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PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF Pantoea stewartii CAUSING BRONZING DISEASE IN MALAYSIAN JACKFRUIT (Artocarpus heterophyllus)

NORSYAHIDAH BINTI MANAF

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NORSYAHIDAH BINTI MANAF

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

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BY

NORSYAHIDAH BINTI MANAF

A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science

Department of Plant Protection

Faculty of Agriculture

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This project report entitled Phenotypic And Molecular Characterization of *Pantoea stewartii* Causing Bronzing Disease in Malaysian Jackfruit (*Artocarpus heterophyllus*) is prepared by Norsyahidah Binti Manaf and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

Student's name: Student's signature:

Norsyahidah Binti Manaf ……

Certified by:

…….

(Dr. Dzarifah Mohamed Zulperi)

Department of Plant Protection

Faculty of Agriculture

Universiti Putra Malaysia

Date:…….

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LIST OF ABBREVIATIONS

%	percent
°C	degree celcius
Bp	base pair
CABI	Commonwealth Agricultural Bureaux International
DNA	deoxyribonucleic acid
DOA	Department of Agriculture
EDTA	ethylene-diamine-tetraacetic acid
FAO	Food and Agriculture Organization
g	gram
h	hour
kb	kilobase pair
L	liter
M	molar
Mb	megabase pair
min	minutes
ml	milliliter
mm	milimeter
mM	milimolar
ng	nanogram
nm	nanometer
PCR	polymerase chain reaction
rpm	rotation per minute

sec	seconds
TAE	tris-acetic EDTA
Taq	*Thermus aquaticus*
TM	melting temperature
U	unit
UV	ultra-violet
V	voltan/volt
x g	gravity force
µg	microgram
µg/ml	microgram per mililiter
µl	microliter
µM	micromolar
µm	micrometer

PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF *Pantoea stewartii* CAUSING BRONZING DISEASE IN MALAYSIAN JACKFRUIT (*Artocarpus heterophyllus*)

NORSYAHIDAH BINTI MANAF (173165)

BACHELOR OF AGRICULTURE SCIENCE

SUPERVISOR: DR. DZARIFAH BINTI MOHAMED ZULPERI

Abstract

Jackfruit (*Artocarpus heterophyllus*) is one of the popular and the most widely grown fruit crop in Malaysia. Under the Third National Agricultural Policy, jackfruit has been recognized as having the potential to meet domestic demand, for export and as a replacement for imports. However, a disease known as fruit bronzing is frequently found infecting jackfruit. The disease is caused by *Pantoea stewartii* (*P. stewartii*), which is characterized by yellowish-orange to reddish discoloration of the affected pulps and rags in the fruit which can cause serious jackfruit losses. Once infected, the fruit quality is compromised and could discourage the consumers and processors. To date, no studies have been conducted on *P. stewartii* and fruit bronzing of jackfruit in Malaysia. The objectives of this study were; 1) to isolate and identify bacterial strains associated with bronzing disease in local jackfruit by using phenotypic characteristics and 2) to characterize genetic relationship of the bronzing-causing strains of jackfruit via molecular identification and phylogenetic analyses. In April 2016, symptoms of fruit bronzing in jackfruits were observed in Muadzam Shah jackfruit plantation of Pahang

state. To identify the pathogen, 10 diseased fruits resembled bronzing symptom were collected from two fields prone to the disease, excised and cultured onto potato dextrose agar (PDA) medium and incubated overnight at 37 ºC. Four representative bacterial colonies were obtained and selected for further characterization. Bacterial colonies were round, yellow-pigmented, smooth and translucent. All strains were Gram-negative short rod-shaped, positive for Kovacs oxidase and catalase tests, and negative for potassium hydroxide (KOH) reaction. These are characteristics of *Pantoea* spp. For the pathogenicity test, 10 ml of 10^8 CFU/ml bacterial suspension of four selected strains were injected into healthy detached jackfruits of J33 (Tekam Yellow) variety. Fruits that were infiltrated with sterile distilled water served as a negative control. Inoculation with all strains were performed in three replications, as well as the uninoculated control detached fruits. The inoculated fruits produced bronzing symptoms as observed on naturally diseased samples, whereas control fruits remained asymptomatic. Strain cultures were re-isolated and showed the morphological and biochemical characteristics as previously described. The PCR amplification of 16S gene using 27F and 1492R primer set produced a 1400 bp amplicon in each strain. Sequencing analysis revealed that all strains were 99% identical to *Pantoea stewartii* reference strain M073 (Accession no. KJ742705). Species-specific PCR with primers ES16 and ESIG2c showed all strains displaying a 920 bp amplicon. The sequence data were deposited in GenBank (Accession nos. KY195916 to KY195919). Phylogenetic analyses of the ES16/ESIG2c gene sequences clustered all strains into *Pantoea stewartii* subspecies (subsp.) *stewartii* DC283 reference strain (Accession no. AJ311838), with 100% posterior probability. To our knowledge, this is the first report of bronzing disease caused by *Pantoea stewartii* subsp. *stewartii* in jackfruit in Malaysia.

FENOTIP DAN PENCIRIAN MOLEKUL KE ATAS *Pantoea stewartii* YANG MENYEBABKAN PENYAKIT PEMERANGAN DALAM NANGKA MALAYSIA (*Artocarpus heterophyllus*)

NORSYAHIDAH BINTI MANAF (173165)
BACHELOR SAINS PERTANIAN
PENYELIA: DR. DZARIFAH BINTI MOHAMED ZULPERI

Abstrak

Nangka (*Artocarpus heterophyllus*) adalah salah satu tanaman buah-buahan yang popular dan paling banyak ditanam di Malaysia. Di bawah Dasar Pertanian Negara Ketiga, nangka telah diiktiraf sebagai tanaman yang mempunyai potensi untuk memenuhi permintaan dalam negeri, untuk eksport dan sebagai pengganti import. Bagaimanapun, satu penyakit yang dikenali sebagai pemerangan kerap ditemui didalam buah nangka. Penyakit ini disebabkan oleh *Pantoea stewartii* (*P. stewartii*), yang mempunyai ciri-ciri kuning-oren ke merah iaitu perubahan warna yang menjejaskan pulpa dan jerami didalam buah nangka dimana boleh menyebabkan nangka mengalami kerugian yang serius. Apabila buah nangka dijangkiti, ia boleh menjejaskan kualiti buah nangka dan penawaran terhadap buah nangka oleh pengguna dan pemproses. Setakat ini, tiada kajian yang telah dilakukan terhadap *P. Stewartii* dan pemerangan buah nangka di Malaysia. Objektif untuk kajian ini ialah; 1) untuk mengasingkan dan mengenal pasti strain bakteria yang dikaitkan dengan penyakit pemerangan dalam nangka tempatan dengan menggunakan ciri-ciri fenotip dan 2) untuk mencirikan hubungan genetik strain pemerangan penyebab nangka melalui pengenalan molekul dan analisis filogenetik. Pada

april 2016, gejala pemerangan dalam nangka telah diperhatikan di ladang nangka Muadzam Shah di negeri Pahang. Untuk mengenal pasti patogen, 10 biji nangka yang mempunyai penyakit yang menyerupai gejala pemerangan telah dikumpul dari dua tempat yang terdedah kepada penyakit ini, dipotong dan dibiakkan ke media potato dextrose agar (PDA) dan dieram semalaman pada 37 ºC. Empat koloni bakteria diperolehi dan dipilih untuk pencirian lanjut. Koloni bakteria berbentuk bulat, kuning-berpigmen, licin dan lut. Kesemua strain adalah Gram-negatif dengan bentuk rod pendek, positif untuk Kovac xidase dan catalase, dan negatif utuk untuk tindak balas kalium hidroksida (KOH). Ini adalah ciri-ciri *Pantoea* spp. Untuk ujian kepatogenan, 10 ml 10^8 CFU/ml empat jenis bakteria dipilih telah disuntik ke dalam nangka sihat jenis J33 (Tekam Yellow). Buah yang telah disuntik dengan air suling steril dijadikan sebagai kawalan negatif. Inokulasi dengan semua strain telah dijalankan dalam tiga replikasi, serta satu inokulasi buah kawalan. Buah yang disuntik menghasilkan gejala pemerangan sebagaimana yang berlaku ke atas sampel secara semula jadi berpenyakit, manakala buah-buahan kawalan kekal asimptomatik. Kultur strain telah diasingkan semula dan menunjukkan ciri-ciri morfologi dan biokimia seperti yang dinyatakan sebelum ini. PCR amplikasi 16S gen menggunakan set primer 27F dan 1492R menghasilkan 1400 bp amplikon dalam setiap strain. Analisis jujukan mendedahkan bahawa semua strain adalah 99% sama dengan rujukan *Pantoea stewartii* strain M073 (No. kesertaan KJ742705). Spesies-spesies PCR dengan primer ES16 dan ES1G2c menunjukkan semua strain mempamerkan 920 bp amplikon. Data jujukan telah disimpan di GenBank (No. kesertaan KY195916 kepada KY195919). Analisis filogenetik ES16/ES1G2c jujukan gen berkelompok semua strain kepada *Pantoea stewartii* subspecies (subsp.) *stewartii* DC283 rujukan strain (No. kesertaan AJ311838) dengan 100% kebarangkalian posterior.

Untuk pengetahuan kita, ini adalah laporan pertama penyakit pemerangan disebabkan oleh *Pantoea stewartii subsp. stewartii* dalam nangka di Malaysia.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Jackfruit (*Artocarpus heterophyllus*) is a non-seasonal fruit belongs to the family Moraceae (Haq, 2006). This fruit is native to the Western Ghats region of India (Sidhu, 2012). Jackfruit was later introduced to other countries such as Malaysia, Australia, Brazil, Indonesia and Vietnam (Haq, 2006). In Malaysia, the planted area for jackfruit has increased consistently from the year 2009. A suitable weather and best crop management can be influenced number of supply for this fruit. In 2013, the production of jackfruit reach to 32,907.4 tonnes with the planted area is 4,902.9 hectare (DOA, 2015).

There are several diseases of jackfruit and one of them is fruit bronzing. Fruit bronzing disease can give impact to jackfruit production since the disease invades the fruits that are on pulps and rags, and symptoms can only be seen when the fruit is opened. When the jackfruit has been invaded, the quality of fruits become decline and as well as the production (Gapasin *et al*., 2014).

1.2 Statement of the Problem

In Malaysia, the suspected outbreak of fruit bronzing disease of jackfruit in Malaysia was recognized in few plantation areas located in Pahang and Negeri Sembilan circa 2010 (unpublished data, DOA 2010). Jackfruit bronzing is a current major problem that leads to loss of production. Once infected, the fruit quality was compromised and could discourage the consumers and processors. The symptoms of fruit bronzing disease often prevent the sale as fresh market value (Gapasin *et al.*, 2014). As jackfruit has been recognized as one of the fruit types for special attention under the implemented Economic Transfer Programme (ETP) by Malaysian government, constant occurrences of this disease have been the most important and major constraint to the production of jackfruits since it could discourage the consumers and processors (Gapasin *et al*., 2014; DOA, 2013).

1.3 Significance of the Study

Jackfruit (*Artocarpus heterophyllus*) remains the important economic-driven fruit crops in Malaysia for both local and export markets, scrutinizing records on the status of fruit bronzing disease is of significant importance. Up to this point, none of the disease occurrences have been well documented in Malaysia. The results of our study will be an important pioneer documentation of bronzing disease of jackfruit in Malaysia. Considering this matter, our study would be a major platform on generating details documentation of bronzing disease and its causal pathogen *Pantoea stewartii* in jackfruit

crops in Malaysia, by using combination of phenotypic characterization and molecular phylogenomics approaches.

1.4 Objectives of the Study

Our study was carried out with the following objectives:

1. To isolate and identify bacterial strains associated with fruit bronzing disease in local jackfruit by using phenotypic characteristics.

2. To characterize the genetic relationship of the fruit bronzing-causing strains of jackfruit via molecular identification and phylogenetic analyses.

The output from this research perhaps may improve and increase efficiency in the development of accurate molecular diagnostic tests for detection and identification of *Pantoea stewarti*, thus bettering the jackfruit industry in Malaysia.

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