Techniques In Genomic DNA Extraction From Palm Oil Leaves

Introduction

The oil palm (Elaeis guineensis Jacq.) having a place with the family Arecaceae [1], a diploid oil-delivering crop with a genomic size of 1.8 Gb [2], is one of the most important oil-bearing crops in the world. It is a large feather palm having a solitary columnar stem, short internodes, and short spines on both the leaf bases and within the fruit bunches [3]. It has irregular sets of leaflets on the leaf, which gives the palm its characteristic appearance. The palm is monoecious with male or female inflorescence, but hermaphroditic inflorescences sometimes develop in the axils of the leaves [3]. The fruit, which is borne on the large compact bunch, is called a drupe [1]. Distinguishing the different types of oil palms has been controversial. These attempts have been unsatisfactory since in the wild state, each palm represents a hybrid with respect to some of its traits [1]. Oil palm is classified based on the fruit type and fruit form. It has three fruit forms: Dura, Pisifera and Tenera (hybrid fruit form) and different fruit types namely, virescens, albescens, nigrescens and poissoni [1]. Oil palm has benefited immensely from conventional breeding program in Nigeria. This has solely been made possible through the dedicated breeding program put in place by the Nigerian Institute for Oil Palm Research (NIFOR).

The progress made so far has been very limited for two reasons: (1) the long generation time and (2) the outcrossing nature of the crop. With the emergence of deoxyribonucleic acid (DNA) marker technologies, scientists see the possibility that significant success can be achieved if markers are extensively applied by oil palm breeders as exemplified by the cloning of the shell thickness gene [4]. Exploration of DNA marker technologies, combining the knowledge from research in molecular genetics and genomics, offers great possibilities to oil palm breeding [5]. With DNA marker technologies, the underlying genetic basis of phenotypic traits can be studied independent of environmental influences. However, critical to the adoption, application and the domestication of these technologies is an effective DNA extraction method that is less complex than the methods that have been previously applied to extract DNA from palms [6-10]. The basic principles underlying DNA extraction procedures are not very complicated, but the growing numbers of DNA extraction procedures indicate that it is not always simple and the published protocols are not necessarily reproducible for all species [11,12]. The objective of this study was to develop a method for DNA extraction from oil palm leaves that is cost effective and adaptable to low budget laboratories

Background

A number of commercial genomic DNA extraction kits are available to speed up the extraction process. However, the use of commercial kits to isolate oil palm DNA is mostly expensive and often does not give satisfactory results compared to the conventional protocol (Ying and Faridah, 2006). The hexadecyltrimetyhlammomnium bromide (CTAB) protocol described by Doyle and Doyle (1987; 1990) is one of the conventional methods commonly used for the isolation of DNA from plant species (Borges et al., 2012) including oil palm. In contrast to commercial kits, this protocol is time- consuming and laborious and as such, can be a problem if

DNA is to be extracted from hundreds of samples. Therefore, there is a need for a rapid and simple extraction procedure that yields good quality and quantity of genomic DNA. Several protocols for rapid preparation of DNA from plant tissues have been reported (Ausubel et al., 2003; Dhakshanamoorthy et al., 2009; Arif et al., 2010) and can be exploited for extracting DNA from oil palm.

The existing modified CTAB-based protocol applied in our laboratory is a combination of methods described by Saghai-Maroof et al. (1984), Rogers and Bendich (1985) and Doyle and Doyle (1987) which, was published by Weising et al. (1995). This method has sucessfully produced high quality and quantity of DNA but only allows a small number of samples to be processed at a time. The current method yields about 200 – 680 µg DNA g-1 leaf tissue (Rahimah et al., 2006). Since the oil palm tissue is very fibrous, approximately 1 to 2 hr is spent grinding four to six samples using mortar and pestle in liquid nitrogen. As such, one laboratory technician can only handle a limited number of samples per day. Additionally, four days are needed to complete the entire extraction protocol. Due to these drawbacks, initiative was taken to test a published DNA extraction protocol that can be completed within a day, and gives the required quality and quantity.

This study explored the DNA extraction protocol described by Arif et al. (2010). The published protocol of Arif et al. (2010) suggests that grinding the tissue in the extraction buffer (with NaCl) and sterile sand provides acceptable DNA yield suitable for routine molecular biology analysis including PCR amplification. The protocol omits the use of liquid nitrogen (N2), polyvinylpyrrolidone (PVP) and lithium chloride (LiCl) and reportedly produces on average 70 µg DNA g-1 sample. The protocol as described was tested on oil palm tissues, but did not produce sufficient amount of DNA for certain applications and the quality was also slightly below expectation. As such, this study describes minor modifications to the extraction protocol of date palm described by Arif et al. (2010) for routine isolation of acceptable quality and quantity of DNA from oil palm tissues.

Problem Statement

The method stated by Arif et al. was chosen due to the hardy and fibrous nature of the date palm leaves which is quite similar to oil palm leaves. The protocol almost simple and it could provide sufficient DNA yield, and appears convenient for daily extraction of DNA from palm oil samples.

Using the same amount of start-up materials (0.1g) processed using the older protocol, the DNA quality and yield varies and the data was fairly inconsistent. In addition, the original protocol just utilizes 0.1 g of starting tissue with 500 microlitre buffer in 1.5 ml Eppendorf tube. The DNA yield obtained from 0.1 g oil palm tissue was generally less50 microgram, and that is not sufficient for some applications such as restriction fragment length polymorphism (RFLP).

Discussion

DNA Extraction

Initially the method described by Arif et al. (2010) was applied without modification. A total of 10 samples were evaluated. DNA from the same sample was then re-extracted using the modified

method described below:

The 2X CTAB lysis buffer (2% cetylmethylam- monium bromide, 100 mM Tris-HCl pH 8.0, 20 m MEDTA pH 8.0, 1.4 M NaCl, and 2% PVP-40), 7.5 ml, was pre-heated in a glass beaker to 60°C in a water bath. While the buffer was heating, 2 g of frozen leaves was ground using a sterile mortar and pestle with gradual addition of liquid N2. The 250 mg sterile acid sand was added into the mortar prior to the grinding process. The frozen fine powder was left at room temperature for 5 min.

Seventy-five µl each of 0.5 M ascorbic acid, 0.4 M DIECA, and 1% ?-mercaptoethanol were added to the pre-heated 2X CTAB lysis buffer (this step was carried out in the fumehood). The powdered tissue was further thawed by immersing the mortars in warm water. Following this, 7.5 ml of the lysis buffer was added into the mortar and gently mixed using the pestle. The mixture was then transferred into a 15 ml Falcon tube, vortexed briefly and incubated at 60oC for 30 min in a shaking water bath. The samples were then allowed to cool at room temperature for 30 min. This is followed by centrifugation at 4000 rpm for 30 min at 25°C in a swing-bucket rotor of the Eppendorf Centrifuge 5810 R. Six millilitres of the upper aqueous phase was carefully transferred into a new Falcon tube using wide bore pipette. An equal volume (6 ml) of chloroform: isoamyl alcohol (24:1) was added and the tube was vigorously shaken for 5 min to mix the solution thoroughly. DNA was precipitated by centrifugation at 4000 rpm, 30 min, 25°C. About 5 ml of the supernatant was again transferred to a new Falcon tube and treated with 6 µl of RNAse at 37°C or room temperature for 30 min. The 500 µl of 3.0 M sodium acetate and 1 volume (5 ml) of cold isopropanol were added gently, mixed and kept in -20°C freezer for at least 30 min. After centrifugation at 4000 rpm, 4°C for 30 min, the supernatant was discarded. The pellet was resuspended in 2 ml of wash buffer (76% ethanol, 10 mM ammonium acetate) and kept at 4°C for at least 30 min. The wash buffer was carefully poured off and the pellet was dried in a speed vacuum for 10-15 min. Two millilitres of 70% cold ethanol was added and kept at room temperature for 15 min. The ethanol was discarded and the tubes were placed in a speed vacuum to allow complete drying of the pellet. The dried DNA pellet was then carefully transferred into a 1.5 ml eppendorf tube and dissolved in 300-350 µl of TE buffer, depending on size of the pellet, followed by incubation at 50°C in a shaking water bath. After the pellet was fully dissolved, a small aliquot of the DNA samples was electrophoresed in 0.8% agarose gels at 100V for 1.5 hr. The results were visualised after staining the gels in 0.5 µg ml-1 ethidium bromide (EtBr) solution.

DNA Quantification

The total DNA yield and purity index were determined using Multiskna Go (Thermo Sciientific). Five microgram of eachof the extracted DNA was also digested with six (BGIII) and four (HaeIII) base pair cutter restriction enzymes to check its digestibility. The DNA was also tested to evaluate its suitability for simple sequence repeat (SSR) analysis and single nucleotide polymorphism (SNP) genotyping.

Conclusion

In order to obtain higher DNA yields, the Eppendorf tube was replaced with 15 ml volume Falcon tube, so that the starting material could be increased to 2 g. The lysis buffer then was increased to 7.5 ml. Liquid nitrogen may not be necessary in the method by Arif et al. (2010)

because the authors used fresh tissue. But it is not possible in some cases the leaf samples were stored frozen in -80 degree celcius in the freezer befor the extraction process happens. This is due to the sampling site was quite far from the laboratory, so extraction process was not possible in the same day the samples were collected. Because of the distance, the sample must be cleaned and frozen in liquid nitrogen as soon the sample has arrived from the sampling site and kept in in -80 degree celcius in the freezer. The advantage of this procedure over the original method is that it allows larger amount of tissue to be used as starting materials. Higher yield of genomic DNA can be obtained for various analysis and long term storage. The method is quite simple and it could be done in two days. A laboratory technician could easily process up to 12 samples a day. Furthermore, the quality and the yield of DNA is also similar to the conventional method. Therefore, we conclude that the modified method can yield DNA for routine molecular biology studies of oil palm and perhaps also useful for other plant species.

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