PCR MARKER (Mar-M) FOR DETECTION OF *Marasmius palmivorus* CAUSING BUNCH ROT DISEASE OF OIL PALM

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Bunch rot disease or *Marasmius* bunch rot is one of the oil palm fruit diseases caused by Marasmioid mushroom (basidiomycetes fungi) known as *Marasmius palmivorus*. The disease was firstly reported in Malaysia and Indonesia by Van Hall in 1924 and Sharples in 1928 (Turner, 1981) and in Andaman and Nicobar Island, India, in the year 1987 (Reddy *et al*., 1987; Dutta and Krishnendu, 2018). An outbreak of the disease has been recorded in Malaysia during the early period of oil palm cultivation and expansion with a severe loss (Chung, 2012). The outbreak was believed to occur after the pathogen attained a certain inoculum level due to favourable conditions of the pathogen (Turner, 1981; Turner and Gillbanks, 2003), including weather. The changes in the climate pose a potential risk of a disease outbreak; therefore, a mitigation plan to manage the disease is essential. One of the crucial steps in the disease management plant is the ability to accurately identify and diagnose the pathogen. Advances in the development of molecular biology enable rapid, robust, specific, and sensitive tools for detecting plant pathogens (Ward *et al*., 2004).

THE TECHNOLOGY

This technology is a DNA-based molecular marker for detection of *Marasmius palmivorus*, the causal pathogen of bunch rot disease of oil palm.

THE NOVELTY OF THE TECHNOLOGY

The marker is the first species-specific PCR marker to identify and detect the presence of *M. palmivorus*. The marker is developed based on internal transcribed spacer (ITS) regions of ribosomal RNA (rRNA) gene cluster using polymerase chain reaction (PCR) technique. PCR is established and well known molecular protocol, therefore can be easily adopted by any molecular laboratory.

MOLECULAR DETECTION OF *Marasmius palmivorus*

The detection method involved a DNA extraction of pure culture (*Figure 1*) or plant tissues such as fruitlets (*Figure 2*), fruiting body (mushroom) (*Figure 3*), stem or rhizomorphs, collected from the field using commercial DNA extraction kits or conventional methods. Homogenisation is carried out using sterile mortar and pestle with the presence of liquid nitrogen or using miller with beads and extraction buffer. The PCR is carried out in a 30-50 µl volume using standard PCR protocol with specific annealing temperature, followed by fragments separation using gel electrophoresis. The gel is then visualised using a UV imaging instrument. The Mar-M marker will produce PCR amplicon with the size of 650 bp (*Figure 4*). The markers do not have cross-reaction with others 70 isolates of fungi and oomyctes including *Ganoderma boninense, Fusarium* sp. and *Phytophthora* sp.

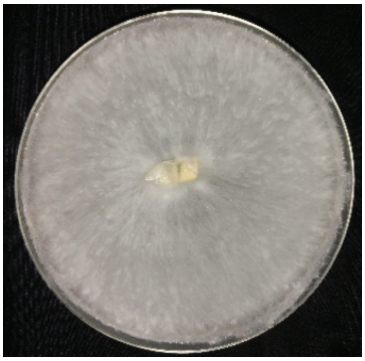

Figure 1. Pure culture of Marasmius palmivorus.

Figure 2. Diseased fruitlets of oil palm.

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Figure 3. Fruiting body or mushroom of Marasmius palmivorus *found on the dead rachis of oil palm.*

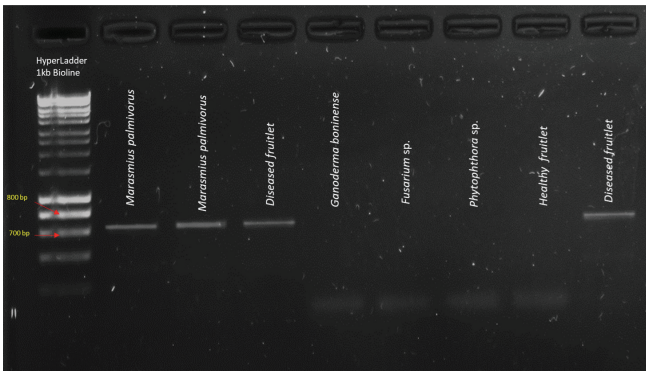

Figure 4. PCR assay with Mar-M marker using DNA extracted from Marasmius palmivorus*, fruitlets and other fungi.*

BENEFIT AND ADVANTAGES

The ability to perform early detection of disease infection will enable mitigation of the disease at the early stage, thereby reducing the potential losses caused by the diseases. The technology offers a rapid detection tool of *M. palmivorus*, so that the oil palm plantation can identify the presence of bunch rot disease to formulate disease management strategies and prevent outbreak that causes severe economic losses. Conventional identification using morphological characterisation of the fungi involved isolation to obtain pure culture of the fungi and microscopic evaluation that usually performed only by a skilled mycologist. Identification using nucleic acid sequence involved a long process, tedious and expensive. PCR is a well-established method that can be carried out using standard thermo-cycler, gel electrophoresis and UV imaging system, which can be found in most molecular laboratories.

ECONOMIC ANALYSIS

The internal rate of return (IRR) for manufacturing the diagnostic kit is estimated at 43.81%, net present value (NPV@10%), RM1 690 000 and discounted benefit-cost ratio (BCR) of 1.33. The discounted payback period is estimated to be five years. The cost of the marker is estimated at RM2000 per box of 96 reactions. The values are subjected to change.

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