UNIVERSITI TEKNOLOGI MARA

DEVELOPMENT OF PCR-BASED DETECTION METHOD FOR FRUIT BUNCH ROT (FBR) DISEASE IN OIL PALM

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MSc

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AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Fruit bunch rot (FBR) disease is considered as a new potential emerging disease of oil palm in Malaysia. The occurrence of this disease has been reported in Perak and Selangor states since 2012. The causal pathogen was identified as *Marasmius palmivorus* Sharples (1928) is known to be as saprophytic apparently become parasitic after it has attained a certain inoculum level of infection. The current study aimed to develop molecular polymerase chain reaction (PCR) detection method for FBR disease in oil palm. The use of molecular diagnostic assay offers specificity and rapid diagnostic for an early disease detection in plantations. Therefore, a total of 39 samples were collected from Perak and Selangor states by random sampling in the oil palm plantations. Basidiocarps and rhizomorphs on diseased oil palm were collected prior surface sterile and 8 pure isolates of *M. palmivorus* were successfully isolated. The identity was confirmed by morphological and molecular identification. An artificial inoculation of *M. palmivorus* on detached oil palm fruit using *in vitro* assay showed positive infection. Ten species-specific primers were designed to amplify the Internal Transcribed Spacer (ITS) of ribosomal gene cluster (rDNA) of *M. palmivorus*. Primers designed in this study were screened for efficiency and specificity before optimized using Gradient PCR for optimum annealing temperature (59 – 64ºC) for PCR amplification. Validation assays performed by cross reaction with 34 different fungi species includes 66 samples from different sources. The results obtained suggests that the new designed primer could be employed to directly analyse sample from the field with the aim of detecting the presence of the pathogen in diseased fruits. Molecular analyses suggested that primer pair MP02 was able to amplify *M. palmivorus* at 64 ºC annealing temperature in 30 cycles of PCR programme. This report for the first time that species specific primer MP02 has revealed to be useful primer in PCR amplification for the detection of *M. palmivorus* rDNA.

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