



UNIVERSITI PUTRA MALAYSIA

***ETIOLOGY OF JACKFRUIT (ARTOCARPUS HETEROPHYLLUS LAM.)
BRONZING DISEASE IN PENINSULAR MALAYSIA***

GAN HWEE YIAM

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**ETIOLOGY OF JACKFRUIT (*Artocarpus heterophyllus* Lam.) BRONZING
DISEASE IN PENINSULAR MALAYSIA**

By

GAN HWEE YIAM

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

November 2017

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DEDICATION

Dedicated to my beloved husband, son, parents, siblings, in laws, supervisors and fellow friends for their endless love, support, understandings, sacrifices, motivation, advice and encouragement.



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UPM

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

ETIOLOGY OF JACKFRUIT (*Artocarpus heterophyllus* Lam.) BRONZING DISEASE IN PENINSULAR MALAYSIA

By

GAN HWEE YIAM

November 2017

Chairman : Associate Professor Wong Mui-Yun, PhD
Faculty : Agriculture

Jackfruit (*Artocarpus heterophyllus* Lam.) is a popular tropical fruit cultivated in Malaysia and is classified under the family of Moraceae. Unfortunately, jackfruit has been found susceptible to the damage bronzing disease since 2008, which can be visualised from the rust coloured specks or streaks appearing on the pulps, rags and seed membrane of the fruit. This predicament has extended to causing the bulbs bear an unpleasant taste and appears asymptomatic from the outer fruit surface. Many possibilities on the potential causative factors have been brought up by several research agencies. Not at least, an important report on similar disease symptoms was described and concluded that the bacteria, namely *Pantoea stewartii* to have caused the bronzing disease in the Philippines recently. At present, research approaches to identify the causal agents and the effect of the disease on jackfruit are still scanty. Thus, the purpose of this study was to identify the biotic causal agent of the jackfruit bronzing disease from samples collected in Peninsular Malaysia using morphological, biochemical and molecular identification, (ii) to conduct pathogenicity test of the suspected causal agents on the susceptible variety 'Tekam Yellow' (J33) and (iii) to elucidate the infection process of jackfruit bronzing by histological examination of infected tissue using scanning electron microscope (SEM). For disease incidence (DI) survey, a total of 59 jackfruits were sampled randomly from five states in Peninsular Malaysia i.e. Perak, N. Sembilan, Pahang, Johor and Selangor in this study. The result showed that the disease incident (DI) of fruit bronzing on jackfruit was high, ranging from at least 20% to 70%. For isolation and identification, a total of 15 samples of fruit with bronzing symptoms were collected from 8 locations in three states in Malaysia. Total of 120 bacteria colonies with dark yellow pigment were observed during isolation plating of all samples. Morphologically, this bacterium is Gram

negative, oxidase negative, appeared as a non-capsulated straight rod with an average of 0.35 (width) x 1.31 (length) μm , facultative anaerobic short-rod. BIOLOG GEN III has identified 26 isolates as *Pantoea* sp. while the molecular data, produced through DNA sequence analysis on the two selected 16S rRNA gene universal primer (B27F and U1492R) using Basic Local Alignment Search Tool (BLAST) of which all the 15 isolates as *P. stewartii* and 1 isolates as *P. dispersa* which showed 99-100% similarity. However, from established phylogenetic tree fifteen isolates were grouped in the same cluster with *P. stewartii* and distinct from *P. ananatis* and *P. dispersa*. The result has further clarified that the isolate that caused fruit bronzing is *P. stewartii*. From pathogenicity test, the two selected *P. stewartii* isolates UPM 001 and *P. stewartii* isolates UPM 002 were confirmed to produce the same type of symptom as observed in the field samples. The pathogens were successfully re-isolated from the bronzing symptom of inoculated parts like rags and bulbs, thus fulfilling Koch's postulates and confirmed that *P. stewartii* as a jackfruit pathogen in Malaysia. Besides, natural opening such as stomata was foreseen to be the possible pathway taken by *P. stewartii* for the intrusion into the fruit.

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

**ETIOLOGI PENYAKIT KARAT NANGKA (*Artocarpus heterophyllus* Lam.)
DI SEMENANJUNG MALAYSIA**

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Nangka (*Artocarpus heterophyllus* Lam.) adalah buah-buahan tropika yang popular di Malaysia dan dikelaskan di bawah keluarga Moraceae. Pengeluaran buah nangka mempunyai potensi komersial yang tinggi untuk pasaran domestik dan juga sebagai laluan pasaran antarabangsa atas peningkatan permintaannya di seluruh dunia. Malangnya, nangka didapati mudah terdedah kepada kerosakan dengan tanda-tanda “karat” atau pemerangan pada bahagian pulpa, jerami and serabut biji nangka sejak tahun 2008. Ini menyebabkan rasa ulas nangka menjadi kurang menyenangkan dan ia sukar dikesani daripada permukaan luar buah. Penyakit pemerangan buah nangka yang disebabkan oleh *Pantoea stewartii* telah dilaporkan di Filipina baru-baru ini. Simptom penyakit pemerangan yang sama juga dikesan di Malaysia tetapi belum ada penyelidikan dijalankan untuk mengenalpasti agen penyebab dan kesan penyakit ini pada nangka. Objektif kajian ini adalah untuk mengenalpasti agen penyebab biotik penyakit pemerangan nangka daripada sampel di Semenanjung Malaysia secara morfologi, biokimia dan molekul, menjalankan ujian kepatogenan agen penyebab yang disyaki terhadap varieti *Tekam Yellow* (J33) dan mengkaji proses jangkitan penyakit pemerangan secara histologi tisu buah menggunakan mikroskop elektron pengimbasan (SEM). Kajian ini mendapati bahawa insiden penyakit (DI) pemerangan buah nangka adalah tinggi, iaitu 20% hingga 70% daripada 59 biji buah nangka yang persampelan secara random dari 5 negeri (Perak, N. Sembilan, Pahang, Selangor dan Johor). Sejumlah 15 sampel buah yang bersimptom pemerangan telah dikumpulkan dari 8 lokasi daripada tiga negeri di Malaysia. Sebanyak 120 koloni bakteria dengan pigmen berwarna kuning gelap diperhatikan semasa pemencilan daripada semua sampel. Bakteria ini tergolong dalam Gram negatif, negatif oksidase, rod lurus dengan purata saiz 0.35 (lebar) x 1.31 (panjang) μm , rod

pendek anaerobik fakultatif. BIOLOG GEN III mengenalpastikan 26 isolat sebagai *Pantoea* sp.. Pengenalpastian mengenai spesies melalui analisa jujuran dan menggunakan BLAST menunjukkan bahawa kesemua 15 isolat sebagai *P. stewartii* and 1 isolat sebagai *P. dispersa* serta menunjukkan 99-100% persamaan. Pohon Phylogenetik yang mengelompokkan 15 isolat dalam kluster sama dengan *P. stewartii* dan berbeza dari *P. ananatis* dan *P. dispersa* membuktikan bahawa *P. stewartii* yang menyebabkan penyakit pemerangan buah. Daripada ujian kepatogenan, simptom yang sama diperhatikan pada buah yang diinokulasi. Terdapat 2 isolat iaitu UPM 001 dan UPM 002 berjaya dipencilkan semula daripada bahagian jerami dan ulas yang memerang dengan patogen yang sama. Oleh itu, kajian ini telah memenuhi Postulat Koch dengan mengesahkan bahawa *P. stewartii* sebagai patogen karat angka di Malaysia. Selain itu, sel *P. stewartii* adalah dijumpai pada dan sekeliling stomata di bawah pemerhatian SEM.

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I certify that a Thesis Examination Committee has met on 20 November 2017 to conduct the final examination of Gan Hwee Yiam on her thesis entitled "Etiology of Jackfruit (*Artocarpus heterophyllus* Lam.) Bronzing Disease in Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

DOA	Department of Agriculture
MP	Minimum Processed
bp	Base pair
CTAB	N-cetyl-N, N, N-trimethyl-ammonium bromide
dNTP	Mixture of deoxynucleoside-triphosphates in equimolar amounts
DNA	Deoxyribonucleic acid
EtBr	Ethidium bromide
M	Molar
rpm	rotation per minute
UV	ultraviolet light
SEM	Scanning Electron Microscope
PDA	Potato Dextrose Agar
NA	Nutrient Agar
NB	Nutrient Broth
TAE	Tris-acetate EDTA
PCR	Polymerase Chain Reaction
kb	kilo base

CHAPTER 1

INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* Lam.) is a type of tropical plant belonged to the family of Moraceae and locally known as “Nangka” in Malaysia. The jackfruit tree bears the world largest fruit which is composite with many starchy seeds enclosed in each of the sweet, aromatic, crispy and edible pulp within the fruit. It can easily distinguish by its short spiny, thick and green to brownish yellow skin and presence of sticky white sap in all part of the plants (Tahir and Jamaluddin, 2008).

Besides being a top preference fruit to Malaysian consumers, jackfruit is an indigenous fruit possesses with great commercial potential. It is a high yielding crop which is non-seasonal, with peak production during the months of June and December (Othman and Subhadrabandhu, 1995). In consequence, jackfruit was identified as one of the high-value non seasonal tropical fruits to be promoted for exportation to Middle East and European countries under the Malaysian Agriculture National Key Economic Areas (NKEA) in year 2013 (“ETP”, 2014). Since then, the production of jackfruits in Malaysia for overseas market has increased tremendously.

Although *A. heterophyllus* is considered as a good commercial crop, new disease, namely jackfruit bronzing disease, with undecided causal agents was reported recently and has affected the production in the country. The productivity constraint of jackfruits which could due to failure of the crop combating biotic and abiotic stress was found worsen by poor farm maintenance and labour force insufficiency.

The first symptom of jackfruit bronzing has been observed at Bukit Goh, Kuantan, Pahang, with affected part in pulps, rags and seed membrane of the fruits, in year 2008. The disease is characterized by yellowish orange bronzing at the initial stage or rust coloured specks on affected parts at all development stages of fruit. This phenomenon causes the pulps to have an unpleasant taste and the fruits appear asymptomatic on the outer surface of fruits. However, similar bronzing symptoms on jackfruits was also reported by Gapasin *et al.* (2014) from Philippines in a published a report.

Research on jackfruit bronzing disease in Malaysia is still of paucity and it is still in its infancy of disease identification which at present is determined simply based on symptoms and simple bacterial culture or colony characterization. Many view of points and conclusion have been contributed by several research agencies in elucidating the possible causal agents of the jackfruit bronzing occurrence.

Amongst them, Department of Agriculture in Malaysia (DOA, 2012) concluded the occurrence of the jackfruit bronzing disease besides caused by a combination of abiotic factors such as weather, soil types, terrain and plant nutrition which could affect the plant health, was also implicated by cosmopolitan fungus, *Rhizopus artocarp*i and latent fungus *Lasiodiplodia* sp. as two main biotic factors. This ubiquitous *R. artocarp*i is universally identified as a causal agent to fruit rot by infecting male flowers and fruits through injury. In the other hand, Malaysian Agricultural Research and Development Institute (MARDI) indicated the disease is caused by nutrient deficiency of iron, copper, and manganese. Federal Land Development Authority (FELDA) pointed out that physiological disorder should be the concern. Hence, an affirmed disease causative agent for jackfruit bronzing disease remained unresolved.

In view of these discrepancies, the objectives of this study were (i) to investigate the occurrence of jackfruit bronzing disease and identify the causal agent using morphological, biochemical and molecular characteristics, (ii) to conduct pathogenicity test on susceptible variety Tekam Yellow (J33), and (iii) to elucidate the infection process of jackfruit tissue bronzing by histological examination of tissue under scanning electron microscope (SEM).

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LIST OF PUBLICATIONS

Hwee-Yiam Gan; Siti Izera I., Mui-Yun, Wong (2017) Jackfruit Bronzing Disease in Malaysia in Proceeding Symposium of Biosecurity 2017, DOA.

Hwee-Yiam Gan; Zainal Abidin M. A.; Siti Izera I. and Mui-Yun Wong First Report of *Pantoea stewartii* as Pathogen Causing Jackfruit Bronzing Disease in Malaysia. (Submitted to *Plant Disease*; submission no.: PDIS-10-16-1538-PDN)

Hwee-Yiam Gan; Siti Izera I.; Yee-Min Kwan, and Mui-Yun Wong. *Pantoea stewartii*, the causal agent of jackfruit (*Artocarpus heterophyllus* Lam.) bronzing disease in Malaysia. (Will be submitted to *Plant Disease*)



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