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# Rapid Isolation of Genome DNA Suitable for PCR from Tropical Almond (*T. catappa)* Plant Populations

B.O. Oboh, L.A. Ogunkanmi and N. Agwu
Department of Cell Biology and Genetics,
Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria

**Abstract:** This study was conducted to develop a rapid and efficient method for the isolation of genomic DNA from the tropical woody tree, *Terminalia catappa* L. Fresh young leaves from 35 trees were sampled for the extraction of genomic DNA. The methodology employed excluded the use of liquid nitrogen and an ultracentrifuge with various modifications in the quantities and reagents used. The result of the extraction showed that genomic DNA of good quality and quantity with a spectrophotometric ratio of between 1.7-2.0 for the trees sampled. Result further showed that the extracted DNA on 1% agarose gel had high molecular weight bands following electrophoresis. Thus, we concluded that the modified protocol used for the extraction of genomic DNA in *T. catappa* which can be easily adapted to other crops produced DNA of good quality and quantity which can be used for PCR based studies.

**Key words:** *Terminalia catappa*, genomic DNA, absorbance, electrophoresis, agarose, ultracentrifuge

## INTRODUCTION

Tropical almond (*Terminalia catappa* L.) is an important tree crop belonging to the family Combretacea. It is also called wild almond, India almond, sea almond, etc and widely grown in tropical region of the world as an ornamental tree. The world's production of this fruit is about 700,000 tons annually. The sweet variety, *Prunus delcis vardulas*, is the source of edible almond nuts. The nut (seed) which contains high quantity of essential amino acids is a good source of dietary protein, with crude protein content of 25.81% (Ezeokonkwo and Dodson, 2004; Nwosu *et al*., 2008). It is native to most countries of Southeast Asia especially in the tropical and subtropical zones. It however occurs in the West African region in areas with high rainfall (1000-3500 mm) and elevations below 300-400 m from Senegal to West Cameroon (Thomson and Evans, 2006). It is cultivated in Nigeria as a shade tree, for its fruits and seeds, for medicinal uses, as wind breakers and supplements for livestock feeds in both savannah and tropical regions (Nwosu *et al*., 2008). The medicinal uses of the leaves include: the treatment of liver related diseases, sickle cell disorders, cancers, anaemia (Oboh *et al*., 2008), tuberculosis (Adeleye *et al*., 2008) and fungal infections (Masoko and Eloff, 2005). *Terminalia sericea* has recently been selected as one of the 50 most important medicinal plants in Africa by the Association for African Medicinal Plant Standards (Masoko and Eloff, 2005).

Population studies of *T. catappa* have suggested variation in fruit size, fruit colour and leaf characteristics (Lepfosky, 1992; Oboh *et al*., 2008). These studies have usually been based on the classification of qualitative and quantitative characters. The need to study the germplasm of crops using molecular methods in addition to quantitative methods have been recommended by a number of authors, including: Samal *et al*. (2003) and Taamalli *et al*. (2006). However, it has been shown that DNA extraction protocols are specific for different plant species and the extraction is not always simple and reproducible (Pandey *et al*., 1996; Porebski *et al*., 1997).

Protocols for extracting genomic DNA have to contend with 2 major challenges: (1) preventing the oxidation of phenolic substances that can react with nucleic acids and proteins and (2) eliminating polysaccharides that interfere with the enzymatic manipulations of DNA in molecular biology research (Vallejos, 2007). Woody-tree crops have a high level of polysaccharides which pose a problem in DNA extraction as they have been found to inhibit the activity of DNA modifying enzymes, inhibit RAPD reactions and also interfere with the quantification of nucleic acids by spectrophotometric methods (Varadarajan and Prakash, 1991; Wikie *et al*., 1993; Pandey *et al*., 1996). The problem of polyphenol and polysaccharides is further aggravated if green over matured tissues are used rather than etiolated leaves (Sharma *et al*., 2000). There is

**Corresponding Author:** B.O. Oboh, Department of Cell Biology and Genetics, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria

apparently no published specific protocol for the extraction of genomic DNA from *T. catappa* and lack of a rapid and easy DNA extraction method is commonly encountered in many laboratories in developing countries. Ogunkanmi *et al*. (2008) modified and reviewed Dellaporta protocol for DNA extraction from the preserved tissues of the *Capsicum* sample which was believed to contain high level of polysaccharides. The modified protocol yielded a high quality DNA suitable for PCR and RAPD analyses and does not require phenol-chloroform extraction.

Therefore, this study aims at developing a rapid protocol for extracting pure genomic DNA from young leaves of *T. catappa* that would be suitable for PCR studies.

## MATERIALS AND METHODS

**Plant material:** Fresh young leaves from 35 *T. catappa* trees were harvested early in the morning from two different locations isolated by a distance of 3-5 km. Twenty trees were from location A (University of Lagos, Lagos, Nigeria) and 15 trees from location B (Federal College of Education, Lagos, Nigeria) all within South Western, Nigeria. This study was conducted in the year 2007.

**DNA isolation:** The isolation protocol was a modified Dellaporta *et al*. (1983) method. From the fresh young leaves harvested, 0.35 g was finely ground in a mortar and pestle in 2 mL of preheated (65°C) extraction buffer (1 M Tris HCl pH 8.0, 0.5 M EDTA pH 8.0, 5 M NaCl). Two hundred microliter of 20% SDS was added to the crushed tissue which was put into a clean autoclaved centrifuge tube. It was thoroughly mixed and incubated at 65°C for 20 min at intervals of 4 min in a water bath. Samples were left to cool down to room temperature and 500 µL of ice-cold 5 M potassium acetate was added. Tubes were shaken to obtain a homogenous mixture. The samples were incubated on ice for 30 min and later centrifuged at 4,000 rpm for 20 min in a bench centrifuge. The aqueous phase (supernatants) was transferred to a new tube. Equal volume (about 1.5 mL) of ice-cold propanol was added into the tube and was mixed gently to precipitate the DNA. Samples were incubated overnight at -20°C and centrifuged at 4,000 rpm for 10 min to pellet the DNA. The supernatant was discarded and the pellets were resuspended in 1 mL of high salt TE (10 mM Tris HCl pH 8.0, 1 mM EDTA pH 8.0) to dissolve the pellets. Two microliter of RNase was added and incubated at 37°C for 1 h. One microliter of ice-cold propanol was added and mixed gently to obtain the DNA pellets. The pellets were washed twice with 70% ethanol, air-dried for 2 h and resuspended in 200-400 µL of low salt TE (10 mM Tris HCl pH 8.0, 1 mM EDTA pH 8.0). It was stored at 4°C until ready for use.

**Analysis:** The quality of DNA extracted was checked by means of electrophoresis in 1% agarose gel and visualized by staining with ethidium bromide under ultraviolet light. It was photographed with the gel documentation system (V/tec).

The purity of the DNA was estimated by determining the absorbance ratio using spectrophotometer model Becham at $OD_{260}$nm and $OD_{280}$nm. The concentration of DNA in each of the samples was measured in µg $mL^{-1}$ as:

$$\text{Concentration of DNA} = OD_{260}\ \text{net} \times \text{dilution factor} \times \text{constant}\ (50\ \mu g\ mL^{-1})$$

## RESULTS AND DISCUSSION

The quality of the genomic DNA when tested on 1% agarose gel showed high molecular weight bands following electrophoresis (Fig. 1, 2). All the 35 samples from both locations had distinct bands showing the presence of DNA except for few samples which were re-extracted and checked for DNA purity.

DNA purity as measured by the ratio of absorbance at 260 and 280 nm gave a range of between 1.7-2.0 for most of the samples in the two locations (Table 1, 2). However, sample 19 in location A (Table 1) and sample 2 in location B (Table 2) had ratios lower than 1.7. The

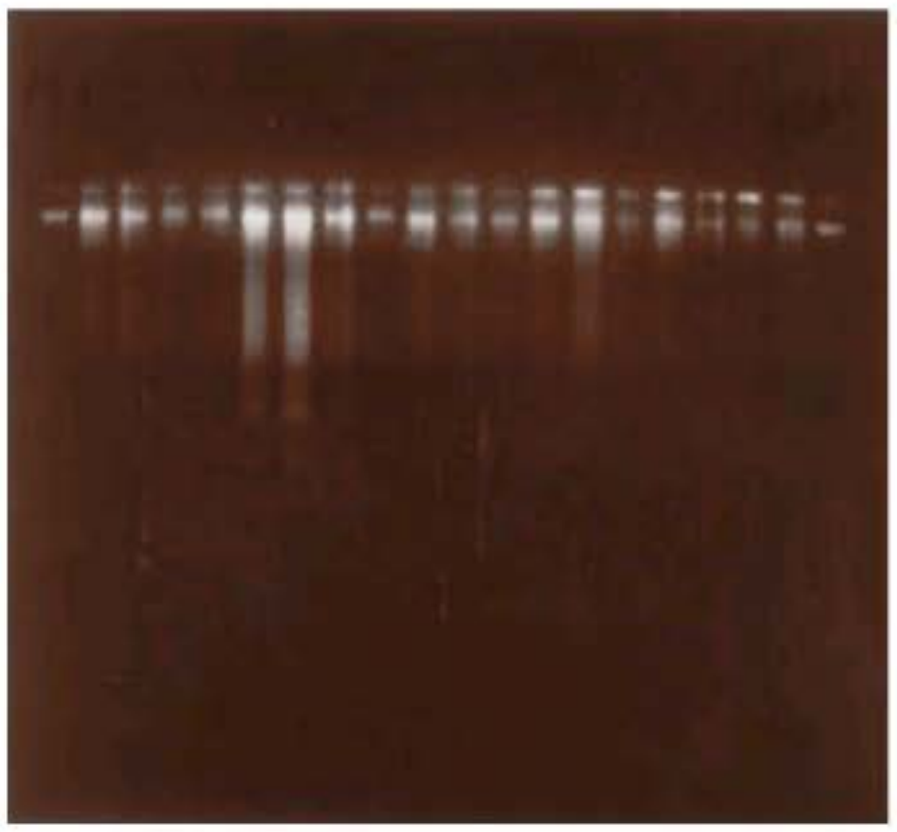

Fig. 1: Quality check (electrophorogram) of DNA samples extacted from the leaves of 20 *Terminalia catappa* trees within the University of Lagos, Akoka; Lagos (location a) on 1% agarose gel

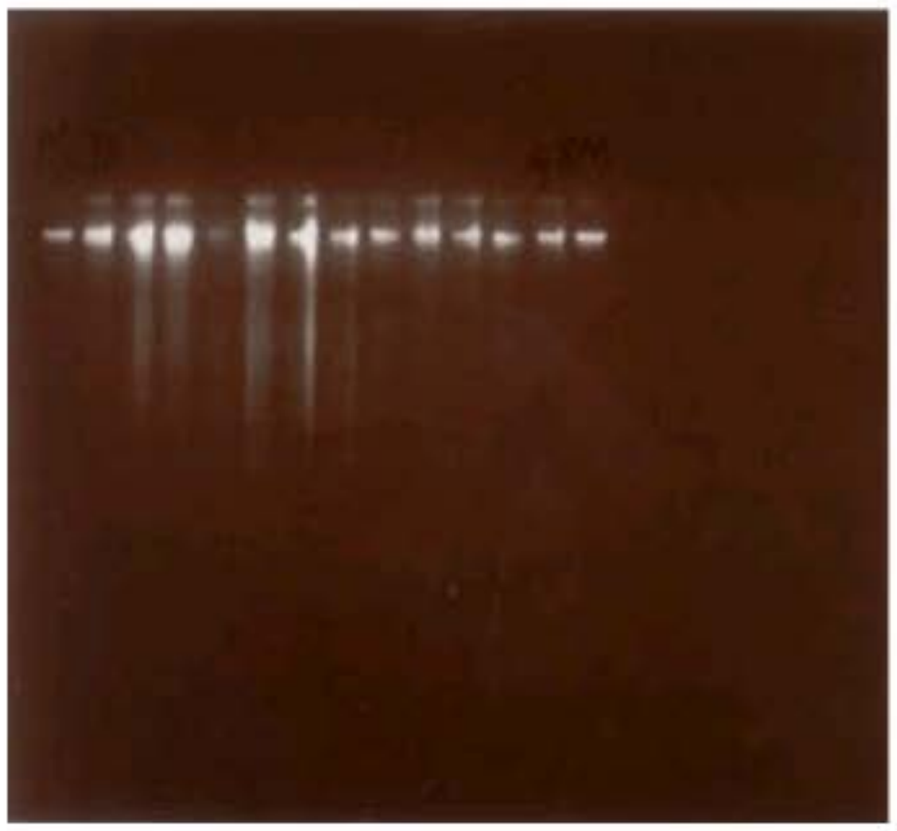

Fig. 2: Quality check (electrophorogram) of DNA samples extacted from the leaves of 15 *Terminalia catappa* trees within the Federal College of Education (technical) Akoka; Lagos (location b) on 1% agarose gel

Table 1: Spectrophotometric measurements for purity of DNA extracted from 20 *Terminalia catappa* trees within the University of Lagos, Akoka (location A)

| Samples No. | $OD_{260}$ABS | $OD_{280}$ABS | Ratio |
|---|---|---|---|
| Blank | 0.000 | 0.000 | 0.000 |
| 1 | 0.032 | 0.018 | 1.823 |
| 2 | 0.068 | 0.035 | 1.942 |
| 3 | 0.043 | 0.024 | 1.826 |
| 4 | 0.050 | 0.027 | 1.920 |
| 5 | 0.024 | 0.013 | 1.769 |
| 6 | 0.068 | 0.036 | 1.941 |
| 7 | 0.070 | 0.038 | 1.861 |
| 8 | 0.144 | 0.084 | 1.797 |
| 9 | 0.101 | 0.058 | 1.843 |
| 10 | 0.071 | 0.041 | 1.763 |
| 11 | 0.034 | 0.021 | 1.667 |
| 12 | 0.087 | 0.049 | 1.867 |
| 13 | 0.051 | 0.031 | 1.778 |
| 14 | 0.036 | 0.021 | 1.789 |
| 15 | 0.009 | 0.009 | 1.000 |
| 16 | 0.031 | 0.017 | 1.737 |
| 17 | 0.045 | 0.026 | 1.792 |
| 18 | 0.041 | 0.026 | 1.727 |
| 19 | 0.027 | 0.018 | 1.643 |
| 20 | 0.041 | 0.023 | 1.809 |

concentration of DNA extracted from the samples showed variation among trees in a location and between locations (Table 3, 4).

The Dellaporta *et al*. (1983) protocol was modified for use in this study and was found to be suitable for DNA extraction. It is a quick, simple, inexpensive method that does not utilize environmentally hazardous reagents for the isolation of genomic DNA from fresh young leaves.

Table 2: Spectrophotometric measurements of DNA extracted from 15 *Terminalia catappa* trees within the Federal College of Education (technical) Akoka, Lagos (location B)

| Samples No. | $OD_{260}$ ABS | $OD_{280}$ ABS | Ratio |
|---|---|---|---|
| Blank | 0.000 | 0.000 | 0.000 |
| 1 | 0.031 | 0.020 | 1.688 |
| 2 | 0.025 | 0.016 | 1.643 |
| 3 | 0.083 | 0.048 | 1.814 |
| 4 | 0.033 | 0.020 | 1.813 |
| 5 | 0.039 | 0.023 | 1.750 |
| 6 | 0.055 | 0.031 | 1.793 |
| 7 | 0.053 | 0.031 | 1.759 |
| 8 | 0.085 | 0.047 | 1.864 |
| 9 | 0.043 | 0.027 | 1.667 |
| 10 | 0.056 | 0.032 | 1.863 |
| 11 | 0.044 | 0.028 | 1.652 |
| 12 | 0.073 | 0.041 | 1.865 |
| 13 | 0.141 | 0.075 | 1.840 |
| 14 | 0.059 | 0.034 | 1.800 |
| 15 | 0.042 | 0.025 | 1.773 |

Table 3: Concentrations of DNA extracted from the leaves of 20 *Terminalia catappa* trees within the University of Lagos, Akoka (location A)

| Samples No. | Constant | Dilution factor | Concentration (ng $mL^{-1}$) |
|---|---|---|---|
| Blank | 50 | 250 | 0.0 |
| 1 | 50 | 250 | 387.5 |
| 2 | 50 | 250 | 850.0 |
| 3 | 50 | 250 | 525.0 |
| 4 | 50 | 250 | 600.0 |
| 5 | 50 | 250 | 287.5 |
| 6 | 50 | 250 | 825.0 |
| 7 | 50 | 250 | 837.0 |
| 8 | 50 | 250 | 1662.5 |
| 9 | 50 | 250 | 1175.0 |
| 10 | 50 | 250 | 837.5 |
| 11 | 50 | 250 | 375.0 |
| 12 | 50 | 250 | 1050.0 |
| 13 | 50 | 250 | 600.0 |
| 14 | 50 | 250 | 425.0 |
| 15 | 50 | 250 | 162.5 |
| 16 | 50 | 250 | 412.5 |
| 17 | 50 | 250 | 537.5 |
| 18 | 50 | 250 | 475.0 |
| 19 | 50 | 250 | 287.5 |
| 20 | 50 | 250 | 475.0 |

Table 4: Concentrations of DNA extracted from the leaves of 15 *Terminalia catappa* trees within the Federal College of Education (technical) Akoka, Lagos (location B)

| Samples No. | Constant | Dilution factor | Concentration (ng $mL^{-1}$) |
|---|---|---|---|
| Blank | 50 | 250 | 0.0 |
| 1 | 50 | 250 | 337.5 |
| 2 | 50 | 250 | 287.5 |
| 3 | 50 | 250 | 975.0 |
| 4 | 50 | 250 | 362.5 |
| 5 | 50 | 250 | 437.5 |
| 6 | 50 | 250 | 650.0 |
| 7 | 50 | 250 | 637.5 |
| 8 | 50 | 250 | 1025.0 |
| 9 | 50 | 250 | 500.0 |
| 10 | 50 | 250 | 675.0 |
| 11 | 50 | 250 | 475.0 |
| 12 | 50 | 250 | 862.5 |
| 13 | 50 | 250 | 1725.0 |
| 14 | 50 | 250 | 675.0 |
| 15 | 50 | 250 | 487.5 |

The method is also rapid, reproducible and reliable in terms of time taken and amount of plant sample required. It is a very useful technique in third world countries that lack sophisticated equipment as the study has shown that with simple equipment, DNA extraction of good quality and quantity is possible.

In this study, liquid nitrogen was totally excluded in the extraction process rather harvested tissues were ground in the preheated buffer in a mortar and pestle into a paste. Despite this, the results from the agarose gel and spectrophotometer revealed that the cell constituents were properly released into the buffer and DNA subsequently isolated. Collected fresh tissue could also be successfully stored in the buffer for sometime especially in areas where materials and resources are not available as suggested by Bhattacharjee *et al*. (2004) and Ogunkanmi *et al*. (2008).

Furthermore, no ultracentrifuge was used in this study rather a bench centrifuge of 4,000 rpm was used. The time of spinning was however increased from 5 to 20 min and it was not a continuous span.

Despite these modifications and the variations in quantities of reagents used, the DNA isolated when tested on 1% agarose gel showed high molecular weight bands. The purity as measured by the spectrophotometer gave a ratio of 1.7 to 2.0 for most samples indicating good quality DNA with little or no contaminant. DNA concentration from the samples also showed that quite a good quantity was extracted which is good enough for molecular marker study such as RAPD, AFLP or any other PCR based analysis.

## CONCLUSION

This study has helped to confirm that the protocol used for DNA isolation in this tree crop, *T. catappa*, can produce good quality and quantity DNA for further PCR based studies and that the method can be easily adapted to other crops especially when facilities are limited.

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