



UNIVERSITI PUTRA MALAYSIA

***IDENTIFICATION OF FUNGAL DISEASE, THE CAUSAL AGENT OF
POSTHARVEST FRUIT ROTS DISEASE ON PAPAYA (*Carica papaya* L.)***

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FACULTY OF AGRICULTURE
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By

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

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CERTIFICATION

This project report entitled Identification of fungal disease, the causal agent of postharvest fruit rots disease on papaya (*Carica papaya* L.) is prepared by Nor Atikah binti Azmi and submitted to the Faculty of Agriculture in fulfilment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science.

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ABSTRACT

Fruit rot of papaya (*Carica papaya* L.) is a postharvest disease in Malaysia. Symptoms of fruit rot, commonly appear during storage are characterized as dark with a wide margin of watery tissue and a surface wrinkled owing to the eruption of the pycnidia. This results in economic loss for fresh market of up to 90% and reduced the domestic market as well for export industry. Morphological criteria such as cultural, conidial and appressorial characters were used to differentiate taxa into species complexes, but cannot separate *Lasiodiplodia* species within a complex. To date, the genus had 30 species known from culture infecting host plants that are more than 500 hosts. *Lasiodiplodia theobromae* have been identified as a species causing fruit rot on papaya within the *Lasiodiplodia* species complex using ITS sequence data and phylogenetic species of *Lasiodiplodia* species causing fruit rot on papaya was constructed. The morphological observations of *Lasiodiplodia theobromae* was based on characters such as host preference, conidial size and shape, growth rate in culture, colour of the cultures, presence and absence of setae as well as teleomorph. Here, the identity of causal agent of fruit rot of *Carica papaya* has been confirmed with the application DNA sequencing of ITS regions of the isolates. Through BLAST finding, the identity of the isolates is 99 % and consisted of 547 bp. By combining DNA-based molecular technique with morphological evidence, recent studies on papaya will improve our understanding of the *Lasiodiplodia* species complex causing fruit disease on papaya.

ABSTRAK

Reput buah betik (*Carica Papaya* L.) merupakan penyakit lepas tuai di Malaysia. Tanda-tanda yang menunjukkan reput buah betik selalunya dapat dilihat semasa penyimpanan. Ianya mempunyai ciri-ciri seperti warna gelap di atas kulit betik, ditambah dengan sel tisu yang berair dan permukaan yang berkedut disebabkan oleh pertumbuhan “pycnidia”. Keadaan ini akan menyebabkan kerugian pada ekonomi pasaran sehingga 90% dan mengurangkan pasaran domestik dan juga untuk industri eksport. Karakter morfologi seperti pertumbuhan, “conidial” dan “apressorial” telah digunakan untuk membezakan “taxa” ke dalam spesies kompleks, tetapi tidak boleh memisahkan spesies *Lasiodiplodia* di dalam kompleks. Setakat ini, genus ini mempunyai 30 spesies yang diketahui dari budaya yang menjangkiti tumbuhan perumah lebih daripada 500. *Lasiodiplodia theobromae* telah dikenal pasti sebagai spesies yang menyebabkan reput buah-buahan pada betik menggunakan data jujukan ITS dan filogenetik *Lasiodiplodia* spesies menyebabkan reput buah-buahan pada betik telah dibina. Pemerhatian morfologi *Lasiodiplodia theobromae* adalah berdasarkan ciri-ciri seperti perumah, saiz conidial dan bentuk, kadar pertumbuhan koloni, warna koloni, kehadiran dan ketiadaan setae serta “teleomorph”. Identiti penyebab penyakit reput buah betik (*Carica papaya*) telah disahkan dengan penjujukan DNA permohonan-kawasan ITS daripada pencilan. Melalui BLAST, identiti pencilan adalah 99% dan terdiri daripada 547 bp. Dengan menggabungkan teknik molekular berasaskan DNA dengan bukti morfologi, ianya akan meningkatkan pemahaman kita mengenai kompleks spesies *Lasiodiplodia* yang menyebabkan penyakit reput buah pada betik.

CHAPTER 1

INTRODUCTION

Carica papaya or papaya is well known as tropical fruits were actually a native fruit originated mainly from Southern Mexico and Central America. This species is then widely spread to Africa and Asia region. This may be because of tropical temperature is between 21 °C and 33 °C that is a suitable temperature for papaya tree to grow. It is originally planted as a home garden plant, but because of the commercial value, its level as a home garden plant has been up as a commercial crops to many countries especially in tropical region. As it is easy to cultivate throughout the year, papaya has its own contribution to the country's economy especially in developing country. Papaya fruit is ranked third with 11.22 Mt or 26.58% of the total tropical fruit production. Asian regions itself produces around 52.55% of papaya between 2008 to year 2010. In 2010 to 2011, through market survey by ThamaraiKannan and Sengottuvel (2012), India (4,713,800 mt), Brazil (1,871,300 mt), Nigeria (703,800 mt), Indonesia (695,214 mt) and Mexico (616,215 mt) are the top five of major countries that producing papaya in the world. The top five of the major countries that had been exporting papaya are Mexico (210,635 mt), Belize (30,137), Malaysia (25,686 mt), Brazil (25,562) and India (15,435 mt).

Even though the papaya production is increasing, the industry faced two major problems which are the papaya ringspot disease caused by a virus and post-harvest losses that caused by fungal diseases. This fungal disease will show the symptom when the papaya is in its ripening stages. There are many fungal diseases that lead to

post harvest losses in many fruits production such as anthracnose disease, soft rot disease, fruit rot disease. There are several common pathogens that caused post-harvest diseases such as *Alternaria sp.*, *Fusarium sp.*, *Penicillium sp.*, *Aspergillus sp.*, *Colletotrichum sp.* and *Botrytis sp.* according to Aktar (2011) that cited from Adaskaveg *et al.* (2002). Sangchote (1998) mentioned that *Lasiodiplodia theobromae*, *Dothiorella sp.* and *Phomopsis sp.* are the pathogens that can infect the fruit during and after harvest. The infection may start from the wounded parts and the development of the symptoms can be seen as the fruit ripen. The other parts of the plant also may be infected although the symptoms cannot be identified or seen. *Lasiodiplodia* species had been found that it may caused papaya with fruit rot disease where researcher had justified that this *Lasiodiplodia* species had infected on many host. Other than fruit rot disease, Netto *et al.*, (2014) said that *Lasiodiplodia theobromae* had been reported as the causal agent of stem-end rot of papaya in Brazil. Adaskaveg *et al.* (2002) stated that *Lasiodiplodia* rot may occur on the skin of injured fruits. It is because of the conidia of the pathogen cannot penetrated the uninjured skin.

Differentiation of a pathogen on their morphology is the first step to determine the pathogen. However, the traditional method used by looking at the morphology of the fungus cannot determine the species that caused the disease accurately unless it is done by a reliable staff which are experienced. Rodríguez-Gálvez *et al.* (2016) in his journal mentioned that identification using only morphology is impossible in *Lasiodiplodia* species. The morphological characteristics of *Lasiodiplodia sp* usually is mixed with other species in their group (Muniz *et al.*, 2012) and in some cases, their morphology characteristics may look similar between each other that will make

the identification by morphology is very difficult (Ni *et al.*, 2012). The wide range of host involving *Lasiodiplodia* species had made them fall into a complex of different species (Alves *et al.*, 2008). Thus, it is difficult to differentiate them using morphological identification studies only.

Amplification of the ITS regions of the ribosomal DNA (615 bp) by polymerase chain reaction and sequencing of the amplified ITS fragments can determine the specific fungus that responsible for the disease. The isolates may be found out earlier compared to traditional morphological analysis that required several days to identify the fungus. According to Taylor and McCormick (2008), although the used of the internal transcribed spacer have its own limitation, it is still the most effective single locus for fungi identification at the species to genus level. The application of PCR along with ITS will help in mitigated the problems with limited morphological variation and the pathogen culture. Plus, the phylogeny analysis will help in defining the control strategies by the pathologist.

This study were conducted to fulfil these objectives which are i) to distinguish *Lasiodiplodia* species causing fruit rot on papaya from others within the *Lasiodiplodia* species complex using ITS sequence data and ii) to clarify phylogenetic species concept of *Lasiodiplodia* species causing fruit rot on papaya.

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