UNIVERSITI TEKNOLOGI MARA

ASSESSMENT ON THE USE OF FTA-STORED DNA METHOD FOR DETECTION OF RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) P12 AND P15 FROM *Elaeis guineensis*

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MSc

June, 2021

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

Flinders Technology Associates (FTA) card is a model tool set employed to serve as an alternative way to preserve the sample and simplifies the steps for the DNA samples collection, transportation and purification. The delivery and storage of oil palm fresh tissue require a lower temperature to avoid DNA degradation. Hence, sample collection and preservation become challenging especially to less equipped laboratories. The main objective of this study was to assess the quantity and quality of isolated DNA from FTA-Stored of oil palm (*Elaeis guineensis*) leaves tissues for detection of random amplified polymorphic DNA (RAPD) P12 and P15 which are DNA markers for dura (D), pisifera (P) and tenera (T) classification. Samples were collected from young oil palm tree via cutting the leaves with harvesting stick and taken to the laboratory. DNA was extracted from fresh samples and FTA-Stored samples using DNeasy plant mini kit (Qiagen USA, coded as Kit D) and EZ.NA Spin Column (Bio Basic Inc. Canada, coded as kit E) respectively. The concentration and purity of the isolated DNA was determined using Scandrop2u. The detection of P12 and P15 DNA markers using PCR-RAPD was carried out using three types of samples, (i) DNA from fresh leaves samples, (ii) DNA from FTA-Stored samples and (iii) Direct FTA-Stored samples. Two primer set were used in the PCR-RAPD: (i) Primer P15 (5'- TTGGCACGGG -3') and (ii) P12 (5'-TCTGGTGAGG-3') with an expected amplicon size of 700 bp, 800 bp 1000 bp and 600 bp, 750 bp, 1100 bp respectively. The amplification and presence of P12 and P15 DNA markers in the DNA of fresh leaves samples, DNA from FTA-Stored samples and direct FTA-Stored samples were determined using agarose gel electrophoresis. The statistical analysis T-Test was carried out to test for significance difference. The quantity of DNA extracted from fresh samples from both kits gave a concentration range of 20 ± 2.60–233 ± 60.50 µg/µl with a purity range of 0.90 ± 0.10–2.07 ± 0.20. The DNA FTA-Stored samples extracted from both kits also gave a concentration range of 34.5 ± 4.90–233.31 ± 105.80 µg/µl with a purity range of 0.95 ± 0.20 - 1.80 ± 0.11. The t-test result indicates that there is no significant difference between fresh samples and FTA-Stored samples. PCR products obtained from the fresh samples extracted from kit D using RAPDP15 indicates that 2 samples out of 3 gave band of 800 bp , 600 bp and 450 bp while kit E did not show bands, RAPDP12 showed band for only one sample extracted from kit D with size of 1100 bp, 750 bp and 500 bp and kit E give two bands out of 3 with size of 750 bp and 600 bp. FTA-Stored samples amplified with RRAPD P15 using kit D give 2 bands out of 3 with a sizes of 1100 bp, 800 bp and 700 bp and kit E show band for all three samples with size of 800 bp and 500 bp, RAPDP12 Kit D showed a single band of 1100 bp, 750 bp and 600 bp while kit E didn't show the band. The result from direct PCR indicates that 2 samples amplified using RAPD P15 only give a band of 1100 bp, 700 bp and 500 bp while none of the samples amplified using RAPD P12 showed a band. The result indicates that FTA-Stored samples have the highest concentration and purity range compared to fresh samples. Based on agarose gel electrophoresis photo, the bands from PCR amplicons of FTA-Stored samples were more distinct than fresh samples. The present research work explains suitability of using FTA cards for DNA storage.

Keywords: Oil Palm; Flinders Technology Associates; Polymerase Chain Reaction; Random Amplified Polymorphic DNA; Gel Electrophoresis

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