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FREEZE-DRYING OF OIL PALM (*Elaeis guineensis*) LEAF AND ITS EFFECT ON THE QUALITY OF EXTRACTABLE DNA

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ABSTRACT

The use of molecular genetic markers in plant breeding and genetic resource management in oil palm requires the analysis of large numbers of samples and the availability of rapid and efficient DNA extraction methods. This often requires leaf samples to be stored in an ultra low temperature freezer for future use. This takes up valuable freezer space. Thus, freeze-drying is proposed as an alternative. The freeze-dried tissue can be ground into dry powder for efficient storage in freezers. This paper describes a method to freeze-dry oil palm leaf and compares the quality of genomic DNA extracted from non-freeze-dried and freeze-dried leaves. The effects of storage temperature (–20°C and 4°C) and duration (up to 18 months) on the DNA stability in the freeze-dried leaf were also evaluated. The freeze-dried leaf yielded high molecular weight DNA of sufficient purity and quality for molecular biology applications. The study demonstrated that freeze-dried oil palm leaves can be stored at -20°C and 4°C for at least 18 months with no DNA degradation. DNA prepared from the freeze-dried leaves was also acceptable for both RFLP and SSR analyses. Freeze-drying oil palm leaf provides an economical solution to long-term storage and handling because of the reduced weight and space requirement. In addition, up to four times as many samples can be processed for DNA isolation per day per person using freeze-dried powder of oil palm leaves, compared to using fresh tissue or tissue frozen in liquid nitrogen.

Keywords: DNA, RFLP, SSR, *Elaeis guineensis*, freeze-dried leaf.

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is a perennial monocot belonging to the family Palmae and tribe Cocoineae. It gives the highest oil yield per hectare of all the economic oil crops (Corley and Tinker, 2003). It is an important crop for Malaysia and contributes significantly to the national economy (Yusof, 2002). In order to continue harnessing the biological potential of oil palm and to maintain its competitive edge, molecular genetic studies are extensively carried out in the Malaysian Palm Oil Board (MPOB).

The isolation of intact, high molecular weight genomic DNA is essential for many applications, including restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR) or microsatellite, analyses. Currently, the most common procedure for isolating oil palm DNA is to use fresh leaf. However, most of the DNA extraction protocols for plant tissues require manual grinding of the material in liquid nitrogen. As the grinding is time-consuming and cumbersome, only a limited number of samples can be processed. For long-term preservation of the tissues for later analysis, they must be stored at constant, ultra low temperatures. Such storage is expensive, so it is important that the storage method is optimized.

Freeze-drying, or lyophilization, is a convenient way of storing plant materials. The tissues can be harvested when in prime condition, washed, freeze-dried and stored for later processing. The dry plant materials can be disrupted very efficiently by dry milling or grinding, and, also, the DNA in a non-hydrated state is less susceptible to shear damage.

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Nucleolytic degradation is also minimized as the DNA is hydrated immediately in the presence of chelating agents and detergents which inhibit nuclease activity (Lichtenstein and Draper, 1985).

The suitability of lyophilized plant tissues for molecular studies has been demonstrated in other crops such as cotton (Saha *et al.*, 1997) and tea (Jaiprakash *et al.*, 2003). Saha *et al.* (1997) reported that high molecular weight genomic DNA suitable for restriction enzyme digestion and as a template for polymerase chain reaction (PCR) amplification was isolated from freeze-dried leaf and root tissues of cotton (*Gossypium hirsutum* L.). The study also found that the total proteins of leaves and roots were unaffected by the freeze-drying based on a comparison of polypeptide profiles by denaturing polyacrylamide electrophoresis. However, freeze-drying completely degraded the RNA in the leaf and root tissues of cotton. Nevertheless, using an RNA isolation protocol that uses guanidine hydrochloride with minor modifications, Jaiprakash *et al.* (2003) successfully isolated high quality RNA from freeze-dried tea (*Camellia sinensis*) leaves that was found to be useful for all downstream applications such as RT-PCR and Northern blot analysis.

In this report, a method to freeze-dry oil palm leaf tissues is described. The effects of the storage temperatures (-20°C and 4°C) on the integrity of the DNA in the freeze-dried tissues were assessed. A second goal of the study was to evaluate the integrity of DNA extracted from freeze-dried tissues stored for up to 18 months. The suitability of the DNA for RFLP and SSR analyses was also evaluated.

It is envisaged that freeze-drying will be an economical solution to long-term storage of biological materials. The stored materials can be as good as the fresh tissue for molecular genetic studies.

MATERIALS AND METHODS

Plant Material

Leaf samples from two clones (A356 and A364) of oil palm (*Elaeis guineensis* Jacq.) were provided by Advanced Agriecological Research (AAR). The leaflets were sampled from three fronds (fronds 6-8). The midrib was removed and the lamina was cut into approximately 6 cm long pieces. The samples were placed in sealed bags and immediately plunged into liquid nitrogen and stored at -80°C until further use. These were the non-freeze-dried tissues.

Freeze-Drying

Leaf samples frozen in liquid nitrogen from the above were wrapped in muslin cloth (25 cm x 25 cm). The shelf of the freeze-dryer (Freeze Dry System with Stoppering Tray Dryer/Freezone 6.0, Labconco Incorporation, Kansas City, MO) was pre-cooled to -34°C using the manual system control. The frozen leaf samples wrapped in muslin cloth were evenly distributed across the entire surface of the temperature-controlled shelf.

Freeze-drying was performed under automatic system control with the conditions controlled by a pre-determined programme. The freeze-drying cycle was as follows: shelf temperature at -34°C for 16 hr, -10°C for 22 hr and 25°C for 10 hr. The condenser was cooled to -46°C and the vacuum maintained at $< 133 \times 10^{-3}$ mbar throughout the freeze-drying cycle. After completion of the cycle, the freeze-dried tissues were sealed in plastic bags and stored at -80°C until use.

Mechanical Tissue Disruption

The freeze-dried leaf tissues were placed in a 1 litre Waring stainless steel container (model SS610), and ground to a fine powder using a Waring Blender Model 32BL80 (Dynamic Corporation of America, New Hartford, CT). For processing small samples, mini stainless steel containers (model MC2 or MC3) were used. To reduce the potential for carry-over, the sample handling tools and containers were vacuum cleaned thoroughly after each sample was processed.

The powdered tissues were immediately sealed in plastic bags to prevent prolonged exposure to moisture, and stored at 4°C, -20°C and -80°C.

DNA Extraction

DNA from the leaf samples was extracted and purified using the method described by Doyle and Doyle (1990). Leaf samples (4 g non-freeze-dried and 0.4 g freeze-dried leaves) were ground to powder in liquid nitrogen using a sterile mortar and pestle. The powdered tissue was transferred to a 50 ml centrifuge tube containing 20 ml modified CTAB buffer (2% CTAB w/v, 20 mM EDTA pH 8.0, 1.4 M NaCl, 100 mM tris-HCl pH 8.0, 5 mM ascorbic acid, 4 mM diethyldithiocarbamic acid sodium salt and 2% polyvinylpyrolidone-40) and 80 µl 2-mercaptoethanol. The mixture was placed at 60°C for 30 min, after which an equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed thoroughly. The mixture was centrifuged at 10 000 rpm for 15 min at 20°C and the upper aqueous phase was transferred to a sterile 50 ml centrifuge tube. The DNA in this aqueous phase was precipitated by adding 0.6 volume of ice-cold isopropanol. The solution was stored at -20°C for 1 hr and centrifuged at 12 000 rpm at 4°C for 15 min. The DNA pellet obtained was washed in 5 ml wash buffer (76% ethanol, 10 mM ammonium acetate) and centrifugation. The pellet

was dried using a speed-vac centrifuge (Savant Oligo Prep™ OP120) for 1 hr and suspended in 4 ml TE buffer (10 mM tris-HCl pH 8.0 and 1 mM EDTA pH 8.0). The DNA solution was treated with 5 µl RNase (10 mg ml^{-1}) at room temperature for 20 min. Subsequently, 0.5 volume of 7.5 M ammonium acetate, pH 7.7 was added and the mixture was placed on ice for 20 min. After centrifugation at 12 000 rpm for 15 min at 4°C, the supernatant was transferred into a clean and sterile 30 ml corex tube. The DNA in the supernatant was re-precipitated by adding 2.5 volumes of ethanol and stored at -20°C for 1 hr. The DNA was recovered by centrifuging the mixture at 12 000 rpm for 15 min at 4°C. The DNA pellet was washed with 5 ml 70% ethanol, dried and dissolved in 1 ml TE buffer.

The DNA concentration of the samples was determined by diluting a small sub-sample (10 µl) in 990 µl sterile water (100x dilution) and the optical density at $A_{260,}$ A_{280} and A_{350} was determined using a spectrophotometer. The integrity of the DNA was further examined by electrophoresing an approximate 5 µg of DNA in 0.9% agarose gel in 1x TAE buffer (0.04 M tris base, 20 mM acetic acid, 2 mM EDTA). The DNA was loaded onto the gel with 1/9 volume loading dye gel solution, FOG (0.1 M EDTA, 25% ficoll, 0.1% orange G and 50% glycerol). The digestibility of each of the DNA sample extracted was also checked by restriction digestion of 5 µg DNA with *Eco*RI (six base pair cutter) and *Hae*III (four base pair cutter).

The digestion was carried out as described below. The digested samples were run next to the undigested samples (for integrity testing). Also loaded was a 1 kb DNA ladder (New England Biolabs) and 2 µl gel tracking dye (25% ficoll type 40, 1% orange G, 1% bromophenol blue, 1% xylene cyanol and 0.1 M EDTA) in separate wells, and the gel was run at 100 V for 60 min until the bromophenol blue reached the end of the gel tray. The gels were photographed under UV light (254 nm; Model Fotodyne Incorporated) using a Polaroid camera.

Restriction Enzyme (RE) Digestion of DNA for Southern Analysis

An aliquot of 20 µg genomic DNA was transferred into a 1.5 ml microcentrifuge tube and sterile water was added to a final volume of 100 µl to form the first master mix. A second master mix consisting of the following was prepared: 20 µl of the appropriate 10x restriction buffer (supplied with the enzyme), 4 µl 0.1 M spermidine trihydrochloride (pH 7.0), 2 µl 100x Bovine Serum Albumin (BSA), 60 U restriction endonuclease *Rsa*I and sterile water to a final concentration of 100 µl. The second master mix was transferred to the microcentrifuge tube containing the first master mix to give a final volume of 200 µl digestion mix. The digestion mix was incubated overnight at 37°C. Subsequently, 20 µl 3 M sodium acetate (pH 4.8) and 440 µl ice-cold absolute ethanol were added to the digested DNA and allowed to precipitate at -20°C overnight. The DNA was recovered by centrifuging at 14 000 rpm at 4°C for 45 min using a high speed centrifuge (Eppendorf 5810R). The supernatant was aspirated out and the resulting DNA pellet was washed with 70% ice-cold ethanol by centrifuging at 14 000 rpm at 4°C for 15 min. The DNA pellet was then dried using a speed vac centrifuge (Savant Oligo Prep™ OP120) for 10 min. The dried DNA pellet was then dissolved in 35 µl TE buffer, followed by addition of 5 ul of the loading dye, FOG. The samples were stored at 4°C (not longer than 72 hr) until required.

Electrophoresis of Genomic DNA for Southern Analysis

Gel electrophoresis was performed using 1% agarose gel. The agarose gel was prepared by adding 2.5 g SeaKem LE agarose powder to 250 ml 1x TPE buffer (90 mM tris-phosphate buffer, 2 mM EDTA pH 8.0). The gel was boiled in a microwave oven with frequent stirring until the powder dissolved and the solution was left at room temperature for about 5 min until the temperature cooled down to about 60°C. The gel was then cast in a 25 cm x 20 cm platform and allowed to solidify at room temperature.

The electrophoresis was carried out using a horizontal gel electrophoresis system (Horizon 20-25, Whatman Biometra) at 100 V for 5 min, followed by 25 V overnight. The gel contained 5 ng 1 kb DNA ladder (New England Biolabs) and 10 µl gel tracking dye in separate wells. Usually the gel was run until the bromophenol blue in the gel tracking dye reached the end of the gel tray. The gel was then stained with 20 µl ethidium bromide (10 mg ml^{-1}) in 200 ml electrophoresis buffer (1x TAE) for 30 min. The stained gel was viewed under UV light and photographed using a Polaroid camera. Prior to blotting, the gels were briefly washed with distilled water.

Southern blotting was carried out using the VacuGene XL Vacuum Blotting System (GE Healthcare). The DNA was transferred onto Hybond™-N+ nylon membrane (GE Healthcare) at a vacuum pressure of 50 mbar for 30 min using 0.4 M sodium hydroxide (NaOH) as the transfer buffer. The blotted membranes were washed with 2x SSC buffer (0.3 M NaCl, 30 mM trisodium citrate, pH 7.0) before being fixed for 20 s at 254 nm using a UV- crosslinker (Spectrolink XL-1000, Spectronics Corporation). The membranes were left to air dry and stored at 4°C until required.

RFLP Probe

The RFLP probe (G240) used was a complementary DNA (cDNA) clone obtained from a young etiolated seedling cDNA library constructed previously as described by Cheah (1996). The probe was provided by MPOB Biological Resource Centre (MBRC).

Labelling of Selected DNA Probe

The DNA probe to be labelled was diluted to a concentration of 5 ng μl^{-1} in TE buffer. The probe was then labelled using the Megaprime DNA Labelling System (GE Healthcare). A total of 50 ng probe was placed in a clean and sterile 1.5 ml microcentrifuge tube. To it was added 5 µl primer solution (supplied with the labelling kit) and sterile water to a final reaction volume of 50 µl. Denaturation of the solution was carried out by heating in a boiling water bath (100°C) for 10 min, and then it was immediately chilled on ice.

A brief spin was carried out to bring the contents to the bottom of the tube. Subsequently, 10 µl labelling buffer (provided in the labelling kit), 5 µl α^{32}P-dCTP (3000 Ci/mmol stock) and 2 µl Klenow enzyme (1 U μl^{-1}) were added to the mixture (to a final volume of 50 µl) and incubated at 37°C for 1 hr. The reaction was stopped by adding 25 µl blue dextran/orange G solution (1% blue dextran w/v, 1% orange G w/v in TE, pH 8.0). The labelled probe was separated from the unincorporated nucleotides by purification through a Sephadex column as described in Sambrook *et al.* (1989).

Southern Hybridization

Pre-hybridization and hybridization were carried out in glass tubes in a rotisserie oven at 65°C. The membranes were pre-hybridized for about 3 hr in a solution containing pre-hybridization buffer as follows: 5x SSPE solution (3 M NaCl, 0.2 M sodium phosphate, 20 mM EDTA pH 8.0), 0.5% SDS, 5x Denhardt's solution (0.1% ficoll, 0.1% polyvinylpyrolidone, 0.1% albumin bovine fraction V) and 100 µg ml^{-1} denatured herring sperm DNA. The pre-hybridization buffer was removed and replaced with hybridization solution containing 5x SSPE (3 M NaCl, 0.2 M sodium phosphate, 20 mM EDTA pH 8.0), 0.5% SDS and 100 µg ml^{-1} denatured herring sperm DNA. Labelled probes were denatured by heating in a boiling water bath for 10 min and plunging into ice water, before adding to the hybridization buffer. The probe was added to a concentration of about 1-3 x 10^6 cpm ml^{-1}. Hybridization was carried out overnight at 65°C.

The hybridized membranes were washed twice in 2x SSC (0.3 M NaCl, 30 mM trisodium citrate, pH 7.0) and 0.1% SDS at 65°C for 15 min each time, followed by once in 1x SSC (0.15 M NaCl, 15 mM tri sodium citrate pH 7.0) and 0.1% SDS at 65°C for 10 min. The membranes were then autoradiographed at -80°C using X-ray films with intensifying screens for seven to 10 days. The X-ray films were developed using 0.22x Kodak's GBX developer for 5 min, rinsed in distilled water, followed by a final wash with 0.2x Kodak GBX fixer for 5 min.

Labelling of Microsatellite Primers and Analysis on Acrylamide Gel

One primer pair was 5' end labelled at 37°C for 30 min using T4 polynucleotide kinase (Invitrogen, USA). The labelling reaction mixture contained 50 pmoles primer, 3 µl γ-^{33}P dATP (GE Healthcare, 3000 Ci/mmol) and 1 U T4 polynucleotide kinase in a total volume of 25 µl.

Subsequently, PCR was carried out in a 25 µl reaction containing 1 U *Taq* polymerase (Invitrogen, USA), 50 mM tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 µM primers, 0.2 mM dNTPs (Invitrogen, USA) and 50 ng template DNA. PCR was performed in a Perkin Elmer 9600 thermocycler heating program as described by Billotte *et al.* (2001):

94°C, 3 min (1 cycle);
93 °C /59 °C/72 °C, 30 s each (5 cycles);
93 °C/57 °C/72 °C, (35 cycles), 30 s each and
72 °C, 2 min (1 cycle).

After PCR was completed, the reaction was stopped by adding 25 µl formamide buffer (0.3% bromophenol blue, 0.3% xylene cyanol, 10 mM EDTA pH 8.0, 97.5% deionized formamide). The 3.5 µl of the PCR reaction mixture was subjected to electrophoresis in 6% denaturing acrylamide gel containing 7 M urea using 0.5x TBE buffer at a constant power of 40 W for 3 hr. The gels were then dried and exposed to X-ray film (Kodak) for three to four days at -80°C. Sizing of each allele was done using AFLP molecular weight ladder (Invitrogen, USA).

RESULTS AND DISCUSSION

Extraction of DNA

In this study, the yields of DNA from non-freeze-dried leaf and freeze-dried leaf tissues were compared. As shown in *Table 1*, the non-freeze-dried (NFD) tissue yielded about two-fold more DNA than the freeze-dried (FD) tissue. The higher yield from the NFD leaf was probably due to more effective tissue disruption by the protocol as the tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. The FD samples, on the other hand, were simply ground to powder using a

blender. Nevertheless, the average yield obtained from the FD samples, 215 μg/0.1 g tissues, would be sufficient for most molecular biology experimentation. The advantages of using FD samples are:

- saves cost as it uses less liquid nitrogen;
- saves effort and time, as grinding in a blender takes only 5 min, compared to manual grinding which can take up to 45 min; and
- increases the throughput of samples that can be processed in a single day. In this study, it was observed that four times as many samples can be processed per day per person, using FD oil palm leaves.

However, if a higher DNA yield is required, the blended FD leaf samples can be further manually ground to a finer state. As shown in *Table 1*, this increased the yield to about 550 μg/0.1 g tissue, much closer to the NFD yield. This will, however, increase the cost and reduce the number of samples that can be processed. An alternative approach is just to obtain more DNA by extracting more FD tissue (0.5 g tissue) with the same buffer volume. This approach does not require additional manual grinding.

The method used to prepare DNA from the FD tissue was similar to that used for the NFD tissue except that grinding the tissue under liquid nitrogen in a mortar and pestle was not done for the FD samples. During DNA preparation, the pre-warmed modified extraction buffer (20 ml) was added immediately to the pre-weighed FD powder (0.4 g) placed in a 50 ml centrifuge tube. This was to minimize exposure to moisture which could have affected the stability of the FD material. The subsequent DNA extraction procedure was the same for both the FD and NFD tissues.

Quality of DNA

An important aim of this study was to evaluate the integrity of the genomic DNA extracted from FD leaf of oil palm. The quality of DNA extracted from the NFD and FD leaves was compared. For this purpose, 5 μg undigested DNA, *Eco*RI-digested DNA and *Hae*III-digested DNA were run on an agarose gel and visualized by ethidium bromide staining. The results in *Figure 1* confirmed that the DNA prepared from the FD leaf tissues was intact and of high molecular weight. The DNA prepared from the FD leaf was completely digested by the restriction enzymes, *Eco*RI and *Hae*III, indicating that there were no impurities inhibiting digestion.

There was no difference in the quality of the DNA extracted from the NFD and FD leaves. The purity of the DNA as determined by the A_{260}/A_{280} ratio (1.8) was high, as shown in *Table 1*. Thus, the freeze-dried oil palm leaf was able to provide high molecular weight DNA of suitable quality, comparable to that obtained from NFD and frozen leaf.

Effects of Storage Temperature and Duration on DNA Yield and Quality

This study also investigated two storage temperatures (-20°C and 4°C) on the yield and quality of DNA from FD leaves of oil palm after various times in storage. This was accomplished by dividing the powdered FD leaves into equal portions (0.4 g / sample) which were then sealed in plastic bags and stored at -20°C and 4°C. The samples were then taken at 2, 4, 6, 12 and 18 months for DNA extraction. The DNA integrity was confirmed by agarose gel electrophoresis and ethidium bromide staining as well as by the absorbance at 260/280 units. As shown in *Figure 2*, there was no significant difference between the results for storage at -20°C (*Figure 2a*) and 4°C (*Figure 2b*). Both the differently stored tissue, at all sampling times, yielded high molecular weight DNA readily digestible by the restriction enzymes, *Eco*RI and *Hae*III. Thus, the DNA in the FD tissue can remain stable for up to 18 months even at 4°C storage.

The DNA yield was also not substantially affected by the storage temperature (*Table 2*). However, there was a slight deterioration in the quality of the DNA after longer storage. The A_{260}/A_{280} ratios for DNA from the FD tissue stored for 18 months at -20°C and 4°C were 1.6 and 1.5, respectively, which were lower than those obtained for shorter storage periods. This was probably due to some degradation of the DNA, although this was not obvious in the gel pictures.

Moisture and oxygen are two important factors which can affect the stability of FD materials. Their effects are temperature dependent. The FD leaf can be maintained at refrigerator temperatures, *i.e.* 4°C

TABLE 1. YIELDS AND PURITY OF GENOMIC DNA EXTRACTED FROM NON-FREEZE-DRIED (NFD) AND FREEZE-DRIED (FD) LEAVES OF OIL PALM

Sample	Ave. yield of DNA#	Purity (A260/A280)
NFD[1]	680 μg/g	1.8
FD[2]	215 μg/0.1 g	1.8
FD (additional grinding)[3]	550 μg/0.1 g	1.8

Notes: [1] DNA extracted from non-freeze-dried (NFD) leaf using the liquid nitrogen extraction method.
[2] DNA extracted from freeze-dried (FD) and powdered leaf without use of liquid nitrogen.
[3] DNA extracted from freeze-dried (FD) and powdered leaf, after additional grinding in liquid nitrogen
The figures are average yield of DNA from six experiments.

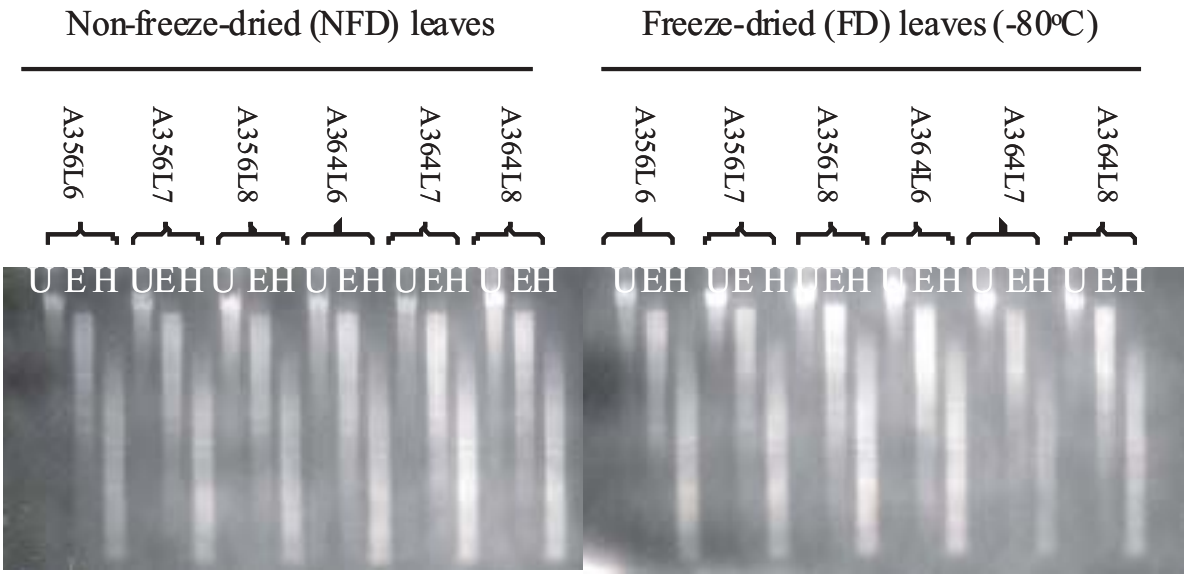

Figure 1. Quality of deoxyribonucleic acid (DNA) extracted from non-freeze-dried (NFD) and freeze-dried (FD) leaves. Agarose gel electrophoresis of 5 μg undigested DNA (U), 5 μg DNA digested with EcoRI *(E) and 5 μg DNA digested with* HaeIII *(H).*

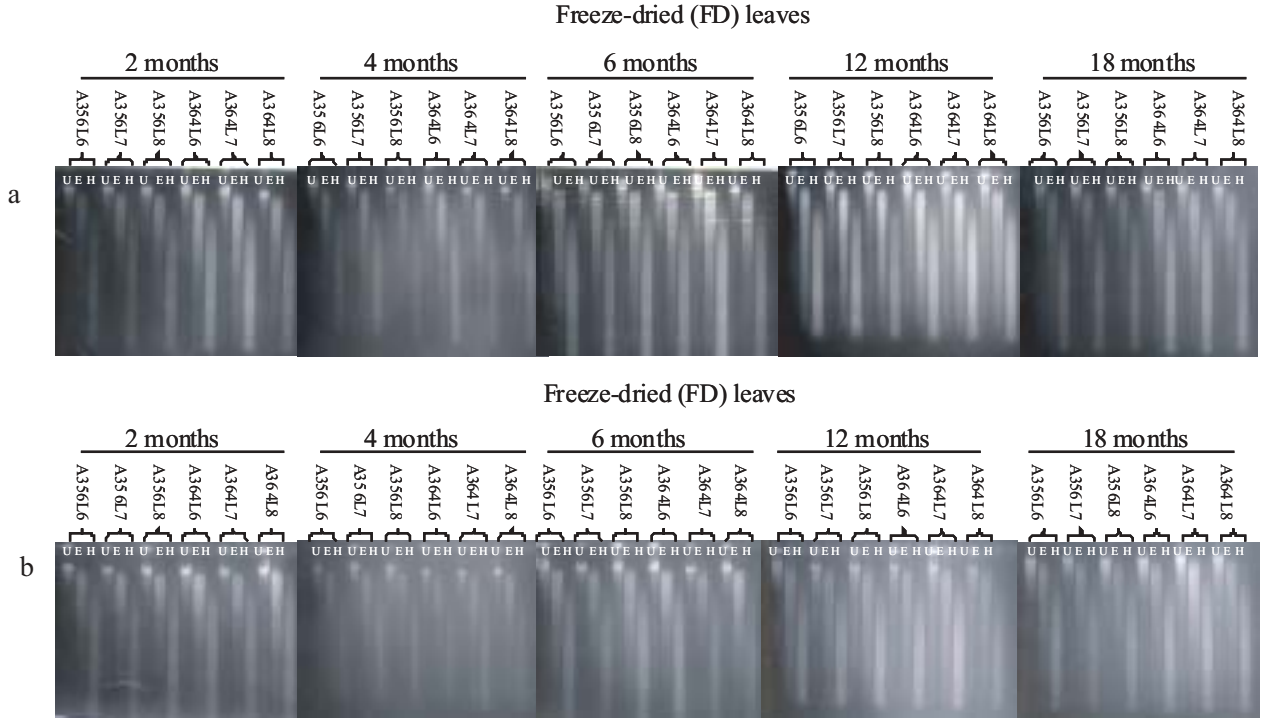

Figure 2. Quality of deoxyribonucleic acid (DNA) extracted from freeze-dried (FD) leaf tissues of oil palm stored at -20°C (a) and 4°C (b). The samples were removed at various time intervals for testing. Ethidium bromide was used to stain 0.9% agarose gel of 5 μg undigested DNA (U), 5 μg DNA digested with EcoRI *(E) and 5 μg DNA digested with* HaeIII *(H).*

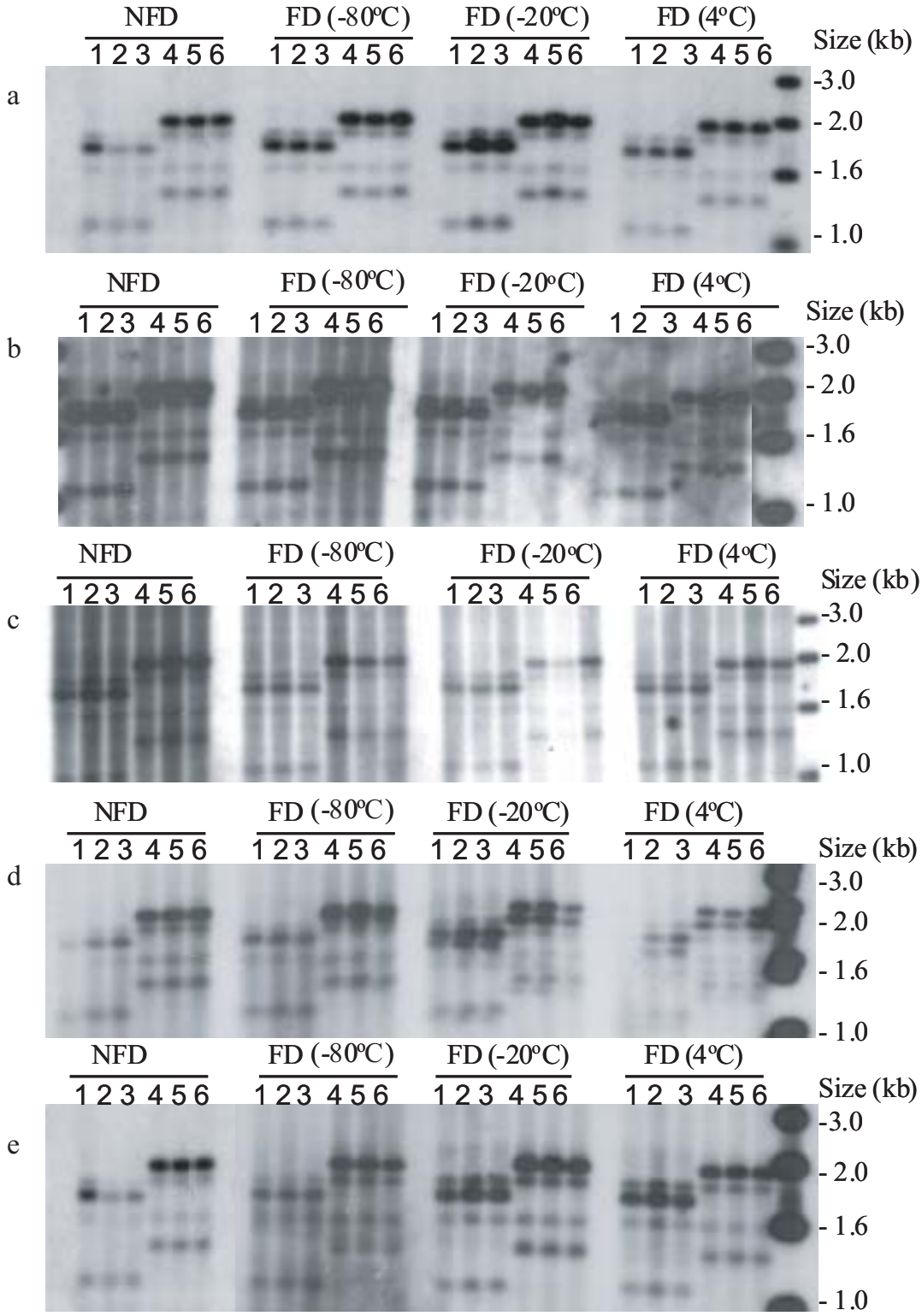

Figure 3. Southern blot analysis of DNA extracted from freeze-dried (FD) and powdered oil palm leaf compared to non-freeze-dried (NFD) leaf and freeze-dried (FD) controls (stored at -80°C). Genomic DNA was digested with the restriction enzyme RsaI *and probed with G240. Total DNA was prepared from freeze-dried (FD) and powdered leaf stored at -20°C and 4°C for two months (a), 4 months (b), 6 months (c), 12 months (d) and 18 months (e). Samples 1-3 are for genomic DNA from clone A356 and samples 4-6 for genomic DNA from clone A364. These results showed that intact DNA suitable for RFLP analysis can be extracted from freeze-dried (FD) and powdered oil palm leaf stored for up to 18 months at -20°C and 4°C.*

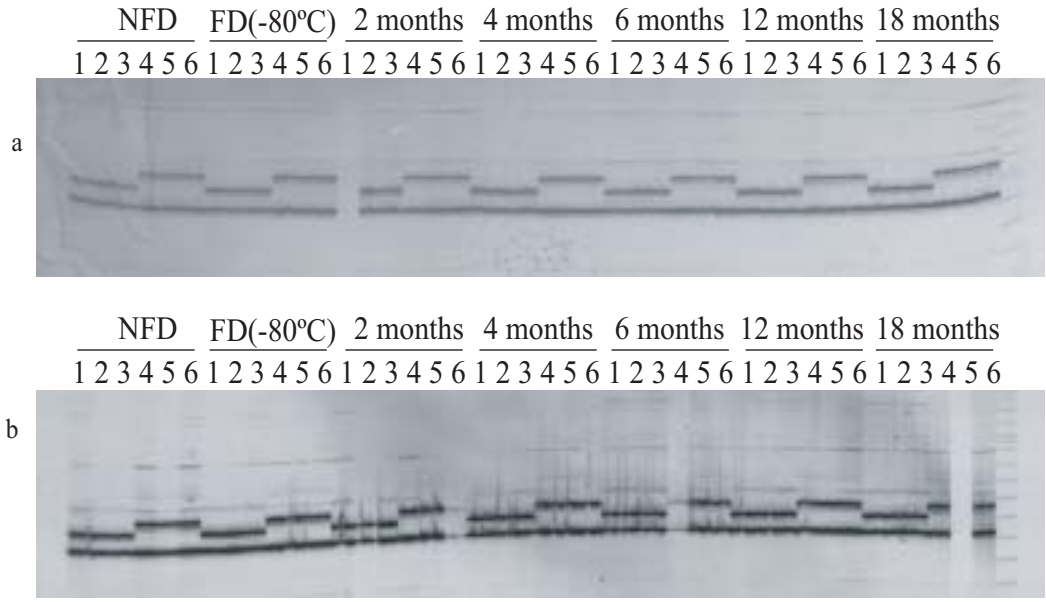

Figure 4. SSR analysis of DNA extracted from freeze-dried (FD) and powdered oil palm leaf compared to the non-freeze-dried (NFD) and freeze-dried (FD) controls (stored at -80ºC). Total DNA was prepared from freeze-dried (FD) and powdered leaf stored at -20ºC (a) and 4ºC (b). The leaves were extracted for DNA after 2, 4, 6, 12 and 18 months storage. Samples 1-3, genomic DNA from clone A356; samples 4-6, genomic DNA from clone A364.

TABLE 2. YIELDS AND PURITY OF GENOMIC DNA EXTRACTED FROM FREEZE-DRIED (FD) LEAVES OF OIL PALM STORED AT DIFFERENT TEMPERATURES (-20°C and 4°C) AND FOR DIFFERENT STORAGE DURATIONS

	Storage temp. (-20°C)		Storage temp. (4°C)	
Strorage duration (months)	Ave. yield of DNA* (μg/0.1 g freeze dried tissue)	Purity (A_{260}/A_{280})	Ave. yield of DNA* (μg/0.1 g freeze dried tissue)	Purity (A_{260}/A_{280})
0	259	1.7	276	1.7
2	200	1.7	199	1.7
4	193	1.7	200	1.7
6	297	1.7	283	1.7
12	264	1.8	268	1.7
18	230	1.6	228	1.5
Ave. yield of DNA =	240.5		242.3	

Note: * DNA extracted from freeze-dried (FD) and powdered leaf without use of liquid nitrogen.

and -20°C, for long periods as clearly shown by this study. However, storage in even lower temperatures can probably extend their shelf life (Labconco, 2004).

Applications of DNA from Freeze-Dried Leaf Tissues in RFLP and SSR Analyses

The integrity and quality of the genomic DNA extracted from FD oil palm leaf were further validated by RFLP and SSR analyses. In the RFLP analysis, 20 μg genomic DNA were digested with the restriction enzyme, *Rsa*I and probed with an oil palm cDNA probe (G240). As shown in *Figure 3*, the DNA extracted from the FD tissue gave comparable results to the NFD tissue. RFLP analysis using DNA isolated from the FD tissues stored at -20ºC and 4ºC produced identical hybridization profiles at all storage durations of 2, 4, 6, 12 and 18 months (*Figures 3 a-e*).

Similar results were also observed for the SSR analysis. As shown in *Figure 4*, similar bands were produced at -20ºC and 4ºC storage temperatures and at all storage durations. The results clearly indicate that high molecular weight genomic DNA suitable for restriction enzyme digestion and template for PCR amplification can be isolated from FD leaf of

oil palm. This study also revealed that DNA extracted from FD tissues stored up to 18 months at temperatures as high as 4°C maintained their integrity and suitability for use in molecular genetic studies. Freeze-drying the oil palm leaf allows for its efficient storage in freezers, where space is limited. Furthermore, even a 4°C refrigerator and a -20°C freezer are suffice for storage for up to 18 months. This can help relieve space in -80°C freezers, which are usually required to store NFD or frozen leaf tissues.

Freeze-drying can also be employed in oil palm research stations, which normally lack ultra low temperature freezers, for initial processing and storage of leaf samples for molecular biology applications. This can help the stations to rescue materials from important palms which have unexpectedly fallen due to disease or other factors for future research or reference.

CONCLUSION

The results of this study showed that freeze-drying of oil palm leaf is an acceptable method for space-efficient storage of the material for genomic DNA extraction. The freeze-dried tissue can be stored at temperatures as high as 4°C for up to 18 months, with no evidence of DNA degradation. The ability to freeze-dry oil palm leaf for DNA extraction provides an economical solution for the long-term storage of the tissue, as it frees precious freezer space.

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