, formed intercalary or terminal, single or in pairs, or in clumps, (7–)10–13(–15) × (7–)9–13(–14) µm (av. 12 × 11 µm), brown, rough-walled.

Culture characteristics: Colony on PDA showing optimal growth at 25 °C with an average growth rate of 4.2–4.9 mm/d. Colony reverse livid red becoming white towards the margin, becoming completely livid red to bay with age. Colony surface cottony greyish rose becoming vinaceous with age and white toward the margins. Aerial mycelium abundant, cottony, with high sporulation and lacking exudates. Sporodochia formed abundantly on CLA after 7 d, pale orange to orange.

Geography and host: Katingan, Central Kalimantan, *Musa* sp. var. Pisang Awak (ABB).

Pathogenicity: Non-pathogenic on Cavendish (AAA).

Material examined: **Indonesia**, Desa Tewang Menyangen, T. Sangalang, Katingan, Central Kalimantan (113°6’552”E, 1°41’83”S), on infected pseudostem of *Musa* sp. var. Pisang Awak (ABB), 23 Jun. 2014, N. Maryani (**holotype** InaCC F974).

Notes: This banana isolate of *F. longipes* displays some unique characteristics which differ slightly from *F. longipes vide* Leslie & Summerell (2008), which include the presence of microconidia and chlamydospores. This species is more similar to *F. equiseti* as described by Nelson *et al.* (1983), except for the length of the long curvature of the macroconidia. Additionally, the chlamydospore formation also differs from the original description of *F. longipes*.

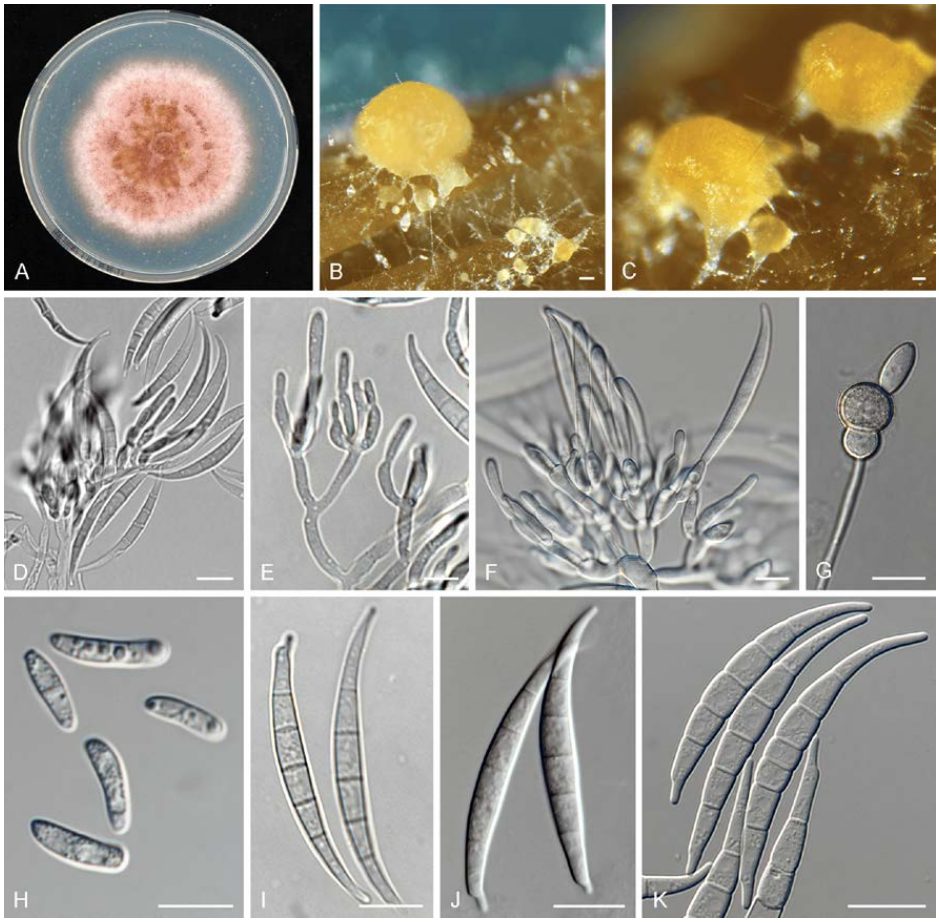

Fig. 11. *Fusarium longipes* (InaCC F974). **A.** Culture grown on PDA. **B–C.** Sporodochia on carnation leaves. **D.** Sporodochial conidiophores. **E–F.** Branched conidiophores. **G.** Falcate-shaped macroconidia. **H.** Microconidia **I.** Chlamydospores. Scale bars B–K= 10 μm.

DISCUSSION

This study further expands our knowledge on the diversity of *Fusarium* species isolated from banana plants displaying symptoms of Fusarium wilt in Indonesia, the centre of origin for this economically important crop. It is not surprising that 90 % of the isolates recovered from the samples were members of FOSC, as the diseased pseudostem of banana served as source of isolation (Maryani *et al*. 2019). However, the remaining isolates were tentatively identified as members of other *Fusarium* species complexes, which included the FIESC, FSSC and FFSC. Remarkably, only *Fusarium* species were isolated, while no other fungal genera could be recovered from the banana samples. This indicates a marked dominance of *Fusarium* in diseased banana plants. It is well known that *Fusarium* is commonly associated with higher plants, being ubiquitous in terrestrial ecosystems, especially in the tropics, where most diseases on perennial crops are induced by this genus (Ploetz 2006). It has also been suggested

that for any *Fusarium* associated disease found in plants, many other *Fusarium* species also reside in the same host as endophytes (Leslie & Summerell 2006). Moreover, the samples were collected from locations in Indonesia where bananas are grown in mixed backyard ecosystems with other tropical crops (Maryani *et al*. 2019). This ecological niche enhanced the chance that a much higher diversity of *Fusarium* species would be discovered than expected.

We were able to identify a total 20 isolates collected from pseudostems of banana plants displaying symptoms of Fusarium wilt that did not belong to FOSC. These isolates were found to belong to three different *Fusarium* species complexes of which eight represented novel phylogenetic species in the FFSC and FIESC. Information regarding *Fusarium* spp. other than *F. oxysporum* in banana is scarce, since the majority of studies point to the specific detection and control of pathogenic strain of *F. oxysporum* (Ploetz *et al.* 2015, O'Donnell *et al.* 1998, Ordonez *et al*. 2015, Maryani *et al.* 2019). However, some studies have reported an abundance of *Fusarium* species in asymptomatic banana plant organs. Zakaria *et al.* (2011) identified *Fusarium oxysporum*, *F. semitectum* and *F. solani* (current name *Neocosmospora solani*) in healthy roots of wild banana plants (*Musa acuminata*) in Malaysia and *Fusarium concentricum* was reported in *Musa sapientum* from Costa Rica (Nirenberg & O'Donnell 1998). Moreover, a higher diversity of *Fusarium* species have been reported from banana fruits, which included *F. chlamydosporum, F. equiseti, F. proliferatum, F. sacchari, F. subglutinans* and *F. verticilloides* (Zheng *et al.* 2014, Moretti *et al.* 2004, Jimenez *et al.* 1996). Two of these species, *F. proliferatum* and *F. verticilloides,* were also found in this study.

Pathogenicity tests demonstrated that the Indonesian isolates were not pathogenic on the Cavendish banana variety Grand Naine. Moreover, our results indicate that these species more likely play an endophytic role, which is consistent with previous knowledge on asymptomatic/ healthy banana plants (Zakaria & Rahman 2011). A similar case has been reported on vanilla stem rot disease in Indonesia. Pinaria *et al.* (2010) isolated 12 *Fusarium* species from symptomatic vanilla stems. Pathogenicity tests indicated that none of these caused any disease on vanilla plants, with the exception of *Fusarium oxysporum* f. sp. *vanillae*. In another study, *Fusarium oxysporum* f. sp. *vasinfectum* was found to be the only species that caused Fusarium wilt of cotton amongst 20 *Fusarium* species isolated from wild *Gossypium* in Australia (Wang *et al.* 2004).

The highest diversity of isolates obtained in this study belonged to the FIESC. This species complex displays a remarkable abundance of phylogenetic species diversity which include both animal and plant associated pathogens, plant endophytes and soil inhabitants (Leslie & Summerell 2006, O'Donnell *et al.* 2009, Villani *et al.* 2016). Many of the FIESC have been isolated from various plants displaying disease symptoms, but their pathogenicity was never established (Leslie & Summerell 2006). Previous studies have reported the presence of FIESC in banana fruits and roots, as well as causing storage rot of bananas (Leslie & Summerell 2006, Zakaria *et al.* 2011, Zheng *et al.* 2012). However, this study represents the first report of

FIESC from the pseudostem of bananas, indicating that members of this species complex have been isolated from every part of the banana plant. Thus far, species of the FIESC have been found to be more abundant in banana fruit, indicating a hemibiotrophic fungal lifestyle in plants (Bacon & Yates 2006), and therefore these are often found in stored banana fruits, which are a very suitable environment for toxin producing fungal species like most FIESC members (Desjardins 2006).

The second most diverse *Fusarium* species complex found in this study was the FFSC. Five species where identified from banana, including the common plant pathogenic species *F. proliferatum* and *F. verticilloides*. Additionally, two novel species, *F. lumajangense* and *F. desaboruense*, were also identified in this study. The FFSC is known to include species able to cause disease in a variety of important agronomic crops, especially in the tropics (O'Donnell *et al.* 1998b). Each of the novel species identified in this complex were closely related to recognized plant pathogens: *F. lumajangense* is phylogenetically and morphologically closely related to *F. mangiferae*, a species causing mango-malformation on mango (*Mangifera indica*), and *F. desaboruense* is closely related to *F. sacchari*, the causal agent of "pokkah boeng" disease on sugar cane (Handojo *et al.* 1989, Britz et al. 2000). The plant pathogenic species *F. proliferatum*, a well-known pathogen on maize, sorgum, mango and asparagus, and *F. verticilloides*, a pathogen on maize (Handojo *et al.* 1989, Britz *et al*. 2002, Ploetz 2006b) and notorious producer of fumonisins (Desjardin 2006), were isolated at low frequency. Interestingly, all the hosts mentioned above are present in Indonesia as important cultivated crops. Moreover, Indonesian bananas are mainly produced in small scale household plantations and co-cultivated with other crops such as rice, maize, sugarcane, and other perennial tropical crops (Maryani *et al.* 2019). This complex agroecosystem from which our banana samples were obtained might explain the presence of FFSC species in banana plants affected by Fusarium wilt.

Members of the FFSC isolated in this study were not pathogenic to the banana variety Cavendish, which is similar to what has been stated for other crops. *Fusarium fujikuroi, F. sacchari, F. subglutinans* and *F. verticilloides* have been reported from rice affected by "Bakanae" disease, although, only *F. fujikuroi*, is known to cause the disease (Zainudin *et al*. 2008, Amatulli *et al*. 2010). A similar set of species in FFSC was also found in sugarcane, maize, and vanilla (Ploetz 2006b, Pinaria *et al.* 2010), thus suggesting that members of FFSC are associated with these crops without inducing disease. Moreover, their presence suggests an endophytic life style, causing no harm to the host plants or perhaps acting as secondary invaders or saprobes as the isolates were obtained from diseased plants. However, banana plants might serve as an intermediate host, as suggested by Handojo *et al.* (1989) for "Pokkah boeng" disease on sugar cane.

A single isolate was found to belong to the FSSC, identified as *F. longipes* based on phylogenetic inference, a species abundant in tropical areas as a soil inhabitant or as a

saprophyte (Blackhouse & Burgess 1995, Onyike & Nelson 1993). However, to our knowledge, our finding is the first report of this species from banana since the report of Reinking & Wollenweber (1927). They described *F. longipes* from mature living leaves of *Musa sapientum* in Honduras. Here, however, this species was cultured from the diseased pseudostem of banana variety Pisang Awak (ABB) on Kalimantan. This species appears to be commonly recovered from both healthy and diseased plants, suggesting that *F. longipes* could be endophytic in banana. This hypothesis was also further supported by the pathogenicity test conducted in this study. *Fusarium longipes* is known to be isolated more frequent during a higher rainfall period and under high temperatures (Burgess *et al.* 1988, Backhouse & Burgess 1995). This is consistent with our findings where *F. longipes* was recovered from banana plants growing at a relatively high temperature (35 °C) and humidity (62 %). With morphological distinctions from the previous description of *F. longipes*, InaCC F974 found in this study might represent a novel species. More isolates and additional gene regions are needed to capture the possible diversity in morphology and phylogenetic relationships.

Our current study highlights the diversity of *Fusarium* species in banana plants exhibiting Fusarium wilt. While only *Fusarium* spp. in the FOSC has been shown to be a true pathogen (Stover 1962, Maryani *et al.* 2019), the role of the remaining species in banana plants requires further investigation. Whether these *Fusarium* species are true endophytes of the various varieties of banana sampled in this study, possible saprophytes or secondary pathogens should still be determined experimentally. Isolation from asymptomatic plants of similar banana varieties would provide possible evidence of an endophytic lifestyle of the*Fusarium* species reported here. Moreover, the pathogenicity of each species on their respective host varieties needs to be tested in the future. Such studies would also reveal whether banana plants serve as intermediate hosts for a particular *Fusarium* species. Lastly, there is no doubt that tropical areas including Indonesia should receive more attention when studying *Fusarium* biodiversity.

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Chapter 6

General discussion

Agriculture has shaped the course of human history and civilization. In many cases, plant diseases and abiotic threats such as drought caused huge politic and economic upheavals with significant social impact as they threaten food security, especially when staple crops are affected (Zadoks 1998, Koeppel 2007, Gurr *et al.* 2011). Banana is one of the most consumed fruits and also a staple in many countries and was and is currently threatened by Fusarium wilt of bananas or Panama disease, one of the most destructive plant diseases (Simmonds 1962, Stover 1962, Ploetz 2015a). Controlling this disease that already spread and devastated thousands of hectares of banana plantations in many countries is of great importance. A major constituent of the manifold attempts to manage Fusarium wilt is the generation of resistant germplasm through effective and strategic breeding programs. Therefore, a diversity study of the causal fungi of Fusarium wilt on banana is critical to evaluate the incidence and efficacy of resistance and to develop management strategies.

Indonesia is the main centre origin of banana. It holds the primary diversity of wild and cultivated banana (Simmonds & Shepherd 1955, Nasution 1990, Perrier *et al.* 2011). Generally, centres of host diversity coincide with centres of diversity of pathogens due to co-evolution (Grünwald & Flier 2005, Stukenbrock & McDonald 2008, Thrreau *et al*. 2009, Ali *et al*. 2014). Here, we focus on Fusarium wilt of bananas that is caused by a suite of the soil born fungus, previously collectively named *Fusarium oxysporum* f. sp. *cubense* (Foc). Since its first identification, many studies were conducted on Foc collections that were developed by sampling from – mostly – global monoculture plantations. However, the necessity of high diversity sampling was always pointed out (O'Donnell *et al*. 1998, Groenewald *et al*. 2006, Fourie *et al*. 2009, Mostert *et al*. 2017), but such collections were thus far not available or inaccessible. This thesis describes genetic diversity studies of *Fusarium* spp. associated with Fusarium wilt in native banana germplasm across the Indonesian archipelago, the centre of diversity of the genus *Musa* L. (Simmonds & Shepherd 1955). This is the first and most comprehensive study addressing sympatric speciation of *Fusarium* spp. pathogenic on banana. We provide new insights in the taxonomy and systematics of these fungi (**Chapter 2**), studied its genetic diversity and population structure (**Chapter 3**) and diversity for pathogenicity (**Chapter 4**) and reveal other species hitchhiking with pathogenic *Fusarium* spp. in affected banana plants (**Chapter 5**). Taken together, these results shed new light on the evolutionary history of these pathogens that changes our view on the *Fusarium* spp. – banana pathosystem.

A required change of the taxonomical status of the *Fusarium* spp. associated with Fusarium wilt in banana

The systematics and taxonomy of *Fusarium* species has historically been an area raising substantial controversy (Nelson *et al*. 1994, Aoki *et al*. 2014). *Fusarium* species have been through several revisions, thus *Fusarium* taxonomists were divided into "splitters", "lumpers"

and "moderates" based on their philosophy in determining *Fusarium* species (Gordon *et al.* 1994, Leslie & Summerell 2006). Previous taxonomy studies of this genus were exclusively based on morphology, hence the division within the genus into sections and lower taxa was according to different taxonomic systems (Wollenweber & Reinking 1935, Snyder & Hansen 1941, Booth 1971, Gerlach & Nirenberg 1982, Nelson *et al*. 1983) until the first sequenced-based *Fusarium* phylogeny study was introduced and established the demarcation of this genus (Guadet *et al.* 1989). Since then, a sequenced-based phylogeny was broadly used resulting in precise and species identification in the genus. With the current progress of whole genome sequencing and phylogeny, the genus *Fusarium* is suggested to comprise multiple species complexes (Geiser *et al.* 2013, O'Donnell *et al.* 2013). The *Fusarium oxysporum* species complex (FOSC) is one of the largest groups and accommodates agronomically important plant pathogens, but its subdivisions await formal description. Many of the agriculturally important pathogens in the FOSC are designated by host and cultivar specificity known as *formae speciales* and physiological race, respectively (Snyder & Hansen 1940, Armstrong & Armstrong 1981). However, both refer to the biology or physiology of the pathogen, which facilitates plant pathologists, but these terms have no taxonomical relevance. Combining taxonomical and pathogenicity data would reduce the confusion and facilitate the debate between pathologists and mycologists on which characteristics are necessary for systematic (Shoemaker 1981). O'Donnell *et al.* (1998) used multi-gene based phylogenetics for the FOSC and later showed that the strains causing Panama disease were distributed over two of its four clades, suggesting polyphyletic or multiple evolutionary origins of these strains. With the advent of contemporary genome analyses, three housekeeping genes viz. the translation elongation factor 1-alpha (*tef1*), RNA polymerase II largest subunit (*rpb1*), and RNA polymerase II second largest subunit (*rpb2*) were proposed for phylogenetic studies (O'Donnell *et al.* 2015). By adopting this suggestion and expanding the sample size of FOSC from Indonesia we discovered one additional clade in the FOSC, denoted as clade 5 (**Chapter 2**).

The consensus is that members of FOSC are asexual (Nelson *et al.* 1983, Gordon & Martyn 1997, Leslie & Summerell 2006). However, it is unknown. Many other fungi were considered to be asexual, including *Puccinia striiformis* of wheat (Jin *et al.* 2010), *Septoria passerinii* (Ware *et al.* 2007) and *Aspergillus fumigatus* (O'Gorman *et al.* 2009) until their teleomorph were discovered or generated. Hence, the best statement would be that sexuality in members of the FOSC is unknown. A good hypothesis might be that if sexual exchange is considered, also considering that they carry the mating type alleles *mat1-1* and *mat1-2,* Indonesia is the niche to explore. Another generally accepted opinion was that the morphological structures of FOSC members are the same. Hence, no morphological description of this species complex is available, despite numerous *formae speciales* and races that have been discovered. To my knowledge Nelson *et al.* (1983) were the last to provide a

formal description of *Fusarium oxysporum* species. Combining Genealogical Concordance Phylogenetic Species Recognition (GCPSR), morphology and pathogenicity properties, we discovered nine phylogenetic species within Foc. Names and formal descriptions of these *Fusarium* spp., causing Fusarium wilt in bananas, were assigned (**Chapter 2**) and we conclude that the known *Fusarium oxysporum* species with pathogenicity to banana – thus previously named Foc – comprise several species (a complex) in the FOSC. It contains a unique new species *F. odoratissimum* that includes all Tropical Race 4 (TR4) strains that are highly pathogenic on Cavendish banana (**Chapter 4**). In contrast, several species - also indigenous to Indonesia - contain Race 1 strains that are highly pathogenic on Gros Michel banana (**Chapters 2 & 4**). Hence, our study contributes to the taxonomy and systematic placement of Fusarium wilt pathogens of banana across the FOSC, where four out of the five recognized clades (O'Donnell *et al*. 1998, O'Donnell *et al*. 2004, **Chapter 2**) include *Fusarium* spp. associated with this important banana disease.

I do realize that taxonomy is always in flux, particularly when our knowledge increases and influences our understanding (Rosman & Palm-Hernández 2008). Our conclusion that several phylogenetic species compose the complex of Fusarium wilt pathogens of bananas, formerly known as Foc, should gather mycologists and plant pathologists to consider my proposal to call it the *Fusarium* of Banana Complex (FuBC) (**Chapter 2**). The term "complex" is used to group/ accommodate several species with unifying characters of pathogenicity on bananas even though they are not monophyletic. This name provides more information on morphology, biology, and evolutionary relationships. The process I developed and executed could be exemplary to further look into other *formae speciales* (Lievens *et al.* 2013) to further resolve the intricate FOSC.

Identifying Fusarium wilt pathogens of banana

The FOSC comprises pathogenic and non-pathogenic strains. Thus, it is essential to correctly identify these characteristics, also for FuBC species. One could argue that *Fusarium* spp. isolated from a diseased plant likely belong *formae speciales* of that host (Wang *et al.* 2014, Pinaria *et al.* 2015). However, many strains that are isolated from roots appear to be non-pathogenic inhabitants (Snyder & Smith 1981, Fravel *et al.* 2003, O'Donnell *et al.* 2009). Hence, extra caution in required as the chance to capture non-pathogenic strain or root inhabitants is high, particularly in the FOSC that contains many root dwelling fungi (Beckman 1987, Lievens *et al*. 2013). The golden standard for the identification of pathogenic strains is based on Koch's postulates comprising isolation and subsequent inoculations to reproduce the symptoms and again find isolation for comparative reason. This, however, is unfortunately frequently overlooked due to tedious and time-consuming procedures or simply because it is impossible to obtain host plants or pure fungal cultures (Hermanto *et al.* 2009, Mostert *et al.* 2017). The KNAW-SPIN program provided a platform to circumvent such limitations and hence

we demonstrated sympatry by showing that many *Fusarium* spp. are pathogens of banana (**Chapters 2, 3, & 4**).

Fusarium species can be recovered from a very wide range of hosts (Leslie & Summerell 2006). It is important to carefully record the correct symptoms and administer host plants, genotypes, and geographical origin and thus limit the species that could be distinguished. We started this study with a comprehensive sampling expedition of Fusarium wilt affected bananas in Indonesia (**Chapter 2**). In order to narrow down the number of isolates and to avoid non-pathogenic root inhabitants we sampled vascular strains of the diseased plants and collected accurate information on the source and location of each sample. Using the well-established *Fusarium* Multi-Locus Sequence Typing database (*Fusarium* MLST, http://www.westerdijkinstitute.nl/fusarium/), partial sequences of *tef1* were used as query for molecular identification of most strains. Upon the identification of FOSC members we also used *rpb1* and *rpb2* which provided a strong signal for further phylogenetic species recognition (Geiser *et al.* 2004, O'Donnell *et al.* 2013, O'Donnell *et al.* 2015) (**Chapter 2**). Once a robust identification was established, further molecular characterization (i.e. using genetic markers) and phenotypes were used to finalize the data set (**Chapters 3 & 4**). Despite the cheap and easy mode of current genome sequence information, it is in many cases not necessary for species recognition, but serves other goals such as phylogeographic inferences (Zheng *et al.* 2018).

Many *formae speciales* of the FOSC have traditionally been identified by using vegetative compatibility groups (VCGs) (Puhalla 1985, Leslie 2013). However, it is a laborious technique, especially for diverse populations where each individual isolate needs to be tested against a series of testers (numbers of VCGs). In many cases it is not possible and will take months or, in our case given the size of the collection, even years. Moreover, VCGs can contain both pathogenic and non-pathogenic strains towards a common host (Leslie 2006, Fourie *et al.* 2009). We demonstrate that VCGs and members of the FuBC do not align. Thus, a single species can contain more VCGs (**Chapters 2 & 3**). With unknown underlying genotypes, VCG analyses might be suitable as a research tool, but not as an identification or diagnostic protocol and therefore further molecular identification is needed (Kistler 1997, Leslie & Summerell 2006). It is therefore very important that a molecular diagnostic for TR4 strains, i.e. *F. odoratissimum* has already been developed (Dita *et al.* 2010) and commercialized. Recently, an even more advanced Loop mediated isothermal amplification (LAMP) diagnostic has been developed (Salacinas *et al.* 2018) and we confirmed that it correctly identified each and every isolate of *F. odoratissimum* irrespective of its geographical origin (**Chapter 4**). However, developing molecular diagnostics that could distinguish FUCB constituents in the FOSC would be very beneficial for disease management. Ideal diagnostics of pathogenic strains would be based on DNA sequences that related to pathogenicity (host-specific) rather

than conserved genes or anonymous molecular markers (Recorbet *et al.* 2004, Lievens *et al.* 2008).

Extensive genetic variation of *Fusarium* spp. associated with banana in Indonesia

Until recently, there was no concern about durability of resistance to Fusarium wilt in banana. The globally grown Cavendish cultivars saved the industry due to their excellent resistance to the strains that caused the epidemic in the Americas (Koeppel 2007, Ploetz 2015b). Outbreaks in South-East Asia were neglected, and awareness was only erupting after the identification of TR4 in Jordan (García-Bastidas *et al.* 2014). Now, there is a broadly shared concern about accessibility and durability of resistance in the field, not only for large-scale plantations but also for subsistence farming. The extensive genetic diversity of the FuBC (**Chapter 2, 3 & 4**) complicates this concern. Thus far, only a limited number of varieties is known to carry resistance to a particular species of the FuBC (Hwang & Ko 2004, Paul *et al.* 2011, Smith *et al.* 2014). In regions where hundreds of cultivated varieties are available, like in Indonesia, subsistence farmers simply choose resistant varieties to replace susceptible ones. This practice makes disease control difficult and influences the evolution of the pathogen in such environments. As discussed above, over the course of history of Foc diversity studies, various genetic or physiological markers were used to determine variation, including physiological race, VCGs, production of volatile compounds and mating types (Ploetz 1990, Moore *et al.* 1991, Moore *et al.* 1993, Fourie *et al.* 2009, Cunha *et al.* 2015). However, ideal genetic markers are selectively neutral, polymorphic within populations, locus specific, reproducible, unambiguously to score and affordable i.e. easy to implement at reasonable cost (Brown 1996, Milgroom 2015). In **chapter 2**, I used DNA sequences of *tef1, rpb1* and *rbp2* for phylogenetic analyses of the FuBC and new phylogenetic species were identified and new taxonomic names were assigned for each species. Members of the FuBC reproduce clonally, hence species-specific mutations occur and are maintained without recombination into other genetic backgrounds (Kistler 1997). Therefore, the phylogenetic tree is accurate and can be used to represent the species and their relationship or ancestry. However, the variation between and among species needs to be assessed by other, genome-wide and many molecular markers. Therefore, genotyping-by-sequencing, using Diversity Array Technology (DArTseq) markers was chosen and to increase the resolution of these analyses. Moreover, markers can be mapped adequately to the *F. odoratissimum* TR4 reference genome and provide good coverage (**Chapter 3**). Cluster analyses of DArTseq markers provide an excellent resolution to discriminate genotypes within the species of FuBC. Thus, each species might contain one or more genotypes.

Genotype diversity is an important concept of plant pathogens that have a significant component of asexual reproduction in their life history (McDonald & Linde 2002). Knowledge of genotype diversity of pathogens has direct applications in agriculture related to disease

management and in our understanding of host pathogen biology. *Fusarium* spp. affecting banana are known to be highly diverse (Ploetz 1990). However, reports on this diversity have been inconsistent (**Chapter 3**) and incomplete due to the absence of indigenous populations from the centre of origin of banana. I demonstrate that the hitherto widest global diversity panel (24 VCGs) (Katan 1999, Ordóñez *et al.* 2015) comprises different species (**Chapter 2**) present in Indonesian populations (**Chapter 3**). Thus, Indonesian collection captured the widest diversity of *Fusarium* spp. affecting banana and supports the view that the most informative study on systematics and population biology of the pathogen is in the primary centre diversity of banana (Leslie & Summerell 2006).

Co-evolution of plants and pathogens in their native environments results in high genetic diversity such as exemplified by the wheat pathogen *Zymoseptoria tritici* in the Fertile Crescent (Stukenbrock *et al.* 2007, Stukenbrock & McDonald 2008), the rice blast pathogen *Magnaporte grisea* in the Himalayan foothills and Asia (Talbot 2003, Tharreau *et al.* 2009) and the coffee rust pathogen *Hemileia vastatrix* in Ethiophia and East Africa (Schumann & D'Arcy 2012). This complies with our identification of eight out of nine *Fusarium* spp. in the FUCB in Indonesia (**Chapter 2**), which also comprise 10 out of 19 globally identified genotypes (Ordóñez *et al.* 2015; **Chapter 3**). However, *Fusarium* spp. also co-evolve in other parts of the world where banana was introduced i.e. in Africa (**Chapter 2**) and Latin America, with the potential emergence of new genotypes (Ordóñez *et al.* 2018).

Pathogenicity is a phenotypic characteristic that can be used to assess genetic variation between and within *Fusarium* spp. affecting banana. The hitherto physiological race nomenclature of FuBC assembled isolates with pathogenicity to Gros Michel as Race 1 strains, while isolates pathogenic on both Gros Michel and Cavendish bananas were classified as Tropical Race 4 (TR4) (Stover & Waite 1960, Su *et al.* 1986). In my study, six out of nine species contained Race 1 isolates (**Chapters 2 & 4**), indicating convergent pathotype evolution in genetically distinct species. *F. tardichlamydosporum* contained both Race 1 and Race 2 isolates (**Chapter 2**), suggesting that both pathotypes recently evolved from a common ancestor. However, despite the high number of isolates, diverse hosts and varying geographical origins, all TR4 strains collapsed into a single new species, *F. odoratissium*. This suggests that TR4 has evolved recently, but we cannot easily explain why this species is found across the Indonesian archipelago. It is likely that TR4 emerged and escaped from Sumatera to Taiwan and has spread locally, regionally, and intercontinentally from South-East Asia, particularly driven by anthropogenic factors due to the vast global Cavendish monoculture export production (Ordóñez 2015, Zheng *et al.* 2018). This crop has thereby become a vehicle for international TR4 dissemination, which projects a grim scenario onto the international export trade, as TR4 will almost surely surface in prone areas that are yet free of the pathogen. The risk is even higher for areas where the crop was introduced and diversity is consequently low such as the

American continent where the least number of species is present compared to Africa and Australia where banana is a native plant (De Langhe & De Maret 1999; **Chapter 2**).

Sources of variation

As mentioned above sexuality is unknown in the FOSC and was never observed or reported neither under field nor laboratory conditions. Thus, genetic diversity must result from non-sexual recombination or simply from mutation, which slows down adaptation (Leslie 2013, Milgroom 2015). However, genetic diversity was found in many different *formae speciales* of *Fusarium oxysporum,* thus in contrast to the general concept that the absence of sex prevents recombination or genetic exchange. Yet, there is clearly tremendous variation of phenotypic characters in *Fusarium oxysporum,* which is exceptional for an asexual fungus and these include characteristics such as host specificity (*formae speciales*), cultivar specificity (race), VCGs and many others. However, neither of these indicate genetic or evolutionary relatedness of these species as we demonstrated in **chapters 2** and **3**, albeit that *F. oxysporum* f. sp. *ciceris*, the causal agent of Fusarium wilt of chickpea, could be an exception as this *formae speciales* is monophyletic (Jiménez-Gasco *et al.* 2002).

I assume that co-evolution is one of the main sources driving diversification in the FuBC in Indonesia where hundreds of banana varieties are grown. In natural ecosystems, the antagonistic interaction between plants and pathogens results in dynamic co-evolutionary processes in which plants evolve to recognize pathogens and pathogens adapt to circumvent plant defence responses (Takken & Rep 2010, Möller & Stukenbrock 2017). This process occurs since the beginning co-occurrence of plants and pathogens and plant domestication shapes the genetic architecture of plants and pathogens. In asexual fungi, mutations or genomic rearrangements could be the primary sources of co-evolutionary processes that underlie diversity. Chromosomal number or size variants were observed in Foc isolates (Boehm *et al.* 1994). This karyotype diversity is likely the result of active families of transposable elements in the genome of *Fusarium oxysporum*, which contribute to chromosome variants (Davière *et al.* 2001, van Dam *et al.* 2018).

The polyphyletic structure of the FuBC, and likely many other *formae speciales*, was identified by using the DNA sequence of conserved genes and indicates that the evolutionary proximity of species with pathogenicity towards unrelated hosts is smaller than between species infecting a common host. Indeed, horizontal gene transfer (HGT) events have been reported in many FOSC species (van der Does & Rep 2007, Ma *et al.* 2010, Vlaardingerbroek *et al.* 2016, van Dam & Rep 2017), particularly in *Fusarium oxysporum* f. sp. *lycopersici* (Fol) where it has been well studied. The tomato - Fol pathosystem complies with the gene-for-gene (GFG) mechanism of host – pathogen interactions. The main virulence factors are located on a pathogenicity chromosome that can be shuttled among Fol strain and even between different *Fusarium oxysporum* ff. spp. As root and soil inhabitants, many *Fusarium oxysporum*

ff. spp., comprising pathogens and non-pathogens, co-inhabit the same niche, which increases the chance for HGT. We collected *Fusarium* isolates from many different banana-planting systems in Indonesia. These various ecosystems are made up by manifold plant species, including host and non-host plants (**Chapter 2**), thereby creating a conducive environment for HGT to occur as pointed out by Vlaardingerbroek *et al.* (2016). The variation of the genome size and chromosome numbers in FuBC species (Miao 1990), reflect the high possibility of HGT within and between species. Future studies on the genomic compositions of each species of the FuBC could reveal whether HGT occurred or is required for the pathogenicity of the constituent *Fusarium* species on bananas.

Native populations of pathogens should be capable of sustaining more genetic variation as they will not be subjected to uniformity and intense selection pressure associated with agricultural monocultures (Stukenbrock & McDonald 2008, Summerell *et al*. 2010). Such ecological settings are exemplary for Indonesian banana farming systems (**Chapter 2**). Despite the limitation of an absent sexual cycle, asexual fungi can undergo anastomosis and subsequently parasexual exchange (mitotic crossing over), which does occur in native populations of plant pathogens (Talbot 2003). Brankovics *et al.* (2017) recently suggested parasexuality for species in the FOSC, but Buxton (1962), who was intrigued by the wide variation of culture characteristics and pathogenicity of *F. oxysporum* f. sp. *cubense* on different banana cultivars, already demonstrated that strains pathogenic on Gros Michel bananas could undergo asexual recombination under laboratory condition. Finally, Taylor *et al.* (1999) conclude that little resolution, low support for clades and incongruence of individual gene genealogies are indicators of asexual recombination of FuBC species under natural conditions. In my analyses of extensive data sets we used gene genealogies for a phylogenetic species concept (**Chapter 2**) and observed low support for *Fusarium purpurascens* and *F. phialophorum*; a condition consistent with recombination (Koenig *et al.* 1997, Taylor *et al.* 1999). Nonetheless, we described them as distinct phylogenetic species due to sequence support and because they were previously recognised as a lineage/cryptic species (O'Donnell *et al.* 1998, Fourie *et al.* 2009). Moreover, Fourie *et al.* (2009) observed that FuBC species harbour either of the two-mating type idiomorphs *mat1-1* and *mat1-2* but that some lineages contain both alleles and are therefore functionally heterothallic. This suggests that these species have been or will be able to undergo sexual reproduction.

Pathogenicity variation

Over the past years several approaches determined genetic variation in FuBC species, but the underlying mechanism remain poorly understood (Moore *et al.* 1993, Bentley *et al.* 1998, Groenewald *et al.* 2006, Fourie *et al.* 2009, Mostert *et al.* 2017). Here, we demonstrate that high resolution genotyping provides a solid identification of individual species and genotypes in natural populations (**Chapters 2 & 3**), which should be generally adopted as a

standard identification and characterization method for *Fusarium* species involved in the complex banana – *Fusarium* spp. pathosystem. The next important step is to determine genetic variation for pathogenicity towards diverse banana accessions, which is critical for appropriate disease management and indispensable for developing resistance breeding strategies. Well-characterized strains are necessary for any forward strategy for improving disease resistance (Russell 1978, Brown 2002), and the banana – *Fusarium* spp. pathosystem is no exception. However, previously, pathogenic variation in the FuBC was not fully assessed due to the low experimental amenability, and the absence of differential banana varieties (Ploetz 2015a). Neither the interaction nor the genetic basis of pathogenicity is well understood in this pathosystem. Until recently, not a single gene for resistance to Fusarium wilt had been mapped. Dale et al. (2017) successfully identified and transferred a major gene for resistance from *M. acuminata* ssp. *malaccensis,* the wild diploid species endemic in Sumatra*,* to Cavendish banana. Sutanto (2014) isolated and characterized resistance gene analogues (RGAs) from Pisang Rejang and Calcutta4. Defence gene analogue (DGAs) were also isolated from local varieties Klutuk Wulung, Pisang Kepok, Ambon Hijau and Rejang (Sutanto 2014), making them prime interest targets for future gene identifications.

A new standardized phenotyping protocol enabled the screening of hundreds of banana accessions, facilitating the analysis of large segregating populations under greenhouse conditions (**Chapter 4**, Ahmad *et al.* in prep., Garcia-Bastidas *et al.* in prep.). These are the first cornerstones for an increased understanding of genetic diversity for pathogenicity in the FuBC, as genomic resolution without appropriate phenotyping tools is an emptyhanded precision. Our contribution is substantial as we screened 78 isolates across the FuBC to four banana accessions and confirmed large differences in pathogenicity that overall overlapped the so-called Race 1 and TR4 pathotypes, which all belong to the newly described species *F. odoratissimum,* as well as quantitative variation (**Chapter 4**). Contrary to the tomato – Fol pathosystem (Houterman *et al.* 2009), the genetic basis of the host- pathosystem for hundreds of *Fusarium oxysporum* ff. spp. is unclear (Armstrong & Armstrong 1981) and only a few comply with GFG, such as *F. oxysporum* f. sp. *melonis* - melon, *F. oxysporum* f. sp. *cucumerinum* -cucumber, *F. oxysporum* f. sp. *pisi* - pea, and *F. oxysporum* f. sp. *niveum* - watermelon (Michielse & Rep 2009). Thus, the majority of race determinations solely rely on assays and the underlying genetic basis for pathogenicity and host resistance is unknown. The FuBC – banana system is therefore just at the start of a deeper understanding that should be the basis for progress towards a much more sustainable global banana production. Clearly, genetic studies have to continue and more differential banana accessions should be added to the test panel to further reveal the widest possible pathogenicity landscapes. Compared to other *formae speciales*, FuBC has the least number of host varieties to be used in phenotyping assays (Stover & Waite 1960, Su *et al.* 1986). However, genomic research will also reveal effector proteins that will further professionalize and extend the FuBC pathogenicity map.

Comparative analyses with the closely related Fol – tomato pathosystem revealed homologs of the secreted in xylem (SIX) effector proteins which interact with host receptors (Fraser-Smith *et al.* 2014, Czislowski *et al.* 2018). However, these studies are based on whole genome profiles of FuBC species, which do not unveil their role during pathogenesis. In another study, using only one susceptible banana variety, Guo *et al.* (2014) report that a set of SIX genes is expressed during infection. Recently, Widinugraheni *et al.* (2018, in prep.) showed that SIX1 alleles contribute to aggressivness of *F. odoratissimum*. However, these effectors are not crucial for the presumed GFG that results in complete resistance to *F. odoratissimum* in the wild accessions *M. acuminata* ssp*. malaccensis* to *F. grosmichelii* in Cavendish*.* Hence, further studies are required to demonstrate the role of these and other genes in the defence response. Comparative genomics will be crucial for further identification and understanding of FuBC effector genes, similar to other *Fusarium* pathosystems (Rep & Kistler 2010), particularly since the Race 1 strains belong to six FuBC species, which might be due to HGT. The global dissemination of TR4 is therefore a great concern as HGT may potentially turn other FuBC species into Cavendish killer strains.

As a matter of fact, Gros Michel is susceptible to a broad suite of *Fusarium* species while Cavendish is susceptible to just *F. odoratissimum* (**Chapter 4**). This is one of the best examples of durable resistance (Jonson 1984, McDonald & Linde 2002) as Cavendish is cultivated on Race 1 infested soils around the world for decades. Contrary to the epidemiological dogma's and expectations, selection pressure has until now not resulted in *Fusarium* strains that overcome the resistance of this banana variety. Hence, it is worthwhile and necessary to understand how selection pressure on soil-borne pathogens is being operationalized. Is it for instance, not as strong because they can easily survive as endophytes in non-host plants (Gordon *et al.* 1989). In any case, the current pandemic truly results from new intrusions of another *Fusarium* species. Therefore, increased insight in the epidemiological processes, genetic variability and selection pressure is necessary to anticipate the durability of any source of resistance, including genes from wild banana accessions (Dale *et al.* 2017) with impressive efficacies to TR4 (**Chapter 4**, Zuo *et al.* 2018) such as *M*. *acuminata* ssp. *malaccensis* (Pahang), the cultivated variety Pisang Rejang and many others (Handayani *et al*. 2017).

Diversity of *Fusarium* species hitchhiking with Fusarium wilt pathogens of banana

The incidence and abundance of *Fusarium* species has been well documented for almost all plant species and in soil (Bacon & Yates 2006, Leslie & Summerell 2006, Ploetz 2006). Members of this genus have adapted to a wide range of ecological conditions and hosts. They are the primary cause of root, stem, and leaf diseases of many agriculturally important crops (Marasas *et al*. 2006, Ploetz 2006). Besides being pathogens, many of these fungi are also known to grow as endophytes in non-host plants (Leslie *et al.* 1990, Zakaria *et al*. 2011). In

host-fungal pathogen systems, most attention – obviously – is directed towards causal agents in mycological studies. Albeit that microbiome studies may have a broader view, they usually focus on bacterial communities (Turner *et al.* 2013, Hardoim *et al.* 2015). Consequently, many endophytic *Fusarium* species are generally overlooked and therefore, their role during the early symptomless phase of pathogenesis is unknown. However, once symptoms become apparent, *Fusarium* species isolated from these infected materials are frequently considered to be the causal agents. Without subsequent inoculation studies such assumptions are at least questionable (Brader *et al.* 2017). Reports on non-pathogenic *Fusarium* species from diseased plants are scarce (Pinaria *et al*. 2010, Wang *et al*. 2014). In **chapter 5**, we identified 20 *Fusarium* isolates from banana plant with Fusarium wilt symptoms and concluded that they belong to the *F. fujikuroi* (FFSC), the *F. incarnatum-equisetii* (FIESC), and the *F. sambucinum* species complexes (FSSC). Many of these *Fusarium* species are well-known pathogens associated with other crops, also commonly cultivated in Indonesia (e.g. *F. fujikuroi*, *F. sacchari* and *F. verticilloides* on sugar cane, maize and rice, respectively), but were never reported in banana. Our assumption is that these species enter affected tissue and hence hitchhike with *Fusarium* spp. associated with banana, but they could also facilitate pathogenesis of these species while dwelling as endophytes in banana. Further experimentation should reveal which option is most likely.

Conclusions and direction of future research

Diversity analyses of plant pathogens in their centre of origin are crucial for understanding the complexity of host – pathogen relationship. This thesis gives a first impression of the enormous diversity of the FuBC in the Indonesian centre of origin of banana. However, this is only a start. Extending knowledge on the extensive diversity of the causal fungi should bridge the gap of understanding their co-evolution with hundreds of bananas varieties. Therefore, Indonesian banana germplasm is also a source for resistance, albeit that many local varieties are very susceptible, hundreds of varieties are potentially resistant to one or more constituent FuBC species, which should be explored in much more detail.

The fundamental taxonomical aspects of this study provide basic knowledge that can be used by plant pathologist, mycologist and breeders. The proposed FuBC unifies Fusarium wilt pathogens of bananas and could accommodate taxonomy and pathology standpoints in various other species and *formae speciales* of the FOSC. Finally, many asexual fungi turned out to have a secret sex life. Therefore, attempting sex with FuCB species is a challenging but worthwhile exploration, particularly under natural conditions in Indonesia. Clearly, fungal genetics would facilitate building the required underlying foundation for the genetic improvement of the crop. On the other hand, in case sex would not work out, which is likely, progress can be guaranteed by continuing building a sound basis of multidisciplinary projects in a stimulating environment and optimal critical mass to further explore the beauty and

challenges of the banana – FuBC pathosystem. This should include high-throughput host and pathogen genomics in parallel with the latest genetic technologies to unravel their interaction, including genome editing, functional studies and crop improvement. The current study is an excellent example how curiosity can push progress in many under-investigated and yet significant problems with other *Fusarium* pathogens in tropical crops.

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Summary

Banana is one of the most consumed fruits and also a staple in many countries. Sustaining banana production is important to supply domestic and international markets, which support the livelihood of millions of smallholder farmers. The most beloved fruit was and is currently devastated by one of the most threatening plant diseases in history called Fusarium wilt or Panama disease. The disease is caused by a suite of soil born fungi, previously collectively known as *Fusarium oxysporum* f. sp. *cubense* (Foc). A prime strategy to manage the ongoing pandemic of the disease that has devastated thousands of hectares of banana plantations in many countries is the generation of resistant banana varieties. Therefore, more knowledge on the diversity of the causal fungi is indispensable. Indonesia is the main centre of origin of banana where hundreds of wild and cultivated varieties are grown across the entire archipelago. Generally, the centres of diversity of hosts and pathogens overlap. Thus, Indonesia is the primary region to study the diversity of the pathogens causing Panama disease due to their co-evolution with the banana hosts. This thesis provides some of the most informative studies on the genetic diversity and phylogeny of *Fusarium* spp. associated with Panama disease. It contributes to the knowledge on the systematics of *Fusarium* species with pathogenicity to banana, their genotypic diversity and pathogenicity, as well as on non-pathogenic *Fusarium* spp. hitchhiking with pathogenic species in the interior of banana stems.

In **chapter 1**, I describe the current state of art of banana research and the related *Fusarium* pathogens. Also, the history and early domestication of banana, its production and importance as a fruit crop commodity are described. This is then connected with Fusarium wilt as one of the main constraints, culminating in the importance and urgency of studying the diversity of the causal pathogens in Indonesia.

Chapter 2 describes a sampling expedition to sample Fusarium wilt affected banana plants across 34 geographical locations of the main banana producing areas in Indonesia on Java, Flores, Kalimantan, Papua, Sumatra and Sulawesi. More than 200 *Fusarium* isolates were recovered from many local varieties that suffered from the disease. The identification and characterization of this collection represents the most extensive diversity analysis of *Fusarium* spp. associated with Panama disease. Using multi-gene phylogenetic analyses, I discovered nine distinct genetic lineages with pathogenicity to banana, which were recognised as new phylogenetic species in the *Fusarium oxysporum* species complex (FOSC), to which new names and taxonomic descriptions were assigned. The complexity and current status of Fusarium wilt disease in banana in Indonesia is also discussed.

In **Chapter 3,** I expanded the diversity analyses by using whole genomic profiles generated by genotyping-by-sequencing using Diversity Array Technology (DArTseq) markers. Cluster analyses of Indonesian and global isolates revealed the widest genotypic diversity ever reported in Fusarium wilt pathogens of banana. More than half of the genotypes identified are present in Indonesia, suggesting that *Fusarium* spp. co-evolved with local banana

varieties. I provide strong evidence that the so-called Tropical Race 4 (TR4) that kills Cavendish varieties, likely emerged from Indonesia, and is still evolving with many local varieties. In contrast, Race 1 isolates, which caused the first epidemic in Gros Michel bananas in the previous century display more genetic diversity. Such precise descriptions of genetic diversity are very important to further explore disease resistance in native and wild banana varieties. The reliability of DArTseq markers was demonstrated by their power to discriminate isolates below the *Fusarium* spp. level, which provided an unparalleled robustness of the proposed species concept.

Chapter 4 focuses on the pathogenicity of the Indonesian collection of *Fusarium* spp. described in **chapter 2 and 3**. All isolates were phenotyped on Grand Naine, Gros Michel, the wild diploid *Musa acuminata* ssp. *malaccensis* (Pahang) and the diploid cultivated variety Pisang Rejang. This resulted in a large database with qualitative and quantitative variation for pathogenicity for the ten identified *Fusarium* spp. in Indonesia. Interestingly, TR4 isolates mapped to one species that I named *Fusarium odoratissimum,* whereas Race 1 strains belonged to six species.

Chapter 5 describes the discovery of other non-pathogenic *Fusarium* species hitchhiking with pathogenic *Fusarium* spp. on banana. Multi-gene phylogenies revealed two new species in the *F. fujikuroi* species complex (FFSC), six new species in the *F. incarnatum-equiseti* species complex (FIESC) and one isolate of *F. longipes* in the *F. sambucinum* species complex (FSSC). The latter was never reported to be present in the pseudostem of banana plants. None of these species are pathogenic on banana and hence, these findings give an insight into the endophytic existence of *Fusarium* spp. in non-host plants and their potential role in the Fusarium wilt disease of banana.

In **chapter 6,** the findings in all chapters are synthesized in an overarching discussion. The required taxonomic revision of the Fusarium wilt pathogens of banana is discussed with a proposition to place them in the *Fusarium* of banana complex (FuBC). The adopted approach of identification comprising molecular-based diversity analyses, which excels over phenotyped-based diversity analyses and the evidence of possible sources of genetic variation in these asexual fungi are also discussed. Despite the advances, the FuBC – banana pathosystem lacks a genetic basis and comparative analyses with other *formae speciales* might therefore be very useful as well as genetic studies in the host. With well-characterized *Fusarium* species, such studies become meaningful and will contribute to an overall strategy for developing new and resistant banana germplasm. The thesis ends with a complete overview of all cited references.

Ringkasan

Pisang merupakan salah satu komoditas buah yang paling banyak dikonsumsi dan merupakan makanan pokok di beberapa negara di dunia. Produksi pisang yang berkelanjutan menjadi sangat penting untuk menjaga ketersediaan buah ini baik di pasar domestik maupun internasional yang juga merupakan penunjang kehidupan jutaan petani kecil. Buah yang paling banyak digemari ini pernah dan masih terancam salah satu penyakit yang sangat merugikan sepanjang sejarah yaitu penyakit layu Fusarium atau *Panama disease*. Penyakit ini disebabkan oleh jamur tanah yang dikenal dengan *Fusarium oxysporum* f. sp. *cubense* (Foc). Strategi utama untuk mengantisipasi persebaran penyakit yang telah menghancurkan ribuan hektar perkebunan pisang di beberapa negara di dunia ini adalah dengan menghasilkan jenis pisang yang tahan penyakit. Sehingga pengetahuan tentang keragaman jamur penyebab penyakit ini menjadi sangat diperlukan. Indonesia merupakan daerah asal dan pusat keragaman pisang. Ratusan jenis pisang liar dan pisang budidaya tumbuh subur diseluruh kepulauannya. Pada umumnya pusat keragaman host/ inang dan agen penyebab penyakit yang menyerangnya berada pada tempat yang sama. Dengan demikian, Indonesia adalah kawasan utama untuk mempelajari keragaman jamur penyebab penyakit *Panama disease* dimana diduga *co-evolution* terjadi antara jamur ini dengan inangnya. Disertasi ini menyajikan beberapa studi/ kajian paling informatif pada keragaman genetik dan *phylogeny Fusarium* spesies yang menyebabkan penyakit layu pada pisang, keragaman genotipik dan pathogenisitasnya, serta *Fusarium* spp. tidak menyebabkan penyakit.

Bab 1 memaparkan tinjauan pustaka terkini pada pisang dan hubungannya dengan *Fusarium* yang menyebabkan penyakit. Sejarah domestikasi pisang, produksi dan pentinganya pisang sebagai komoditas buah juga ditinjau. Hal ini kemudian dihubungkan dengan penyakit layu Fusarium sebagai hambatan utama budidaya, sampai kepada pentingnya dan kebutuhan mendesak untuk mempelajari keragaman jamur penyebab penyakit ini di Indonesia.

Bab 2 menjelaskan explorasi penyakit layu Fusarium di Indonesia meliputi 34 lokasi sentra produksi pisang di Indonesia yang ada di pulau Jawa, Flores, Kalimantan, Papua, Sumatra dan Sulawesi. Lebih dari 200 isolat *Fusarium* berhasil diisolasi dari berbagai jenis pisang lokal yang terserang penyakit. Identifikasi dan karakterisasi koleksi ini menunjukkan analisis keragaman paling luas dan lengkap pada *Fusarium* spp. yang berasosiasi dengan *Panama disease*. Dengan menggunakan *multi-gene phylogeny* analisis saya menemukan sembilan *genetic lineage* dengan patogenisitas terhadap pisang dan dikenali sebagai *phylogenetic species* baru di dalam *Fusarium oxysporum species complex* (FOSC), sehingga nama dan deskripsi taksonomi diberikan pada spesies-spesies tersebut. Kompleksitas dan status terkini dari penyakit layu Fusarium pada pisang di Indonesia juga dibahas pada bab ini.

Pada **bab 3**, saya mengkaji lebih jauh analisis keragaman menggunakan profil genom yang dihasilkan dari *genotyping-by-sequencing* menggunakan penanda genetik *Diversity Array Technology* (DArTseq). Analisis klaster strain-strain asal Indonesia dan dunia

menghasilkan keragaman genotip yang paling tinggi yang pernah dilaporkan pada jamur penyebab layu Fusarium pada pisang. Lebih dari separuh genotip yang diidentifikasi berasal dari Indonesia, menunjukkan bahwa *Fusarium* spp. berevolusi bersama dengan beragam pisang lokal. Deskripsi keragaman genetik secara tepat dan akurat sangat penting untuk dapat mengeksplorasi ketahanan penyakit pada pisang-pisang lokal maupun pisang-pisang liar. Keandalan penanda genetik DArTseq ditunjukkan oleh kemampuannya membedakan strain-strain dalam spesies *Fusarium* sehingga menghasilkan analisis yang kuat untuk konsep spesies yang diusulkan pada **Bab 1**.

Bab 4 fokus pada patogenisitas strain-strain *Fusarium* spp. dari Indonesia yang dideskripsikan pada Bab 2 dan Bab 3. Semua strain diuji patogenistasnya pada kultivar pisang Grand Naine, Gros Michel, pisang liar *Musa acuminata* ssp. *malaccensis* (Pahang), dan diploid kultivar pisang Rejang. Percobaan ini menghasilkan data yang cukup besar dengan variasi kuantitatif dan kualitatif patogenisitas pada sepuluh spesies *Fusarium* asal gejala layu panama yang teridentifikasi di Indonesia. Semua ras TR4 terpetakan sebagai satu spesies, *F. odoratissimum*, sedangkan ras 1 terdiri dari enam spesies.

Bab 5 menjelaskan penemuan beberapa *Fusarium* spesies non-patogen membonceng pada *Fusarium* spp. patogen pisang pada satu gejala. *Multi-gene phylogeny* berhasil mengidentifikasi dua spesies baru pada *F. fujikuroi species complex* (FFSC), enam spesies baru pada *F. incarnatum-equiseti species complex* (FIESC) dan satu strain *F. longipes* pada *F. sambucinum species complex* (FSSC). Spesies terakhir yang disebutkan belum pernah dilaporkan ada pada pseudostem pohon pisang. Tidak ada satupun dari spesies ini yang menyebabkan penyakit pada pisang, namun penemuan ini memberikan pengetahuan tentang keberadaan *Fusarium* spp. endofit serta potensial peranannya pada pisang yang teserang layu Fusarium.

Pada **Bab 6**, penemuan-penemuan di semua Bab disintesis dalam sebuah pembahasan yang menyeluruh. Kebutuhan revisi taksonomi patogen layu Fusarium pada pisang dibahas dengan sebuah usulan untuk menempatkannya pada *Fusarium of banana complex* (FuBC). Pendekatan identifikasi yang digunakan meliputi analisis keragaman berbasis molekuler yang mampu mengungguli analisis keragaman berbasis fenotip serta bukti-bukti kemungkinan sumber keragaman genetik yang dapat terjadi pada jamur aseksual ini juga dibahas. Disamping kemajuan yang telah dicapai, dasar-dasar genetik interaksi *pathosystem* antara FuBC – pisang belum diketahui dan komparatif analisis dengan *formae specialis* lain akan sangat bermanfaat termasuk dasar-dasar genetik pada inang. Dengan karakterisasi yang sangat baik yang telah diketahui pada *Fusarium* spp., studi seperti itu akan menjadi sangat bermanfaat dan dapat digunakan untuk mengembangkan jenis pisang baru dan tahan terhadap penyakit.

Samenvatting

De banaan is een van de meest geconsumeerde fruitsoorten en tegelijkertijd een basisvoedsel in veel landen. De instandhouding van de bananenproductie is belangrijk om lokale en internationale markten te voorzien zodat miljoenen kleine boeren in hun levensonderhoud kunnen voorzien. Het geliefdste fruit werd en wordt echter verwoest door een van de bedreigendste ziekten in zijn historie; *Fusarium* verwelkingsziekte of Panamaziekte. De ziekte wordt veroorzaakt door een reeks bodemgebonden schimmels, die voorheen bekend stonden onder de gezamenlijke naam *Fusarium oxysporum* f.sp. *cubense* (Foc). De eerste strategie om de huidige pandemie, die reeds duizenden hectaren bananenplantages in vele landen heeft verwoest, te beheersen is het ontwikkelen van resistente rassen. Daarom is meer kennis van de schimmels die deze ziekte veroorzaken onmisbaar. Indonesië is het belangrijkste oorsprongsgebied van banaan waar honderden wilde soorten en variëteiten in de gehele archipel voorkomen en worden geteeld. In het algemeen overlappen de oorsprongsgebieden van gewassen en hun pathogenen. Daarom is Indonesië een van de primaire gebieden om de diversiteit van de schimmels die Panamaziekte veroorzaken te bestuderen vanwege hun co-evolutie met de waardplant. Dit proefschrift is een van de informatiefste studies naar de genetische diversiteit en de fylogenie van *Fusarium* soorten die worden geassocieerd met Panamaziekte. Het draagt bij aan de kennis en de systematiek van *Fusarium* soorten die pathogeen zijn op banaan, door de beschrijving van hun genetische diversiteit en pathogeniteit, en beschrijft ook andere soorten die banaan normaliter niet aantasten maar die meeliften met pathogene soorten in het vaatsysteem van aangetaste planten.

In **hoofdstuk 1** beschrijf ik de stand van zaken in het *Fusarium* onderzoek bij de aanvang van het onderzoek. De geschiedenis en de domesticatie van banaan komen naar voren, en daarnaast wordt het belang van het gewas als handelsgewas besproken. Dit wordt vervolgens in verband gebracht met *Fusarium* verwelkingsziekte als een van de belangrijkste beperkingen, uitlopend op het belang en de noodzaak om de diversiteit van de daaraan ten grondslag liggende schimmels in Indonesië te onderzoeken.

Hoofdstuk 2 beschrijft een verzamelexpeditie waarin ziekte planten werden bemonsterd in 34 verschillende locaties in de belangrijkste bananen producerende gebieden op Java, Flores, Kalimantan, Papoea, Sumatra en Sulawesi. Meer dan 200 *Fusarium* isolaten werden verkregen uit vele lokale rassen die door de ziekte waren aangetast. De identificatie en karakterisering van deze collectie is een van de uitgebreidste diversiteitsanalyses van *Fusarium* soorten die met Panamaziekte in verband worden gebracht. Door gebruik te maken van fylogenetische analysen die werden uitgevoerd op basis van meerdere genen ontdekten wij negen onderscheiden genetische lijnen met pathogeniteit voor banaan, die wij vervolgens hebben beschreven als nieuwe soorten van het *Fusarium oxysporum* soorten complex (FOSC) met de daarbij behorende namen en beschrijvingen. Daarnaast wordt aandacht besteed aan de complexiteit, de huidige omvang en het belang van Panamaziekte in Indonesië.

In **hoofdstuk 3**, heb ik de voorgaande diversiteitsanalyse uitgebreid door gebruik te maken van sequentietechnieken gekoppeld aan Diversity Array Technology (DArTSeq) waarmee ik profielen van het gehele genoom kon samenstellen. Door dit te combineren met een collectie wereldwijde isolaten resulteerde dit in een van de uitgebreidste diversiteitsanalyses van pathogenen die *Fusarium* verwelkingsziekte in banaan veroorzaken. Meer dan de helft van de geïdentificeerde genotypen zijn aanwezig in Indonesië, hetgeen suggereert dat deze *Fusarium* soorten zijn ontstaan door de co-evolutie met lokale bananenvariëteiten. Wij leveren sterke aanwijzingen dat het zogenaamde Tropische fysio 4 (TR4) dat Cavendish bananen doodt waarschijnlijk in Indonesië is ontstaan en zich nog steeds verder ontwikkeld in vele lokale rassen. In tegenstelling daarmee vertonen fysio 1 isolaten, die in de vorige eeuw de eerste epidemie in "Gros Michel' veroorzaakten, een grotere diversiteit. Het precies beschrijven van genetische diversiteit is van belang voor het opsporen van resistentie in inheemse rassen en wilde soorten. De betrouwbaarheid van DArTSeq merkers werd duidelijk door de hoge resolutie waarmee zij in staat zijn om isolaten binnen soorten te onderscheiden. Dit legt een ongeëvenaarde en robuuste basis onder het voorgestelde soortsconcept.

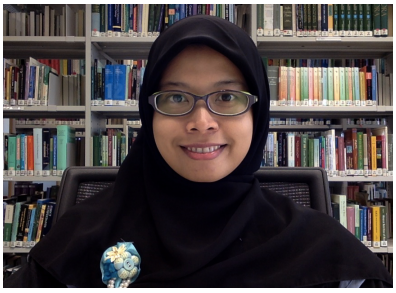
Hoofdstuk 4 concentreert zich op de pathogeniteit van de collectie Indonesische *Fusarium* isolaten die in banaan werden aangetroffen. Alle isolaten werden getest op 'Grand Naine', 'Gros Michel', de wilde diploïde *Musa acuminata* spp. *malaccensis* (Pahang) en de geteelde diploïde variëteit Pisang Rejang. Dit resulteerde in een grote dataset met kwalitatieve en kwantitatieve pathogeniteitsgegevens van de 10 geïdentificeerde *Fusarium* soorten in Indonesië. Het was interessant te zien dat de TR4 isolaten tot één soort behoren, die wij *Fusarium odoratissimum* hebben genoemd, terwijl de fysio 1 isolaten verdeeld zijn over zes soorten.

Hoofdstuk 5 beschrijft de ontdekking dat andere *Fusarium* soorten met de stammen meeliften die banaan wel kunnen aantasten. Genetische analysen op basis van meerdere genen laten zien dat twee van deze nieuwe soorten tot het *F. fujikuroi* soortencomplex (FFSC) behoren, zes nieuwe soorten maken onderdeel uit van het *F. incarnatum-equiseti* soorten complex (FIESC) en een *F. longipes* isolaat behoort tot het *F. sambucinum* soorten complex (FSSC). Deze laatste soort is nooit eerder beschreven in de pseudostam van bananen. Geen van deze nieuwe soorten is pathogeen op banaan en daarom geven deze resultaten inzicht in het epidemiologische en polyfage gedrag van *Fusarium* soorten op andere plantensoorten en de mogelijke rol ervan bij de ontwikkeling van *Fusarium* verwelkingsziekte in banaan.

In **Hoofdstuk 6** worden alle resultaten aaneengesmeed in een overkoepelende discussie. De benodigde taxonomische revisie van pathogenen die *Fusarium* verwelkingsziekte in banaan veroorzaken wordt bediscussieerd en er wordt voorgesteld die isolaten onder te brengen in het nieuwe Fusarium van banaan complex (FuBC). De gebruikte benadering waarin gebruik werd gemaakt van moleculaire diversiteitsanalysen, die beter zijn dan op phenotypering gebaseerde methoden, en de mogelijke oorzaak van de gevonden genetische diversiteit in deze asexuele schimmels worden ook bediscussieerd. Ondanks deze inzichten ontbeert het FuBC-banaan pathosysteem een genetische basis en vergelijkende

analysen met andere *formae speciales* kunnen daarom zeer waardevol zijn naast genetisch onderzoek aan de waardplant. Met goed beschreven *Fusarium* soorten winnen dergelijke studies aan waarde en dragen zij bij aan een alomvattende strategie om nieuwe en resistente bananenrassen te ontwikkelen. Het proefschrift eindigt met een compleet overzicht van de gebruikte literatuur.

Curriculum vitae



Nani Maryani was born on July 29th 1983 in Tangerang, Indonesia and received her primary education in Jakarta. She continued her education at Bogor Agricultural University (IPB) and graduated (2005) in Biology with a specialization in Mycology and a BSc thesis on the formulation of *Acremonium* infected *Aquilaria* plants to produce Agarwood (Gaharu), under the supervision of Dr. Gayuh Rahayu.

After graduation, she joined the Laboratory of Mycology, department of Biology, IPB, and continued the *Acremonium* research. In 2006, she worked as a biology teacher for high school students at the School of Universe, Bogor. In 2007, she took a short training on Teaching and Pedagogy at the University of Ibn Khaldun, Bogor. In 2008, she joined the department of Biology Education, Faculty of Education and Teacher Training, University of Sultan Ageng Tirtayasa (UNTIRTA), Banten, Indonesia. In 2010, she commenced a Master course in Microbiology at IPB and in 2011 she continued her Master 2 Research at the *Institute Nationale des Science Appliqués* (INSA) in Toulouse, France. She studied the phenotypic variation of *Xanthomonas campestris* pv. *campestris*, the causal agent of Black Rot disease of cabbage, for her MSc thesis in the group of Prof. Matthew Arlat (LIPM INRA Toulouse).

By the end of 2013, she continued her study as a PhD at Wageningen University and Research in the Scientific Program Indonesia Netherlands of the Royal Netherlands Academy of Arts and Sciences (KNAW-SPIN), under the supervision of Prof. Gert Kema. The results of her project on exploring Fusarium wilt pathogens of banana in Indonesia are presented in this book. She will continue her career as a researcher and lecturer in the department of Biology Education, UNTIRTA, Banten, Indonesia and hopes to continue her discovery research on *Fusarium* and banana in Indonesia.

List of Publications

Maryani N., Lombard L., Poerba Y.S., Subandiyah S., Crous P.W., Kema G.H.J. (2019). Phylogeny and genetic diversity of the banana Fusarium wilt pathogen *Fusarium oxysporum* f. sp. *cubense* in the Indonesian centre of origin. *Studies in Mycology* 92: 155–194. https://doi.org/10.1016/j.simyco.2018.06.003.

Maryani N., Sandoval-Dennis M., Lombard L., Crous P.W., Kema G.H.J. (2019). New endemic *Fusarium* species hitchhiking with pathogenic *Fusarium* strains causing Panama disease in small-holder banana plots in Indonesia. *Submitted*

Maryani N., Seidl M.F., Crous P.W., Kema G.H.J. (2019). Genotyping-by-sequencing reveals extensive genotypic diversity among sympatric Fusarium wilt pathogens of banana in Indonesia. *Submitted*

Maryani N., Ahmad F., Keizer L.C.P., Crous P.W., Kema G.H.J. (2019). Pathogenic diversity of Indonesian Fusarium wilt pathogens in wild and cultivated bananas. *In submission.*

In books of abstracts

Maryani N., Ahmad F., Schmidt S.M., Lombard L., Poerba Y.S., Crous P.W., Kema G.H.J. (2015). Diversity of *Fusarium oxysporum* f. sp. *cubence* isolated from local banana cultivars in Indonesia. In Book of abstracts of 28th Fungal Genetics Conference, 17-22 March 2015, Pacific Grove, CA, USA.

Maryani N., Lombard L., Poerba Y.S., Subandiyah S., Crous P.W., Kema G.H.J. (2016). Phylogenetic diversity of *Fusarium oxysporum* f. sp. *cubense* in Indonesia. In Book of abstracts of 13th European Conference on Fungal Genetics (ECFG13), 3-6 April 2016, Paris, France.

Maryani N., Seidl M.F., Meijer H.J.G., Poerba Y.S., Subandiyah S., Crous P.W., Kema G.H.J. (2016). *Fusarium oxysporum* f. sp. *cubense* in Indonesia: Diversity and Pathogenicity. In Book of abstracts of Wageningen Indonesian Scientific Exposure (WISE), 28 October 2016, Wageningen, NL.

Maryani N., Seidl M.F, Meijer H.J.G., Kema G.H.J. (2018). Genetic variation of Indonesian *Fusarium oxysporum* f. sp. *cubense* isolates and their pathogenicity on wild and cultivated banana species. In Book of abstracts of 14th European Conference on Fungal Genetics (ECFG), 25-28 February 2018, Haifa, Israel.

Education Statement of the Graduate School
Experimental Plant Sciences

Issued to: Nani Maryani
Date: 29 October 2018
Group: Laboratory of Phytopathology
University: Wageningen University & Research

1) Start-up phase	date
► **First presentation of your project**	
Genome wide diversity analyses of Fusarium oxysporum f.sp. cubense in Indonesia	31 Mar 2014
► **Writing or rewriting a project proposal**	
Genome wide diversity analyses of Fusarium oxysporum f.sp. cubense in Indonesia	31 May 2014
► **Writing a review or book chapter**	
► **MSc courses**	
► **Laboratory use of isotopes**	
Subtotal Start-up Phase	*7.5 **

2) Scientific Exposure	date
► **EPS PhD student days**	
EPS PhD student day 'Get2Gether', Soest, NL	29-30 Jan 2015
EPS PhD student day 'Get2Gether', Soest, NL	09-10 Feb 2017
► **EPS theme symposia**	
EPS Theme 4 Symposium 'Genome Biology', Wageningen, NL	13 Dec 2013
EPS Theme 2 Symposium 'Interaction between plants and biotic agents' & Willie Commelin Scholten Day, Amsterdam, NL	25 Feb 2014
EPS Theme 4 Symposium 'Genome Biology', Wageningen, NL	03 Dec 2014
EPS Theme 2 Symposium 'Interactions between plants and biotic agents' & Willie Commelin Scholten Day, Leiden, NL	22 Jan 2016
EPS Theme 2 Symposium 'Interactions between plants and biotic agents' & Willie Commelin Scholten Day, Wageningen, NL	23 Jan 2017
EPS Theme 2 Symposium 'Interactions between plants and biotic agents' & Willie Commelin Scholten Day, Amsterdam, NL	24 Jan 2018
EPS Theme 1 Symposium 'Developmental Biology of Plants', Wageningen, NL	30 Jan 2018
► **National meetings (e.g. Lunteren days) and other National Platforms**	
Annual meeting 'Experimental Plant Sciences', Lunteren, NL	14-15 Apr 2014
Annual meeting 'Experimental Plant Sciences', Lunteren, NL	13-14 Apr 2015
Annual meeting 'Experimental Plant Sciences', Lunteren, NL	11-12 Apr 2016
► **Seminars (series), workshops and symposia**	
CBS Symposium '2nd International Workshop on Ascomycetes Systematics', Amsterdam, NL	22-24 Apr 2015
CBS Symposium 'Fungi and Global Challenges', Amsterdam, NL	14-15 Apr 2016
Wageningen PhD Council PhD Symposium 'Diversity in Science', Wageningen, NL	26 Apr 2016
31st KNPV Fusarium Meeting, Utrecht, NL	26 Oct 2016
Wageningen Indonesian Scientific Exposure (WISE), Wageningen, NL	28 Oct 2016
Westerdijk Institute Symposium 'Cryptic Speciation in Classifications', Utrecht, NL	01 Sep 2017
► **Seminar plus**	
► **International symposia and congresses**	
Indonesian Student Scientific Conference (ISSC2014), Wageningen, NL	22 Nov 2014
28th Fungal Genetics Conference, Pacific Grove, CA, USA	17-22 Mar 2015
13th European Conference on Fungal Genetics (ECFG13), Paris, France	03-06 Apr 2016
14th European Conference on Fungal Genetics (ECFG14), Haifa, Israel	25-28 Feb 2018
► **Presentations**	
Talk: Indonesian Scientific Students Conference (ISSC2014), Wageningen, NL	22 Nov 2014
Talk: International Conference on Sustainable Agriculture and Natural Resources Management, Surabaya, Indonesia	06-07 Aug 2015
Talk: EPS Theme 2 Symposium & Willie Commelin Scholten Day, Leiden, NL	22 Jan 2016
Talk: CBS Symposium 'Fungi and Global Challenges', Amsterdam, NL	14 Apr 2016
Talk: 31st KNPV Fusarium Meeting, Utrecht, NL	26 Oct 2016
Talk: Westerdijk Institute Symposium 'Cryptic Speciation in Classifications', Utrecht, NL	01 Sep 2017
Talk: 14th European Conference on Fungal Genetics (ECFG14), Haifa, Israel	28 Feb 2018
Poster: Wageningen Banana Day, Wageningen, NL	18 Nov 2014
Poster: 28th Fungal Genetics Conference, Pacific Grove, CA, USA	17-22 Mar 2015
Poster: 13th European Conference on Fungal Genetics, Paris, France	03-06 Apr 2016
Poster: Wageningen Indonesian Scientific Expoosure (WISE), Wageningen, NL	28 Oct 2016
► **IAB interview**	
► **Excursions**	
PhD Excursion, visit to breeding company Enza Zaden, Enkhuizen, NL	12 Jun 2015
PhD Excursion, visit to company Tomato World, Honselersdijk, NL	14 Oct 2016
Subtotal Scientific Exposure	*22.9 **

3) In-Depth Studies	date
► **EPS courses or other PhD courses**	
Course 'Fungal Biodiversity', Utrecht, NL	03-14 Feb 2014
Postgraduate course 'Introduction to R for Statistical Analysis', Wageningen, NL	19-20 May 2014
Spring School 'Host-Microbe Interatomics', Wageningen, NL	02-04 Jun 2014
COMREC Bioinformatics Course, Wageningen, NL	04-06 Feb 2015
Postgraduate course 'Genome Assembly', Wageningen, NL	28-29 Apr 2015
Graduate course 'Phylogenetics: Principles & Methods', Wageningen, NL	17-19 May 2016
Plant Pathogenomics Training School of the COST Action SUSTAIN, Norwich, UK	03-07 Apr 2017
► **Journal club**	
► **Individual research training**	
Subtotal In-Depth Studies	*8.5 **

4) Personal development	date
► **Skill training courses**	
Course 'Social Dutch 1 - level A1', Wageningen, NL	Feb-Mar 2014
Course 'Project and Time management', Wageningen, NL	Mar-Apr 2014
PhD Competence Assessment, Wageningen, NL	21 Jan 2014
Course 'Techniques for Writing and Presenting a Scientific Paper, Wageningen, NL	07-11 April 2014
EPS Introduction Course, Wageningen, NL	20 Jan 2015
Course 'Information Literacy PhD including End-Note Introduction', Wageningen, NL	17-18 Feb 2015
Course 'Scientific Writing', Wageningen, NL	Oct-Nov 2015
Workshop 'Reviewing a Scientific Paper', Wageningen NL	17 Sep 2015
Last stretch of your PhD, Wageningen, NL	30 Sep 2016
Adobe InDesign Essential Training, Wageningen, NL	07-08 Jun 2017
► **Organisation of PhD students day, course or conference**	
► **Membership of Board, Committee or PhD council**	
Subtotal Personal Development	*8.7 **

TOTAL NUMBER OF CREDIT POINTS	***47.6 ****

Herewith the Graduate School declares that the PhD candidate has complied with the educational requirements set by the Educational Committee of EPS which comprises of a minimum total of 30 ECTS credits.

** A credit represents a normative study load of 28 hours of study.*

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