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MORPHOLOGY IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF Lasiodiplodia theobromae CAUSING FRUIT ROT DISEASE OF GUAVA (Psidium guajava L.) IN MALAYSIA

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Morphology Identification and Molecular Characterization of *Lasiodiplodia theobromae* Causing Fruit Rot Disease of Guava (*Psidium guajava* L.) in Malaysia

By

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CERTIFICATION

This project paper entitled, "Morphology Identification and Molecular Characterization of *Lasiodiplodia theobromae* Causing Fruit Rot Disease of Guava (*Psidium guajava* L.) in Malaysia" is prepared by Rohaida binti Che Ludin and submitted to the Faculty of Agriculture in partial fulfilment of the requirement of PRT 4999 (Project) for the award of the degree of Bachelor of Horticultural Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Guava (*Psidium guajava* L.) belongs to the family of Myrtaceae is native to tropical America. Postharvest diseases, which cause serious problems during storage and transportation of guava fruits, are the major factors that limit the thriving guava industry in Malaysia. The fruit rot diseases reduce the fruit quality by altering the consistency, colour, taste and reduce shelf-life. Fruit samples from guava peel showing brownish black lesions of irregular shape and rapid spread of internal decay may appear. Few studies have investigated fruit rot disease on guava, however, only morphological characteristics were used and no molecular characterization was conducted to identify the pathogen identification. The objectives of this study were 1) to identify primary pathogen that caused guava fruit rot based on morphological and cultural characters as well as analyses of nucleotide sequences of the internal transcribed spacer (ITS) region of ribosomal DNA 2) to construct ITS phylogeny based on ITS sequences and their closest relatives by using Maximum Likelihood method. In order to identify the fruit rot-causing agent, 10 symptomatic fruits were collected from Seri Kembangan Selangor farmers market and fungi were isolated from fruit samples. One species, which is *Lasiodiplodia theobromae* was isolated frequently from the infected guava fruit. Colony morphology, and growth rates of the representative isolates were determined from the single conidium isolate obtained from the guava fruits. The pathogen identity was compared based on amplified ITS sequence using Basic Logical Alignment Search Tool (BLAST) nucleotide searches at GenBank. The findings of this study confirmed the true identity of the pathogen associated with fruit rot disease of guava in Malaysia was 99% matched with BLASTn search and ITS sequence was from *Lasiodipldia therobromae*. Correct identification of this pathogen is helpful for more effective management of fruit rot disease of guava. Further screening of effective fungicides and understanding of the epidemiology of this fungal pathogen will help to reduce financial loss to the guava industry in Malaysia.

ABSTRAK

Jambu batu (*Psidium guajava* L.) adalah dari keluarga Myrtaceae yang berasal dari Amerika tropika. Penyakit pasca tuai, yang menyebabkan masalah yang sangat besar semasa penyimpanan dan penghantaran buah jambu batu, adalah faktor yang utama yang menghalang pengembangan industri jambu batu di Malaysia. Penyakit reput buah mengurangkan kualiti buah dengan mengubah struktur, warna, rasa dan mengurangkan jangka hayat. Sampel buah menunjukkan symptom reput buah yang berwarna coklat kehitaman dalam bentuk yang tidak teratur dan reput dalaman buah cepat merebak akan kelihatan. Terdapat beberapa kajian terhadap penyakit reput buah jambu batu, namun, hanya melalui kaedah pengenalpastian morfologi yang dijalankan dan tiada kaedah pencirian secara molekul dijalankan untuk mengenal pasti identiti patogen. Objektif kajian ini adalah untuk 1) mengenal pasti patogen yang menyebabkan reput buah jambu batu berdasarkan penegenalpastian morfologi dan kultura dan juga jujukan nukleotid bahagian *internal transcribed spacer* (ITS) pada ribosom DNA, 2) untuk membina filogeni ITS berdasarkan jujukan ITS dan spesies kulat berdekatan dengan menggunakan kaedah *Maximum Likelihood*. Untuk mengenalpasti agen penyakit reput buah, 10 biji buah yang menunjukkan simptom reput buah telah diambil dari pasar basah Selangor dan kulat-kulat tersebut telah diasingkan daripada sampel buah terinfeksi. Satu spesis *Lasiodiplodia theobromae* telah diasingkan beberapa kali daripada buah jambu batu yang dijangkiti. Morfologi koloni dan kadar pertumbuhan telah dikenalpasti dari wakil kulat yang telah diasingkan daripada satu konidium yang didapati daripada jambu batu. Identiti patogen telah dibandingkan berdasarkan jujukan ITS yang telah dibanyakkan menggunakan *Carian Alat Susunan Logik* (*BLAST*) nukleotida dari GenBank. Kajian ini membuktikan identiti sebenar patogen yang menyebabkan penyakit reput buah jambu batu di Malaysia adalah 99% sepadan dengan keputusan BLAST dan jujukan ITS adalah daripada spesis *Lasiodiplodia theobromae.* Pengenalan yang betul patogen ini sangat membantu untuk pengurusan yang

lebih berkesan terhadap penyakit reput buah jambu batu. Pemeriksaan lebih mendalam terhadap racun kulat yang efektif dan pemahaman terhadap epidemiologi kulat ini akan membantu dalam mengurangkan kerugian dalam industri jambu batu di Malaysia.

CHAPTER 1

Introduction

1.1 Background of Study

Psidium guajava or most known as guava is from family Mytaceae. Its generics name is determined from the Greek expression "psidion" name of the pomegranate, same time its scientific name is came from Spanish word "guayabe" implying guava tree, itself continuously determined from those Arawakan by means of the Cariban through those Tupian. Those Spaniards brought the fruits over those Pacific to the Philippines in 1526, named as "guayabas" or "bayabas" got to be great built (Morton, 1987). Starting with here those fruits spread through Southeast and East Asia and India by Portuguese voyagers in the early seventeenth century. Starting with India the fruit have been disseminated on her neighbors and to Arabia. Today the fruits could be discovered developing for more than fifty nations all around those tropics and subtropics including a part of the Mediterranean territories for example, inside the cutoff points about scope 35ºN and 35ºS of the equator (Lim & Khoo, 1990).

In Malaysia, guava is known as Jambu Batu. In spite of guava have been planted over Malaysia to quite a while it have been main in later a considerable length of time that the crop pulled in with great arrangement of consideration (Lim & Khoo, 1990). Those zone under guava might have been with the goal inconsequential that it did not actually value a posting under incidental harvests in the 1984 Annual Report of the Department of Agriculture, Peninsular Malaysia. Because of demand, guavas are now being grown or cultivated at warm and humid and also subtropical territory. Imperative guava-growing regions are placed in the states of Perak, Johor, Selangor, and Negeri Sembilan. Different states that about lesser importance are Malacca, Kelantan, Terengganu, Perlis, Kedah, Pahang, Sabah and Sarawak

(Anem, 2013. retrieved from *http://animhosnan.blogspot.my/2010/12/guava-clone-in-malaysia.html*).

In Malaysia guava is habitually planted likewise the sole crop. It is not, nonetheless unprecedented to figure the tree developed concerning illustration as inter-crop between rows of other tree grown foods products for example, such that durian, alternately in discrete pieces inside the blended crop orchards. The fruit appearance is round or ovary based on the species with its long from 3 on 14cm. The rough skin generally transformed from green to yellow when ripen. Guava basically have certain types of fragrance odor. Guava flesh might sweet or sour. Those flesh shade starting with white to profound pink with rely on upon species. In Malaysia there are several varieties that give economic importance, which are Gu4, Gu5, Gu7, Jambu Biji, Laknaw, Taiwan, Hongkong pink, Vietnamese and Red Malaysian. The guava skin contrast from pink to red. Guava species Gu4, Gu5, Gu7, Jambu biji and Hongkong pink with diameter from 4. 8 cm to 6. 7 cm and slender skin from 0. 5-1. 5cm will be littler and slender compared to guava Vietnamese, its skin breadth from 10 to 11cm and thickness from 1. 9 cm on 2. 5 cm. There are two top season for fruit collecting which is January until March and October to December (retrieved from *http://www.malaysiafruit.com/guava/guava.php*). The moderate yield of the seeded cultivars can extent to 50 MT/ha/year. By there, there are amount about issues in generation which influence expense and which in turn, influence the competitiveness of the guava both provincially and abroad.

The greater issues that may be posed is because of the pests and diseases. The tropical climate, described by high mean temperatures of 28-30ºC, high relative moistness and overwhelming yearly rainfall about 2000 mm, may be favorable of the fast burgeoning and speedy pest proliferation, weeds and sicknesses. Under such climatic regime, those gainful productions for fruit yields necessitates costly control measures to decrease misfortunes. Ripe guavas simply wound and exceptionally perishable. Fruits for processing might be harvested

using mechanical tree-shakers or plastic nets. Specialists in Kurukshetra University, India, have demonstrated that treatment of harvested guavas with 100 ppm morphactin (chlorflurenol methyl ester 74050) surge shelf life of guavas by controlling contagious fungal decay, and lessening the color, weight, sugars, ascorbic acids and non-volatile organic acids (Morton, 1987). Joined together fungicidal and double-wax covering would extend the marketability by 30 days. Throughout the rainy season in India, and the area of Sancti Spiritus, Cuba, the fungus, *Phytophilous parasitica*, is answerable for much irresistible tree grown fruit rots. *Botryodiplodia* sp. and *Dothiorella* sp. caused stem-end decay in fruits injury during harvesting. *Macrophomina* sp. has been interfaced to fruit rot in Venezuela and *Gliocladium roseum* has been recognized looking into rotting fruits on the market in India (Morton, 1987).

1.2 Justification of the study

In Malaysia, researches on the guava postharvest diseases are very limited. The main focus in Malaysia majority are only on the main crops such as oil palm, rubber, paddy and cocoa. This limitation causing the importance of guava fruit in Malaysia's economic is being neglected.

1.3 Objectives of the study

1) To characterize and identify the causal agent causing guava fruit rot based on morphological and cultural characters

2) To construct internal transcribed spacer (ITS) phylogeny based on ITS sequence and their closest relatives by using Maximum Likelihood method

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