



UNIVERSITI PUTRA MALAYSIA

***CHARACTERIZATION AND PATHOGENICITY OF *Fusarium* SPECIES
COMPLEX ASSOCIATED WITH *Fusarium* WILT OF BANANA IN
MALAYSIA***

ANYSIA HEDY BINTI UJAT

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MALAYSIA**

By

ANYSIA HEDY BINTI UJAT

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Master of
Science**

February 2021

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

CHARACTERIZATION AND PATHOGENICITY OF *Fusarium* SPECIES COMPLEX ASSOCIATED WITH *Fusarium* WILT OF BANANA IN MALAYSIA

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ANYSIA HEDY BINTI UJAT

February 2021

Chairman : Dzarifah binti Mohamed Zulperi, PhD
Faculty : Agriculture

The emergence of the *Fusarium* wilt of banana (Panama disease) caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Fusarium odoratissimum*) which caused loss to the global banana production is known to infect majority banana cultivar. A total of 38 isolates of *Fusarium spp.* related to banana *Fusarium* wilt obtained from the Biological Control Laboratory, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia were cultured on different media for observation of culture characteristics. Based on the culture morphology, isolates are grouped into four different group and a representative isolate were chosen for each group, namely Prk 4-1, Ns 5-2, Phg 3 and Swk 4. Micromorphology shows no significant difference in the macro- and microconidia size. To further identify the isolates, molecular analysis was conducted by using the sequences of Histone H3 gene and TEF-1 α gene. TR4 specific primers were used to confirm the presence of *Fusarium odoratissimum* (TR4). The phylogenetic tree using Histone H3 sequences showed all isolates grouped into *Fusarium oxysporum* species complex while phylogenetic tree using TEF-1 α sequences revealed that the samples were composed of 27 isolates of *Fusarium odoratissimum* (TR4), two isolate *Fusarium gros-michelii* (R1), six unknown *Fusarium oxysporum* species complexes isolates and three *Fusarium fujikuroi* isolates. By using TR4 specific primers, all 27 samples were confirmed to be *Fusarium odoratissimum* (TR4). To study the susceptibility of local banana cultivar, pathogenicity test was conducted on 3 months old banana plantlets consisting of five different local banana cultivars of three different genome types: *Musa acuminata* AAA (Pisang Berangan and Pisang Serendah), *Musa paradisiaca* AAB (Pisang Nangka and Pisang Raja) and *Musa balbisiana* BBB (Pisang Nipah). Observation was recorded for 30 days, and foliar symptoms showed different

severity of disease progression. However, internal symptoms showed that all of the inoculated plantlets were severely infected. Cooking banana (AAB and BBB) of local cultivar were also found to be susceptible to *Fusarium odoratissimum* (TR4). This study shows that there are not only *Fusarium odoratissimum* (TR4) presence in Malaysia but also *Fusarium gros-michelii* (R1) and a possibility of an unknown strain of *Fusarium oxysporum*. Pathogenicity test reveals that local banana cultivar in Malaysia are susceptible to *Fusarium* wilt disease regardless of the genotype of the banana plants. These findings will aid in the substantive documentation of *Fusarium spp.* in the local banana plantation scenario.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

**PENCIRIAN DAN KEPATOGENAN KOMPLEKS SPESIS *Fusarium*
BERKAITAN DENGAN PENYAKIT LAYU *Fusarium* POKOK PISANG DI
MALAYSIA**

Oleh

ANYSIA HEDY BINTI UJAT

Februari 2021

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Kemunculan penyakit layu *Fusarium* pokok pisang (Penyakit Panama) disebabkan oleh *Fusarium oxysporum* f. sp. *cubense* Tropical 4 (*Fusarium odoratissimum*) yang telah menyebabkan kerugian dalam pengeluaran pisang global menjangkiti majoriti kultivar pisang. Sebanyak 38 pencilan *Fusarium spp.* diperoleh dari Makmal Kawalan Biologi, Jabatan Perlindungan Tumbuhan, Fakulti Pertanian, Universiti Putra Malaysia telah dikultur di atas media yang berbeza untuk pemerhatian ciri - ciri kultur. Berdasarkan morfologi kultur, pencilan diasingkan kepada empat kumpulan yang berlainan dan wakil pencilan dipilih untuk setiap kumpulan, iaitu PRK 4-1, NS 5-2, Phg 3 dan Swk 4. Mikromorfologi menunjukkan tiada perbezaan yang ketara dalam saiz makro- dan mikrokonidia. Untuk pengenalan lanjutan pencilan, analisis molecular dilakukan dengan menggunakan jujukan gen Histone H3 dan gen TEF-1 α . Primer spesifik TR4 telah digunakan untuk mengesahkan identiti *Fusarium odoratissimum*. Pohon filogenetik yang menggunakan jujukan Histone H3 menunjukkan kesemua pencilan dikelompokkan ke dalam spesies complex *Fusarium oxysporum*, manakala pohon filogenetik menggunakan jujukan TEF-1 α menunjukkan bahawa pencilan terdiri daripada 27 *Fusarium odoratissimum*, dua pencilan *Fusarium grosnichelii*, enam pencilan merupakan spesies *Fusarium oxysporum* yang tidak diketahui, dan tiga pencilan *Fusarium fujikuroi*. Dengan menggunakan primer spesifik TR4, kesemua 27 pencilan telah disahkan sebagai *Fusarium odoratissimum*. Untuk mengkaji kerentanan kultivar pisang tempatan, ujian kepatogenan dilakukan pada tanaman pisang berusia 3 bulan yang terdiri daripada lima kultivar pisang tempatan yang berlainan dari tiga jenis genom berbeza; *Musa acuminata* AAA (Pisang Berangan dan Pisang Serendah), *Musa paradisiaca* AAB (Pisang Nangka dan Pisang Raja) dan *Musa balbisiana* BBB

(Pisang Nipah). Pemerhatian dicatatkan selama 30 hari dan gejala daun menunjukkan tahap keparahan perkembangan penyakit yang berbeza. Walau bagaimanapun, gejala dalaman menunjukkan bahawa semua tanaman yang diinokulasi telah dijangkiti dengan teruk. Kultivar tempatan pisang masak (AAB dan BBB) juga didapati rentan terhadap *Fusarium odoratissimum* (TR4). Kajian ini menunjukkan bahawa bukan sahaja *Fusarium odoratissimum* (TR4) terdapat di Malaysia tetapi juga *Fusarium grosmichelii* (R1) dan kemungkinan *Fusarium oxysporum* yang tidak diketahui. Ujian kepatogenan menunjukkan bahawa kultivar pisang tempatan di Malaysia rentan terhadap penyakit layu Fusarium tanpa mengira genotip tanaman pisang. Penemuan ini akan membantu dalam dokumentasi substantif *Fusarium spp.* dalam senario penanaman pisang tempatan.



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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

°C	degree Celsius
μL	microlitre
μm	micrometre
ANOVA	Analysis of Variance
bp	base pair
CTAB	Cetyl Trimethyl Ammonium Bromide
DAI	Days after inoculation
ddH ₂ O	double distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agricultural Organisation of the United Nation
GTRGAMMAI	Genera Time Reversible Gamma Inverse
ha	hectare
LAMP	Loop-Mediated Isothermal Amplification
MEGA	Molecular Evolutionary Genetics Analysis
MgCl ₂	Magnesium chloride
ML	Maximum-likelihood
NaCl	Sodium Chloride

PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PP	Posterior probability
RAPD	Random Amplified Polymorphic DNA
RAxML	Randomize Axelerated Maximum-likelihood
RFLP	Restriction Fragment Length Polymorphism
Rnase	Ribonuclease
rpm	rotation per minute
SNA	Spezieller Nährstoffarmer Agar
SNP	Single Nucleotide Polymorphism
<i>spp.</i>	species

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Fusarium oxysporum (*F. oxysporum*) has been identified in native soil from many parts of the world; However, it is not pathogenic to native plant communities (Gordon, 2017). *Fusarium oxysporum* appears to be host specific therefore subdivision *formae specialis* (f. sp.) is being used to reflect plant pathogenic specialisation (Leslie and Summerell, 2006). *F. oxysporum* comprising over 120 *formae specialis* according to their host plant and under each *formae specialis* can be subdivided into physiological race depending on the virulence pattern of host varieties (Agrios, 2004). The host of *F. oxysporum* extends from vegetables such as tomato; *F. oxysporum* f. sp. *lycopersici*, fruits, for example strawberry *F. oxysporum* f. sp. *fragariae*, and even flowers like carnation; *F. oxysporum* f. sp. *dianthii* (Agrios, 2004). *Fusarium oxysporum* f. sp. *cubense* infects banana plant, *Musa* spp. and its family plant, *Heliconia* spp. (Ploetz, 2015a).

Banana *Fusarium* wilt is historically known as a very destructive disease as the Gros-Michel cultivar is not planted anymore due to this disease (Ploetz, 2015b, 2015a). Since the discovery of the disease in the Panama, multiple ways have been used to control and eradicate the disease; however, most of the effort were futile as it could not fully control the spread of the disease. To curb the spread of this disease, a non-susceptible variety of banana, the Cavendish variety was planted to replace the Gros Michel variety. However, a more virulence race of the *F. oxysporum* f. sp. *cubense* arise was reported to attack Cavendish banana plantation across Asia in the 1990s, first detected in Taiwan (Su et al., 1986) raising the concern of global banana producing countries and scientist as the fate of the banana plant could succumb to this disease (Smith et al., 2006).

1.2 Problem Statement

Fusarium wilt of banana is a disease induced by the pathogenic fungus, *Fusarium oxysporum* f. sp. *cubense* (Foc). Among the most significant impact it causes is the economic loss when Foc race 1 it first attacked the “Gros-Michel” plantation. In the early 1990s, the Cavendish variety was susceptible to the attack of the Tropical Race 4 (TR4) of the same fungus. Since then, the disease once again spread across Southeast Asia, especially in Malaysia, Indonesia, Philippines, China, and Taiwan and also in the Northern Territory of Australia (Ploetz, 2015b). In recent years, there are more reports of the attack of Foc TR4 outside

Southeast Asia (Ordoñez et al., 2015; Hung et al., 2017; Maymon et al., 2018; García-Bastidas et al., 2019a).

To manage the spread of this disease, an understanding of the diversity and variation of the pathogenic fungus *Fusarium oxysporum* f. sp. *cubense* is very important. The knowledge behind the diversity of the pathogen and susceptibility of cultivar would help to give insight to controlling the spreading of the disease.

Recent study on the susceptibility of the banana plant is only centered at the commercial banana cultivar, the cavendish cultivar; and in a local scenario, the Berangan cultivar, there are no study on the susceptibility of other local cultivar. As the centre of diversity of the banana plant itself, study on the susceptibility the local banana cultivar against the fungus that cause massive loss to the local banana plantation should also be assessed and address.

1.3 Significant of Study

This study aims to contribute to the biological insight and diversity of *Fusarium oxysporum* f. sp. *cubense* in Malaysia and the susceptibility of local banana cultivar, thus help in controlling the disease prevalent in the country. This study is an important development of sustainable management strategy of *Fusarium* wilt of banana in Malaysia.

1.4 Objectives of Study

The aim of this study was to develop an understanding in the diversity of *Fusarium* spp. in the local plantation and to identify the susceptibility of the local cultivar. Thus, the objectives were.

1. To investigate the genetic diversity of *Fusarium* species complex causing *Fusarium* wilt on banana in Malaysia via phylogenetic analysis of partial sequence of Histone (H3) gene and translation elongation factor 1 alpha (TEF-1 α) gene.
2. To evaluate pathogenicity of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 1 on selected local banana cultivars in Malaysia.

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BIODATA OF STUDENT

Anysia Hedy binti Ujat was born on 17 October 1994 in Bintulu. She completed her formal education with Sijil Pelajaran Malaysia from Sekolah Menengah Bandar Bintulu. In 2012, she pursues tertiary education in Life Science Foundation Programme in Universiti Malaysia Sarawak (UNIMAS). Upon completing her foundation programme, she continues her undergraduate study in UNIMAS in Bachelor of Science (Resource Biotechnology) and graduated in 2016.

In 2017, she was awarded with Sarawak State Bursary Scholarship and continues to pursue her interest in research by enrolling as Master of Science (Plant Pathology) candidate in Universiti Putra Malaysia (UPM) as full-time student under the supervision of Dr Dzarifah Zulperi and Associate Professor Ganesan Vadamalai.

LIST OF PUBLICATION

Journal

Ujat, A. H., Vadamalai, G., Hattori, Y., Nakashima, C., Wong, C. K. F., & Zulperi, D. (2021). Current Classification and Diversity of *Fusarium* Species Complex, the Causal Pathogen of Fusarium Wilt Disease of Banana in Malaysia. *Agronomy*, 11(10), 1955.

Poster and Proceedings

1. Ujat, A. H., Hattori, Y., Nakashima, C., Wong, C. K. F., and Zulperi, D. (2019). Diversity of *Fusarium* Species Infecting Banana in Malaysia. Asian Mycological Congress, Tsu, Mie, Japan.
2. Ujat, A. H., Hattori, Y., Nakashima, C., Wong, C. K. F., and Zulperi, D. (2019). *Fusarium* Wilt of local Banana Variety in Malaysia. International Society for Southeast Asian Agricultural Sciences, UPM, Serdang, Malaysia.

Awards and Honours

1. Tun Taib Scholarship by Yayasan Sarawak

GenBank:

Deposited sequence:

1. His H3 gene (GenBank accession nos: LC545766.1 to LC545798.1)
2. TEF-1 α gene (GenBank accession nos: LC545799.1 to LC545836.1)