Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.2, 2015

Isolation and Estimation of DNA Level in Coconut Leaf (Coccos Nucifera)

AINA, V.O[1] IBRAHIM, M.B[2] ABDULSALAMI, M.S[3] ADEWUMI, A.A.J.[1]
PETER M. WAZIRI[4] ISIYAKU MUHAMMED[4]
1.Dept of Applied Science, College of Science and Technology, Kaduna Polytechnic, P.M.B 2021, Kaduna – Nigeria
2.Central Administration Kaduna Polytechnic, Kaduna
3.Dept of Biological Sciences, Nigerian Defence Academy, P.M.B. 2109, Kaduna – Nigeria
4.Dept of Biochemistry, Kaduna State University, P.M.B. 2339, Kaduna – Nigeria
Corresponding Author Email: vocwummi2006@yahoo.com

Abstract
The DNA Level of Coconut leaf was determined using agarose gel electrophoresis and UV-double beam spectrophotometer. 30μg leaf sample was weighed; chemical homogenization using mortar and pestle was done using lyses buffer and "Morning fresh" detergent. Whole sample was centrifuged at 10,000 rpm for 20 minutes; the supernatant was decamped into clean microcentruifuge tubes. Ethanol (500ml) was added and mixed thoroughly and incubated at room temperature for 30 minutes. DNA is insoluble in ethanol and so will appear as white precipitate at the bottom of the tube. Sample was centrifuged at 10,000 rpm for 5 minutes, thereafter the content was exposed to the atmosphere for 10 minutes to rid off remaining solvent (Ethanol). The pellets were dissolved in 50ml of TE (Tris-EDTA) buffer. The DNA (25ml) was taken and diluted in 1.75ml of TE Buffer and absorbance read at 260nm and 280nm with purity of DNA calculated followed by Electrophoresis. 25ml of the DNA sample was taken and ran on 0.8% agarose gel electrophoresis using a standard marker for 60 minutes. The analysis was done in triplicate with the ratio of absorbance at 260 and 280nm (1.79, 1.76 and 1.84) showing the purity of the DNA sample.
Keywords: *Coccos nucifera,* Percentage Purity, DNA Sample, TE Buffer, Agarose Gel Electrophoresis.

INTRODUCTION

The coconut palm, *Coccos nucifera* is a member of the family Arecaceae (palm family). It is the only accepted species in the genus cocos (Hahn, 1997). The term coconut can refer to the entire coconut palm, the seed, or the fruit, which is not a botanical nut. Found across much of the tropic and subtropics area, the coconut is known for its great versatility as seen in many domestic commercial and industrial users of its different parts. Coconuts are part of the daily diet of many people. When young, the entire fruits are used as melons, when mature only the seeds are used as nuts. The oil and milk derived from it are commonly used in cooking and frying. Coconut oil is also widely used in soaps and cosmetics. The clean liquid coconut water within is a refreshing drink and can be processed to create alcohol. The husks and leaves can be used as material to make a variety of products for furnishing and decorating. It also has cultural and religious significance in many societies that use it (Grimwood *et al,* 1975).

The determination of the concentration of DNA or RNA in solution is a fundamental task in molecular biology. The quantity of DNA is limiting in most experiments, therefore the knowledge of its concentration is critical. Determination of the DNA concentration can be estimated either by qualitatively comparing the fluorescence of DNA bands in an agarose gel to a standard or by spectrophotometric.

Deoxyribonucleic Acid (DNA) is a nucleic acid containing the genetic instructions used in the development and functioning of all known living organisms (with the exception of RNA viruses). The DNA segments carrying this genetic information are called genes. Likewise, other DNA sequences have structural purposes, or are involved in regulating the use of this genetic information. Along with RNA and proteins, DNA is one of the three major macromolecules that are essential for all known form of life.

DNA consists of two long polymers of simple units called nucleotides, with backbones made of sugars and phosphate groups joined by ester bonds. These two strands run in opposite directions to each other and are therefore anti-parallel. In living organisms, DNA does not usually exist as a single molecule, but instead as a pair of molecules that are held tightly together (Berg, *et al*, 2002). These two long strands entwine like vines, in the shape of a double helix.

As a portable source of both food and water, the coconut played a critical role in the ability of humans to voyage, establish trade routes and colonize lands in regions throughout the Cold World Tropics (Harries, 1978). This species continues to have hundreds of uses as a source of food, drink, fiber construction matter, charcoal and coil. (Batugal, 2005). The history of dispersal and cultivation of this species is thus fundamentally intertwined with human history in the tropics.

AIMS AND OBJECTIVES OF THE RESEARCH

These includes:

1. To isolate and estimate the DNA level of coconut leaves (*Coccos nucifera*)
2. To evaluate a basis for genetic analysis in the area of scientific, medical or forensic purposes such as introduction of DNA into each of animals or plants or for diagnostic purposes.

MATERIALS AND METHODS

This research/bench work was carried out between May – November, 2012 in the Biochemistry Laboratories of the Department of Biochemistry, Kaduna State University, Kaduna – Nigeria.

Principle of Extraction

The concentration of DNA sample can be checked by the use of UV spectrophotometry. DNA absorb UV light very efficiently making it possible to detect and quantify at concentrations as low as 2.5µg/*µl*. The nitrogenous bases has an absorption maximum at about 260nm, using a 1-cm light path, the extinction coefficient for nucleotides of this wavelength is 20. Based on this, the absorbance at 260nm in a 1-cm quartz cuvette of a 50*µg/ml* solution of double stranded DNA or a 40*µg/ml* solution of single stranded RNA is equal to 1 or RNA in the sample as follows:

DNA Concentration (*µg/ml*) =

($O.D_{260}$) x (dilution factor) x [50*µg* DNA/*ml*/(1 $O.D_{260}$ unit)]

Coconut leaf (30mg) was weighed and homogenized using mortar and pestle. At pH8, 100*µL* of 0.1M Tris HCl buffer was added, SDS (Sodium Dodecyl Sulphate) (50*µl)* was also added and mixed by inverting the tube. Potassium acetate (mildly acidic) (100*µl* of 5M) was then added, mixed by inverting the tube and placed an ice for 5 minutes. The whole tube was centrifuged at 10,000 rpm for 20 minutes supernatant was decanted into a clean micro centrifuge tube.

Ethanol (500*µl*) was added, mixed thoroughly and incubated at room temperature for 30 minutes. DNA is insoluble in ethanol and so will appear as white precipitate at the bottom of the tube. The sample was centrifuged at 10,000 rpm for 5 minutes with after which the tube content was exposed to the atmosphere for 10 minutes in order to get rid of any remaining ethanol. The pellets were dissolved in 50*µl* of TE Buffer. The DNA (25*µl*) was taken and diluted in 1.75ml of TE Buffer, with absorbance taken at 260nm and 280nm and purity calculated, then followed by electrophoresis. (Lodhir M.A *et al* (1994).

TAE Buffer (400ml 1X) was prepared by adding 40ml 10X TAE Buffer to make a 1.0% agarose gel by adding 1.0g of agarose powder to 50ml 1X TAE buffer in a 250ml Erlenmeyer flask, another 50ml 1X TAE Buffer was added, swirled to suspend the agarose in the TAE buffer. This mixture was placed in a microwave oven and heated for 2 minutes. After proper cooling on bench top, 10.0ul ethidium bromide (10.0*µg*/ml) was added and swirled to mix. A Gel mold was assembled with the 1.0% agarose solution poured carefully into one corner of the mold and allowing for even spread and allowing it to get firm. This was then placed into an electrophoresis chamber with the remaining 500ml 1X TAE Buffer poured to cover the gel completely. In a labeled centrifuge tube, 25*µl* sample DNA was mixed with 5*µl* sample loading dye. In another labeled centrifuge tube, 15*µl* of DNA ladder and 5*µl* sample loading dye were mixed both sample DNA and DNA ladder were centrifuged for 30 seconds at 5000 rpm to remove air bubbles.

Sample and Ladder DNA (25*µl*) each were placed into separate lanes avoiding overfilling the wells. A lid was placed on the chamber and the appropriate terminals connected to a power supply. On a setting of 100V, the DNA samples were ran through the gel until the loading dye was two-thirds of the way through the gel for 1 hour, with the DNA bands identified using an ultraviolet light box. (Lodhir M.A *et al* (1994).

RESULTS

Table 1: Absorbance Values of DNA samples extracted from coconut leaf from three different locations (markets) measured at 260 and 280nm

	ABSORBANCE VALUES			
Location	260nm	280nm	260/280 Ratio	Concentration in *µg/ml*
Karsuwa Benchi	2.45	1.37	1.79	8.7
Central Market	2.77	1.57	1.76	9.83
Kasuwa Monday	2.68	1.46	1.84	9.51

Agarose gel image of DNA isolated from coconut leaf from three different locations

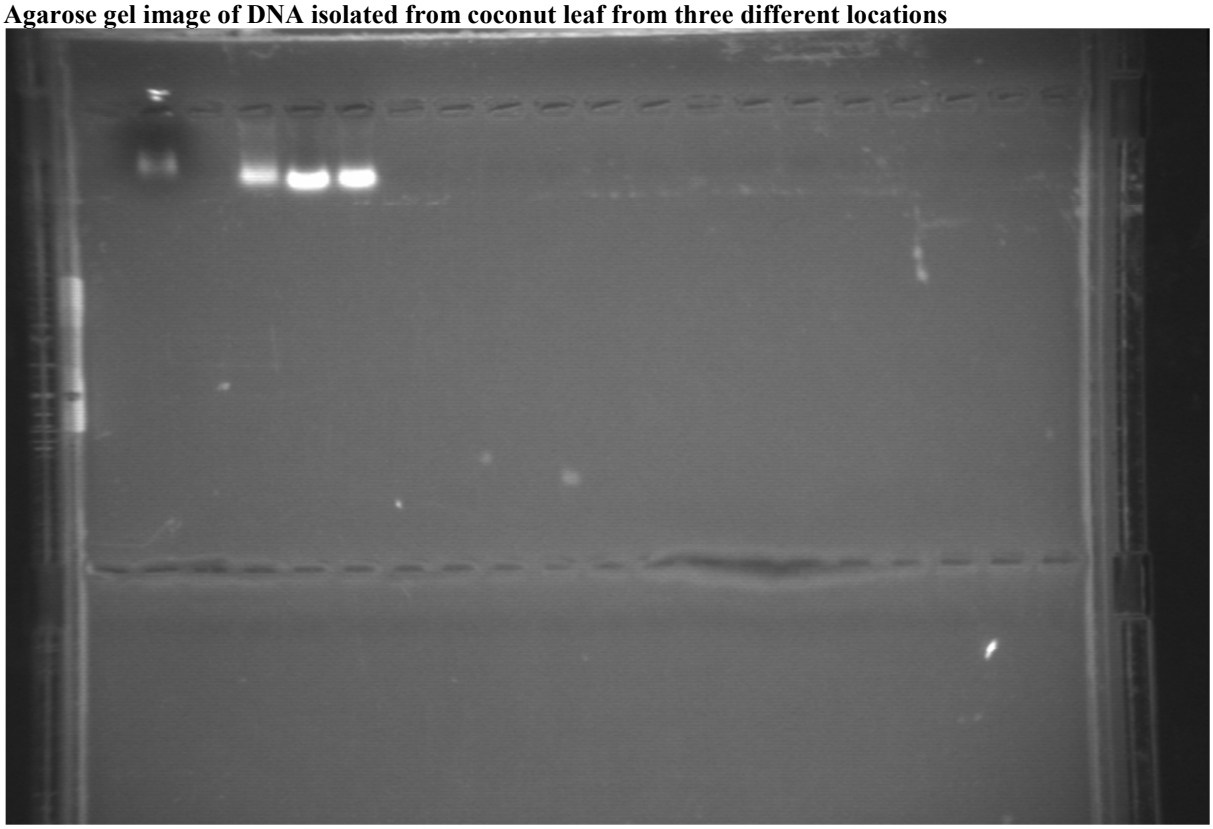

Plate 1

DISCUSSION

From the results obtained, Table 1 shows the absorbance of the DNA samples at 260nm and 280nm. The absorbance at 260nm indicated the presence of DNA whereas at 280nm indicated the presence of proteins. From the values of 2.45, 2.77 and 2.68 obtained at 260nm, it showed that there was significant amount of DNA in the samples from the three different locations and also the values of 1.37, 1.57 and 1.46 obtained at 280nm showed the presence of protein in the DNA sample. From the 260/280 ratio obtained, the values of 1.79, 1.76 and 1.84 indicated the purity of the DNA samples since the range of purity was between. (1.65 – 1.85) (biobzc@hofstra.edu)?

Table 1 also showed the DNA concentration in the three samples 8.7*µg/ml* from Kasuwa Berchi, 9.83*µg/ml* from the Central Market and 9.51*µg/ml* from Kasuwa Monday, being very significant amounts/concentrations which could serve as a good source of DNA for Genetic engineering, Forensics and possibly for cloning.

ACKNOWLEDGEMENT

The co-authors wish to thank and acknowledge with gratitude, the Head of Department, Department of Biochemistry, Kaduna State University, Kaduna – Nigeria for allowing access to reagents and laboratory facilities.

REFERENCES

Batugal (2005). Performance of Coconut Hybrids in some countries of Asia, Africa and Latin America in int. plant Genetic Resour Newslett. 142, 47 – 54.

Berg, J., Tymoczko, J and Stryer, L. (2002): Biochemistry Freeman and Company 0 – 7167 -4955 – 6.

Edwards, KC., Johnson, C. and Thompson, C. (1991): A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucl Acids res 19:23 – 49.

Grimwood, B.E., Ashman, F., Dendy, D.A.V., Jarman, C.G., Lttle, E.C.S and Timmins, W.H., (1975) Coconut palm products – Their processing in developing countries. Rane FAO pp 3 – 4.

Hahn, W.J. (1997). Arecanae: The palms retrieved April 4, 2011 from the Tree of life project website.

Lodli, M.A, Ye, G.N., Weedan, N.F x Reish, B.I. (1994): A simple and efficient method of DNA extraction from grapevine cultivas and vitis species, plant Mol. Biol Report (12) 6 – 13.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Academic conference: http://www.iiste.org/conference/upcoming-conferences-call-for-paper/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar