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Application of Whatman FTA Card Method in Oil Palm DNA Extraction and PCR Analysis

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ABSTRACT

Polymerase Chain Reaction (PCR) analysis in the study of oil palm DNA generally carried out by using DNA template obtained from grinding of leaf samples in liquid nitrogen followed by hexadecyltrimethylammonium bromide (CTAB) protocol. The present study explores the FTA card as a method to retrieve PCR-amplifiable oil palm DNA. Oil palm leaves were cut and crushed before deposited onto the FTA card. An attempt was made by amplifying the EgSHP gene using a punch of FTA card as a DNA template. The successful outcome of PCR was measured by the presence of PCR amplicons on 1% agarose gel electrophoresis, indicate the genotype of oil palm fruit form. This present study demonstrates that the FTA card provides a versatile alternative to the study of oil palm genetics.

Keywords: *oil palm, FTA, PCR*

INTRODUCTION

In Malaysia, the most important economic crop is oil palm which covered about 5.74 million hectares of planted areas with contribution of 29% from the global fats and oils [1]. It is crucial for the oil palm plantation and researchers to encounter fierce competition in producing its oil and fats for the world by using such approaches like breeding and genetic transformation. Generally, the isolation of oil palm DNA is conducted by grinding the leaf sample with liquid nitrogen followed by the CTAB protocol [2-3]. An isolation method without the use of liquid nitrogen has been developed by [4]. However, this method requires the use of fast homogenisation of the leaf tissue in the presence of DNA extraction buffer using mortar and pestle. These existing protocols are time consuming and require more space for the preparation of DNA extraction as well as leaf sample storage.

Flinders Technology Associates (FTA) Card is a modest toolset that can reduce the steps for the collection of DNA samples, transportation, purification and storage. It serves as an innovation toolset that directly processing a genomic DNA to the preparation for polymerase chain reaction (PCR) analysis [5]. FTA card is a paper-based product containing a combination of chemical formulation that will initiate cells to lyse and immobilise the nucleic acids on the paper medium for a long period of storage. The large nucleic acids become physically trapped within the fibres of the FTA matrix and are preserved intact, while the cellular debris can be rapidly removed by simple washes of the inoculated card [6]. The FTA matrix (Figure 1) contain chemical coating that protects DNA from degradation allowing the cards to be stored at room temperature.

Previous study using massive number of maize leaf sample deposited on FTA card showed that PCR product yield was sufficient for reliable scoring using SSR markers in marker assisted backcrossing [5]. An application of FTA card in plant genetic study by [7] reported that the FTA card extraction method yielded less concentrated and less fragmented samples than the CTAB-based protocol. Furthermore, the application of FTA card is easy and relevant especially for the experimental field in remote locales [8]. A successful PCR-amplifiable amphibian DNA from skin swabs applied on FTA cards proved that the FTA technology was an

effective tool in term of time saving and elimination of the DNA extraction step [9]. On the other hand, [6] have evaluated the successful of using FTA filter matrix technology for preparing and storing total genomic DNA from yeasts and molds. Moreover, [10] demonstrated that the blood transferred onto FTA cards followed by elution in Chelex®100 was the best approach for the molecular diagnosis of trypanosomiasis using PCR. In epigenetic study [11], a methylation assessment was successfully conducted based on sperm cells stored on FTA classic cards. The objective of this study was to investigate a potential method to amplify gene of interest from oil palm DNA stored in FTA card matrix and no prior DNA extraction or purification steps are needed.

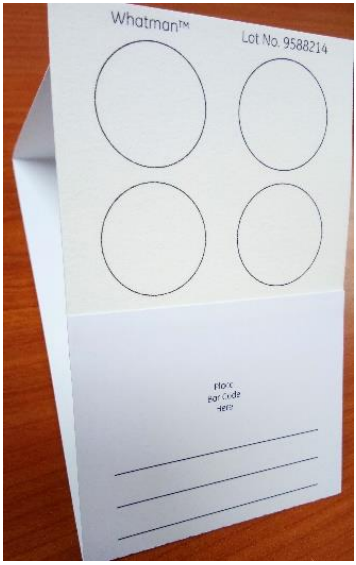

Figure 1: FTA Card Matrices (Source by author)

METHODOLOGY

Collection and Application of Samples

Young leaves were collected from oil palm tree that are grown wildly at an opened and spacious area in Tanjung Karang, Selangor (coded as A) and Kampung Gajah, Perak (coded as B) (Figure2).

Figure 2: Leaf Sample was Taken from Wildly Grown Oil Palm; Sample A and Sample B (Source by author)

The leaves were cut into a small pieces and crushed using a pestle and a mortar (Figure 3: left). The crushed leaf was then placed directly onto the FTA card and covered over by its cover sheet. By using a pestle, a moderate pressure was applied onto the covered sample to position the crushed plant print onto the card matrix. Two crushed plant prints were prepared for sample A and B for this study (Figure 3: right). The samples were then allowed to dry for one hour at room temperature for further use.

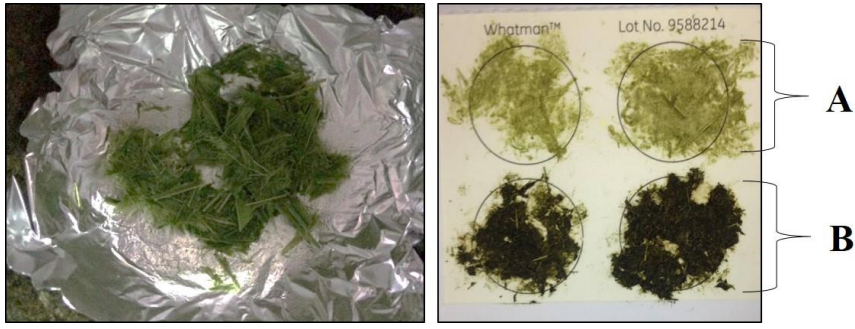

Figure 3: Preparation of Samples Crushed Oil Palm Leaf and Palm Print for Sample A and B (Source by author)

Preparation of Sample DNA for PCR Analysis

One sample disc from each card (three replicates) were punched out from the spot where sample A and B were imprinted using a 2.0 mm Harris Micro punch and denoted as A1, A2, A3, B1, B2 and B3 (Figure 4). Each of them was placed in a 1.5 ml of Eppendorf tube, respectively. Then, 200 µl of FTA Purification Reagent was added into the tube and vortexed for five minutes. All spent FTA Purification Reagent was then removed and discarded by using a pipette. The washing step involving FTA purification Reagent was repeated twice for a total of three washes.

After that, 200 µl of 1xTE Buffer was added to the same tube. The tube was incubated at room temperature for five minutes. All spent 1xTE Buffer was then removed and discarded with a pipette. The washing step involving TE buffer was repeated for a total of two washes. The disc was dried at room temperature for about 45 minutes before the PCR analysis.

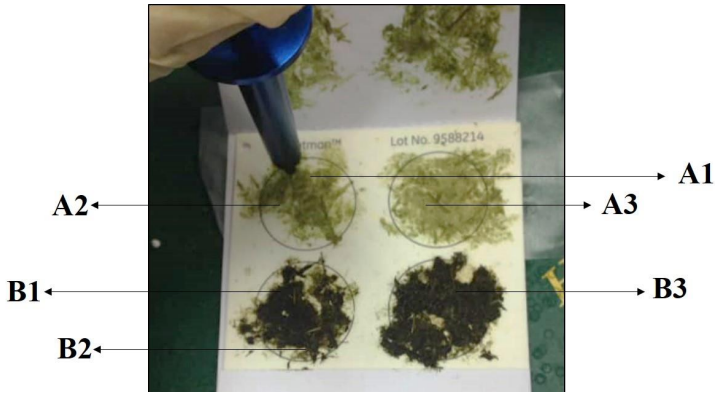

Figure 4: Sample was Punched with 2.0 mm Harris Micro Punch (Source by author)

Amplification of EgSHP Gene

Primers sequences for EgSHP gene were adapted from [12] as follow:

EgSHP-Forward: 5'-TTGCTTTTAATTTTGCTTGAATACC-3'
EgSHP-Reverse: 5'-TTTGGATCAGGGATAAAAGGGAAG-3'

The PCR reactions were performed in a total volume 25 µl containing a dried FTA disc, 6 µl 5xHOT FIREPol® Blend Master Mix (Solis BioDyne, Estonia), 1 µl of 20 µM EgSHP forward primer, 1 µl of 20 µM EgSHP reverse primer, 20 µM of each primer and 17 µl of Molecular Biology Grade Water. The negative control in this study contains similar contents as the PCR preparations, except for the presence of the FTA card. The following PCR conditions were employed: an initial denaturation step at 95°C for 12 minutes followed by 35 cycles of denaturation for ten seconds at 95°C, annealing for 30 s at 46.1°C, extension for 20 seconds at 72°C, and a final extension at 72°C for five minutes then maintained at 4°C.

Detection of PCR Amplicons

PCR amplicons were electrophoresed and visualised in a 1.0% (w/v) agarose gel incorporated with GelStar nucleic acid stain (Lonza, USA). Ten µL of each PCR products were mixed with 6x DNA loading dye (Jena Bioscience, Germany) before loaded into the gel well. A 100bp DNA marker was used as a reference band in this study. The electrophoresis was performed in 1xTBE buffer with the layer of buffer is 2 to 3 mm high above the gel. The DNA band was visualised using Omega FluorTM Plus gel documentation system (VWR Life Science, USA).

RESULT AND DISCUSSION

In order to demonstrate the amplification of gene unique to oil palm in this study, an EgSHP gene marker developed by [12] has been used. This gene is related to oil palm shell in the reference of pisifera genome [13]. Figure 5 shows the PCR products of sample amplified for EgSHP gene in this study. The successful amplification was detected by the presence of PCR amplicons in between 500-600bp on the agarose gel in sample A1 (Lane 1), B1 (Lane 4) and B2 (Lane 5). According to [12] the PCR product size of 550bp has been validated for detection of EgSHP gene in dura, tenera and pisifera fruit types.

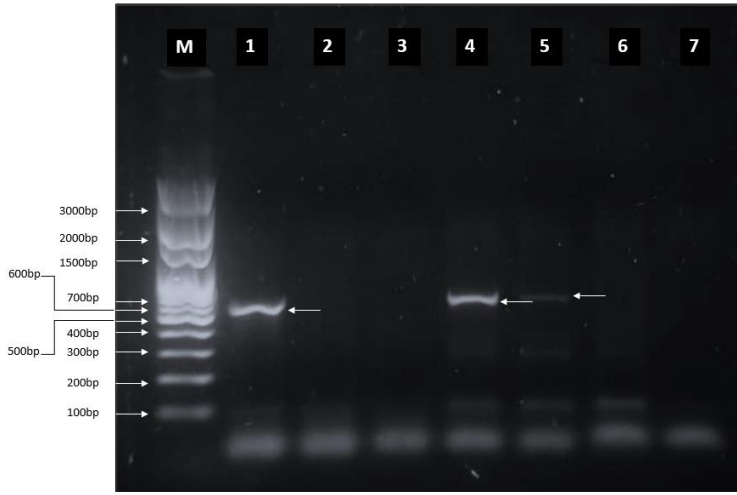

Figure 5: Photograph of 1% (w/v) Agarose Gel Containing PCR Products of Sample Amplified for EgSHP Gene. Lane from Extreme Left: M=100bp DNA Ladder, Lane 1= A1, Lane 2= A2, Lane 3= A3, Lane 4= B1, Lane 5=B2, Lane 6=B3, Lane 7=Negative Control

This is the first study demonstrating the amplification of gene using oil palm DNA stored in FTA card. Hence, it provides such a useful finding when the card provided DNA could be successfully amplified. Out of six sample discs, 50% was successfully amplified. The PCR failure might be due to punches taken from areas of the card which contain insufficient or no DNA. In addition, the use of superfine resolution (SFR) agarose also worked best with simple sequence repeat (SSR) marker gene [14-15]. Storage of PCR ready oil palm DNA onto the FTA card in this study provide an economical solution to the long term storage of oil palm leaf samples and tedious DNA extraction protocol.

CONCLUSION

In conclusion, the usage of FTA card as a sampling method to collect and store the oil palm DNA has been achieved. This has been shown through the successful amplification of the oil palm DNA stored in the FTA card resulting the manifestation of a single band fragment on the 1% (w/v) of agarose gel electrophoresis for the PCR product by using Eg SHP primer. Although the

structures of the oil palm leaf is thick and hardly to crush into tiny pieces, the extraction of oil palm DNA into the FTA card was practicable without compromising the quality of its DNA prior the PCR analysis.

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