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A Rapid and Inexpensive Method of DNA Extraction from Palmyra Palm (*Borassus flabellifer*)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: The study aims to highlight the simple optimisation, inexpensive and rapid procedure for DNA isolation from tough leaves (Palmyra palm) without compromising the yield and purity of DNA.
Study Design: Leaf of palmyra palm (*Borassus flabellifer*) was used to conduct the experiment followed by laboratory analysis, DNA extraction and PCR amplification.
Results and Discussion: The results showed that different buffers examined for the extraction of DNA provided significantly different levels of yield and purity. DNA isolated by lysis buffers C showed satisfactory amplifications in PCR. The fingerprint we obtained by using the DNA extracted by these buffers provided higher resolution than those using buffers.
Conclusion: This study suggests that grinding of Palmyra palm leaves with sterile sand or cover slips and inclusion of SDS, Tween 20, and NaCl (1.4 M) in the lysis buffer without the costly use of liquid nitrogen, PVP and β mercaptoethanol, provides a DNA yield of sufficient purity, suitable for PCR amplification and subsequent use.

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Keywords: Palmyra; DNA; extraction; amplification; quality.

1. INTRODUCTION

Palmyra palm is botanically known as *Borassus flabellifer L.* and belongs to the family *Arecaceae* is dioeciously in nature. The genus *Borassus* is an extensively disseminated palm seen from western Africa and Madagascar to eastern Indonesia and Papua New Guinea [1]. *Borassus flabellifer* L., or palmyrah, is commonly distributed in India, Southeast Asia and rarely in other temperate regions of the world [2]. It is a multipurpose tree of enormous use and the palm is dispersed along the coastal belts of India, northern Sri Lanka and SE Asia and eastern Indonesia [3]. The palm is located from sea level up to 760 m. The palms are scattered in Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Orissa, Maharashtra, Bihar, Madhya Pradesh and West Bengal. Limited numbers of palms are also seen in Gujarat, Assam and Uttar Pradesh [4]. The palmyrah palm has been closely associated with human culture and tradition from the prehistoric era. It is an economically important palm and is used widely as timber and firewood, for making fibres of various kinds, thatching material, furniture and in the food industry for making sugar, starch and many folk medicines [5]. The use of molecular markers to assess genetic diversity is essential to refine and complement classification based on morphological data [6]. Understanding the genetic diversity and similarities existing within populations is vital for effective management of germplasm in a breeding programme as the breeders can use the data in the development of breeding populations and crossing programmes to exploit hybrid vigour. Plant DNA isolation methods require grinding of the plant material in liquid nitrogen [7]. Therefore, any tissue immersed in liquid nitrogen abruptly converts to brittle solid to facilitate its powdery form. Palmyra palm leaves are hard, fibrous and the extraction of genomic DNA from the leaves is difficult [7]. To overcome the preservation related problems and use of liquid nitrogen, use of coverslips/sterile sand is general for grinding the leaves of Palmyra palm. In many developing countries, liquid nitrogen is not easily available and its storage and maintenance is also difficult [7]. There are three main contaminants associated with plant DNA that can cause considerable difficulties when conducting PCR experiments: polyphenolic compounds, polysaccharides and other unknown compounds, these compounds may interfere with the successful isolation of PCR amplifiable DNA [8]. In this present study, for the first time, we are reporting a simple and inexpensive method of DNA extraction from Palmyra palm, which is more suitable for PCR amplification. The study aims to optimize a simple, inexpensive and rapid procedure for DNA isolation from tough leaves (Palmyra palm) without compromising the yield and purity of DNA.

2. MATERIALS AND METHODS

Leaf of palmyra palm (100 mg) was placed in a sterile mortar. Sterile sand (50 mg)/cover slips and 1000 µL of lysis buffer (Table 1; lysis buffers A to C) were added separately to the sterile mortar. Leaf sample was finely crushed using mortar and pestle and allowed to dry at room temperature for about 5 min. Crushed leaf sample with sand (50 mg) or coverslips was transferred into a 1.5 mL Eppendorf tube. The tube was then kept in a water bath at 60°C for 30 min. After mixing by brief vortex, the tube was centrifuged at 10,000 rpm for 5 min. An aliquot of supernatant (200 µL) was transferred to a new tube, taking care to avoid carryover of any dirt or debris. An equal volume (200 µL) of chloroform: isoamyl alcohol (24:1) was added and the tube was shaken gently top to bottom for 5 min followed by centrifugation at 10,000 rpm for 5 min. The supernatant (200 µL) was transferred to a new tube and sodium acetate (3.0 M; 20 µL) plus cold isopropanol (500 µL) was added gently and the tube was kept in the freezer for 5 min followed by centrifugation at 8000 rpm for 10 min. The resulting supernatant was discarded and 500 µL of 70% cold ethanol was added. After centrifugation at 8,000 rpm for 5 min, the supernatant was discarded and the tube contents were air dried at room temperature. DNA was eluted with 30 µL of TE buffer, add 5 µl RNase and kept at 37°C for 1 hr. Store the DNA at 4°C for further use.

2.1 Analysis of Isolated DNA

A long (20×14 cm) 0.8% agarose gel using 1x TAE buffer containing 0.5 µl/ml of ethidium bromide was used for electrophoresis purposes. Gel image was visualized using Proxima C16 UV transilluminator and Opticom imaging system. Gel image analysis and the sizes of DNA bands were determined using 100 base-pair ladders.

Table 1. Constituents of Lysis buffers (100 ml, pH 8.0)

Lysis buffer	Main components	Additives
A	10mMTris+20mM EDTA+ 2% CTAB (w/v)	1.4M NaCl + 1% PVP+1%βmercapto ethanol
B	10mMTris+20mM EDTA+ 2% CTAB (w/v)	1.4M NaCl + 1% PVP+1%βmercapto ethanol + 1% Tween 20
C	10mMTris+20mM EDTA+ 2% CTAB (w/v)	1.4M NaCl + 1% PVP+1%βmercapto ethanol + 1% Tween 20 + 1% SDS

Tris- Trisaminomethane, EDTA –ethylinediamine-tetra-acetic acid, CTAB- cetyltrimethylammonium bromide, NaCl – sodium chloride, PVP- polyvinylpyrrolidone , SDS – Sodium Dodecyl Sulfate

3. RESULTS AND DISCUSSION

3.1 DNA Yield and Purity

Fig. 1 shows the effects of different buffers on DNA yield and purity. Different buffers that were examined for DNA extraction gave significantly different levels of yield and purity. DNA extracted with the buffer C gave a higher yield than buffers A and B (Fig. 1). To assess the purity of DNA with respect to protein contamination since proteins (in particular, the aromatic amino acids) tend to absorb at 280 nm, the ratio of ODs at 260 nm and 280 nm is commonly used. A ratio of ~1.8 is generally accepted as pure DNA [7]. A lower ratio, indicates the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. In the present study the ratio of OD values at 260/280 nm was more or less similar (1.7 to 1.9) for all the buffers except buffer A (F1,). A secondary measure of nucleic acid purity is based on the ratio of OD values at 260 nm and 230 nm. The 260/230 values for pure nucleic acid are often higher than the respective 260/280 values [7]. Generally, 260/230 values lies within the range of 2.0–2.2. A lower value, may indicate the presence of contaminants which absorb at 230 nm. Ethylenediaminetetraacetic acid (EDTA), carbohydrates and phenol absorb near 230 nm. We found that OD values of 260/230 nm for DNA-extraction using buffers C (1.8 to 1.9) were significantly different compared with the others (Fig. 1).

3.2 PCR Amplification

RAPD-PCR is done to examine the amplification of the isolated DNA using different lysis buffers (Fig. 2). DNA isolated using lysis buffers C showed satisfactory amplifications in PCR. The fingerprint obtained by using the DNA extracted by these buffers provided higher resolution than those using buffers A and B. This did not showed up in the expected PCR products.

3.3 Effect of SDS, TWEEN 20, PVP and NaCl

Sodium Dodecyl Sulfate, Molecular Biology Grade (SDS), is a detergent that is known to denature proteins. It is used in nucleic acid extraction procedures for the disruption of cell walls and dissociation of nucleic acid: protein complexes. TWEEN 20 is a nonionic detergent widely used in biochemical applications. It has been used as an emulsifying agent for the preparation of stable oil-in-water emulsions. TWEEN 20 has been used in pre-extraction of membranes to remove peripheral proteins (used at 2% for extraction of membrane-bound proteins). PVP is used to bind the polyphenolic compounds [9], purge polyphenols [10] and may promote precipitation of the phenolic compounds [7,11]. The presence of polyphenolic compounds was observed to be reduced after using PVP in the DNA extraction procedure [12,13]. However, the present study showed that the inclusion of PVP in the lysis buffer did not significantly improve the DNA yield or purity compared with SDS and Tween-20.

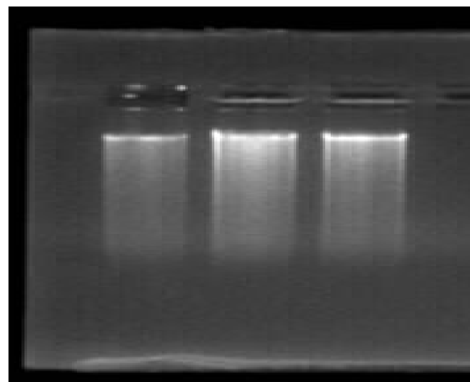

Fig. 1. Comparison of DNA-extraction using different buffers (it is recommended to mark/indicate on the image which is buffered A, B, and C)

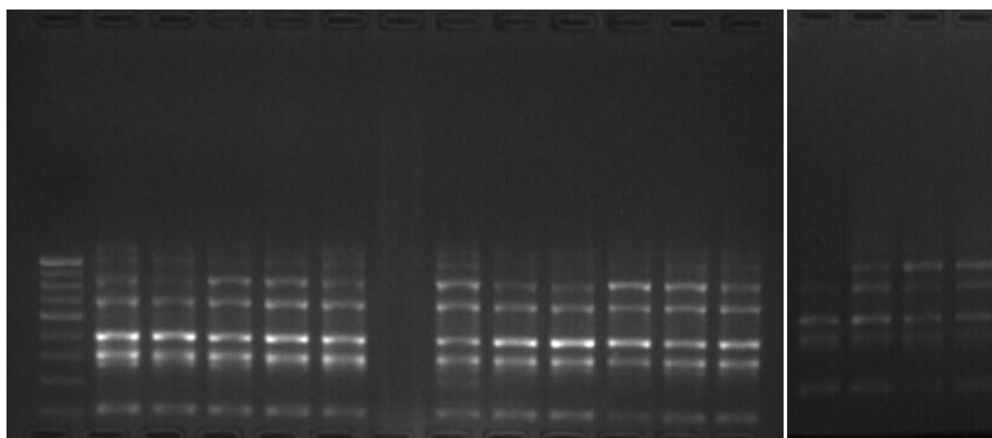

Fig. 2. PCR amplification using isolated DNA by different lysis buffers as a template

4. CONCLUSION

In conclusion, this study suggests that grinding of Palmyra palm leaves with sterile sand or coverslips and inclusion of SDS, Tween 20, and NaCl (1.4 M) in the lysis buffer without the costly use of liquid nitrogen, PVP and β mercaptoethanol, provides a DNA yield of sufficient purity, suitable for PCR amplification and subsequent use.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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