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Preliminary Studies on Isolation of Genomic DNA suitable for PCR from Some African Sapindaceae

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Abstract: For any meaningful genetic research, basic techniques of biotechnology must be in place, one of which is isolation of DNA. Although several protocols exist for the extraction of plant DNA, a major but limiting step in genetic research is poor extraction. This study was conducted in order to isolate pure genomic DNA from some members of family Sapindaceae in Africa using a rapid and efficient method. Fresh and dried young leaves from 56 species were sampled for extraction of genomic DNA. The methodology employed includes a modification in the quantity of plant materials and reagents used and excluded the use of ultracentrifugation techniques. The result revealed genomic DNA with absorbance ratio ranging between 1.4 and 2.0 for all the taxa sampled. Hence, it was concluded that the modified protocol yielded genomic DNA suitable for PCR based analysis.

Key words: Sapindaceae, absorbance, genomic DNA, ultracentrifugation techniques, protocol

INTRODUCTION

Sapindaceae Juss. is one of the flowering plant families in the order Sapindales comprising about 140-150 genera and 1400-2000 species worldwide in form of trees, shrubs and tendril-bearing vines (Watson and Dallwitz, 1992). Several species in the family contain mildly toxic soap-like compounds known as saponins in their leaves, seeds, root, fruits, barks, twigs and/or pericarp hence they serve as foaming agents. Several genera in the family Sapindaceae are cultivated for their brightly coloured fruit or reddish new growth, or as shade trees. Although, a few species are found in Africa, Australia and South America, the majority are native to Asia (APG, 2003) and one third of the species represented in the family belong to the tribe Paullineae Kunth (Buerki *et al*., 2009). Within Sapindaceae, divergence of all the subfamilies occurred in the early cretaceous (Buerki *et al*., 2010). Members are great economic, industrial (Dimmit, 2008) and medicinal (Odugbemi and Akinsulire, 2006; Sofidiya *et al*., 2007; Adesegun *et al*., 2008; Muanya and Odukoya, 2008; Pendota *et al*., 2008; Antwi *et al*., 2009; Ripa *et al*., 2010) value to humans. The attractive fruit of the sub-family Nepheleae Radlk are the most important members of the Sapindaceae (Leenhouts, 1978). A number of authors have shown the need to study the germplasm of crops using molecular methods in addition to quantitative methods including: Samal *et al*. (2003) and Taamalli *et al*. (2006). However, studies have shown that DNA extraction is not always easy and reproducible and the protocols used are specific for different plant species (Pandey *et al*., 1996; Porebski *et al*., 1997). In molecular biology research, the ability to eliminate interference of polysaccharides with the enzymatic manipulation of DNA as well as preventing the oxidation of phenolic substances that can react with nucleic acids and proteins are the major constraints in DNA extraction protocols (Vallejos, 2007). Again, the problem of polyphenols and polysaccharides is further exacerbated by the use of overly matured leaves rather than young leaves (Sharma *et al*., 2000). Therefore, the aim of this study was to identify a rapid protocol for extracting pure genomic DNA from some members of the family Sapindaceae in Africa suitable for PCR.

MATERIALS AND METHODS

Source of plant materials: Herbarium dried and fresh leaves were used for the study. Plant material used for DNA extraction was obtained between July and December 2009 from fields, botanic gardens, forest reserves and this was complemented with herbarium samples. These were

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dried and stored in silica gel prior to DNA isolation. From herbarium specimens, 0.5 cm^2 of plant tissue was removed and either stored in plastic tubes at -20°C or used immediately.

Identification of the plant samples: Voucher specimens were prepared and sent to the Forestry Herbarium, Ibadan for authentication. These were then deposited at the University of Lagos Herbarium for reference purpose.

DNA extraction: Total genomic DNA was extracted using the 2-Cetyltrimethylammonium bromide (CTAB) procedure of Doyle and Doyle (1987) with minor modifications followed by additional purification. Approximately 0.3-0.5 g of plant material was ground in a mortar with 1 mL 10x CTAB extraction buffer (containing 100 mM tris-HCl (Trizma Hydrochloric Acid) pH 8.0, 1.4 M NaCl, 20 mM EDTA and 10% CTAB). The buffer was pre-heated in a water bath at 65° C for 30 min. The slurry was poured into a tube and incubated at 65° C for 20 min with occasional gentle swirling. The incubated materials were de-proteinized once with equal volume (1 mL) of SEVAG (24:1 chloroform: isoamylalcohol) mixing gently but thoroughly. The cap of the tubes were opened to release gas and retightened. They were then rocked using an orbital shaker (100-150 rpm) for 60 min. After rocking, the samples were centrifuged at 4000 rpm at 25°C for 20 min and the samples were separated into 2 layers. The upper layer (aqueous layer) was carefully pipette into a freshly labelled tube and the nucleic acid was precipitated by addition of ice-cold isopropanol for herbarium samples (two-third volume of supernatant) or absolute ethanol for fresh samples (twice the volume of supernatant) down the side of each tube and mixed by gently inverting the tubes 6 - 10 times. The tubes were allowed to stand undisturbed in a rack and stored at -20°C for 24 h. After this re-precipitation, the tubes with contents were centrifuged at 3000 rpm for 5 min at 4°C. The supernatant was discarded gently with great care not to dislodge pellets from the bottom of the tube. The tubes were allowed to drain inverted on a clean paper towel overnight at room temperature. The DNA samples were then eluted in milli-Q water.

Gel electrophoresis: This involved quality check of the DNA samples on 1% agarose gel. The gel was run on 0.5× tris Borate EDTA (TBE) buffer at 75 V for 1 h 30 min. The gel was visualized by staining with 10 mg mL^{-1} ethidium bromide under Ultra Violet (UV) light and photographed with the gel documentation system (UVitec, UK).

Quantification of DNA samples: This involved the determination of the concentration and relative absorbance of each DNA samples using an Eppendorf biophotometer. It was achieved by mixing 55 µL of sterile water with 2 µL of the DNA sample in a cuvette. The cuvette was then placed in an Eppendorf Biophotometer Plus (Germany) and readings were documented at 260 and 280 nm, respectively.

Polymerase chain reaction (PCR) amplification: Here, nuclear DNA region was amplified. The Intergenic Transcribed Spacer (ITS) region AB101> and AB102< (White *et al*., 1990) primer were used and the fragment size amplified was between 1236-1280.

Amplification of selected regions were achieved in a 25 µL reaction mixtures containing 22.5 µL PCR premix, 0.5 µL BSA, 0.5 µL forward primer, 0.5 µL reverse primer and 1.0-2.0 µL total genomic DNA. The amplification of was improved by the addition of 4% DMSO in the total volume of the PCR mix. PCR amplification was carried out in a Gene Amp® PCR System 9700 thermal cycler (Applied Biosystems Inc. (ABI), Foster City, USA) using the following programme: initial denaturation for 3 min at 94°C followed by 35 cycles of denaturation for 1.00 min at 94°C, annealing for 45 s at 52°C and extension for 2 min 30 sec at 72°C. The amplification was completed by holding the reaction mixture for 7 min at 72°C to allow complete extension of the PCR products and a final hold of 4°C. These were then visualized on agarose gel.

RESULTS

Samples were authenticated by Mr. B.O. Daramola at the Forestry Herbarium Ibadan and the voucher numbers are stated below (Table 1).

Taxa assessed were distributed in 21 genera and 56 species i.e., *Allophylus* (13), *Atalaya* (1), *Blighia* (1), *Cardiospermum* (2), *Cyrtanthus* (8), *Deinbollia* (8), *Dodonaea* (1), *Eriocoelum* (1), *Haplocoelum* (1), *Laccodiscus* (1), *Lepisanthes* (1), *Litchi* (1), *Lychnodiscus* (1), *Majidea* (1), *Melicoccus* (1), *Pancovia* (4), *Paullinia* (1), *Placodiscus* (4), *Radlkofera* (3), *Sapindus* (1) and *Zanha* (1).

Deoxyribonucleic acid (DNA) samples were extracted from all the samples collected and deposited in the DNA bank at the Royal Botanic Gardens Kew, London

Table 1: Sources of materials used for the study

Species	Collection date	Country: Exact site	Voucher location	DNA Bank Number
Allophylus abyssinicus	16-Jan-64	Trinderet forest	FHI 20336	MWC 39915
Allophylus africanus	19-Sep-09	Cameroon: Bimbia forest reserve	LUH 1194	MWC 39910
Allophylus bullatus	22-Sep-09	Cameroon: Buea mountain	LUH 1185	MWC 39911
Allophylus dregeanus			FHI 75205	MWC 39918
Allophylus grandifolius	26-Aug-83	Cameroon: Muyuka	HNC 50596	MWC 39919
Allophylus hirtellus	17-Sep-09	Cameroon: Bakingili forest	LUH 1190	MWC 39912
Allophylus macrobotrys	04-Jan-79	Cameroon: Limbe botanic gardens	FHI 95067	MWC 39921
Allophylus rubifolius	24-Nov-86	Cameroon: Ndian falls	FHI 98646	MWC 39924
Allophylus schweinfurthii	7-Oct-68	Cameroon: Kribi	HNC 30321	MWC 39925
Allophylus spicatus	08-Jul-08	Nigeria: Olokemeji forest res.	LUH 3442	MWC 39914
Allophylus talbotii	25-Jul-60	Cameroon: Yaoundé	SFRK 28391	MWC 39927
Allophylus welwitschii	17-Sep-09	Cameroon: Bakingili forest	LUH 1192	MWC 39876
Allophylus zenkeri	14-Apr-62	Cameroon: Batouri	SFRK 6261	MWC 39928
Atalaya capensis	Jan-37	South Africa: Pretoria	GCH 8980	MWC 39950
Blighia welwitschii	17-Sep-09	Cameroon: Bakingili forest	LUH 1192	MWC 39953
Cardiospermum grandiflorum	14-Dec-09	Nigeria: Owena community for.	LUH 1196	MWC 39954
Cardiospermum halicacabum	29-May-75	Nigeria: Dumbi community	ABU 947	MWC 39853
Cyrtanthus angustifolius	20-Jul-81	Gabon: Makoku	FHI 102936	MWC 39837
Cyrtanthus carneus	26-Sep-76	Ghana: Bia national park	GCH 4650	MWC 39855
Cyrtanthus macrobotrys	19-Sep-09	Cameroon: Limbe botanic gardens	LUH 1187	MWC 39839
Cyrtanthus setosus	17-Sep-09	Cameroon: Bakingili forest	LUH 3444	MWC 39838
Crytanthus sp.	17-Sep-09	Cameroon: Bakingili forest	LUH 3445	MWC 39955
Crytanthus sp.	17-Sep-09	Cameroon: Bakingili forest	LUH 3446	MWC 39956
Achyranthes talbotii	14-Dec-09	Nigeria: Aponmu forest reserve	LUH 3447	MWC 39877
Cyrtanthus villager	01-Dec-68	Ghana: Atewa	GCH 38921	MWC 39856
Deinbollia angustifolius	20-Jul-81	Gabon: Makoku	FHI 84378	MWC 39857
Deinbollia grandifolia	09-Jun-79	Ghana: Bakwai	GCH 47068	MWC 39884
Deinbollia insignis	18-Mar-86	Nigeria: Obudu	FHI 102216	MWC 39858
Deinbollia kilimandscharica	17-Aug-65	Ethiopia	GCH 7781	MWC 39840
Deinbolla mollusca	05-Nov-73	Ghana: Bonsa	GCH 45939	MWC 39886
Deinbollia pycnophylla	30-Apr-62	Cameroon: Batouri	GCH 6226	MWC 39888
Deinbollia pynaerti	30-Apr-62	Cameroon: Batouri	GCH	MWC 39945
Deinbollia voltensis	15-Jul-70	Ghana: Kpondai	GCH 40483	MWC 39890
Dodonaea viscosa	02-Jun-09	Nigeria: ABU, Zaria	LUH	MWC 39860
Eriocoelum macrocarpum	16-Sep-09	Cameroon: Limbe botanic gardens	LUH 1195	MWC 39892
Haplocoelum gallaense	20-Jul-86	Cameroon	HNC 59423	MWC 39861
Laccodiscus ferrugineus	19-Sep-09	Cameroon: Bimbia forest reserve	LUH 1183	MWC 39880
Lepisanthes senegalensis	13-Sep-88	Nigeria: Imo	ABU 2619	MWC 39852
Litchi chinensis	30-Apr-10	Madagascar: Antananarivo	LUH 3452	MWC 39867
Lychnodiscus grandifolius	15-Dec-69	Cameroon: Kribi	HNC 31755	MWC 39864
Majidea fosterii	16-Sep-09	Cameroon: Limbe botanic gardens	LUH 1718	MWC 39959
Melicoccus bijugatus	13-Apr-60	Cameroon: Victoria	FHI 52431	MWC 39901
Pancovia atroviolaceus	17-Sep-09	Cameroon: Bakingili forest	LUH 1182	MWC 39843
Pancovia bijuga	06-May-77	Nigeria: Lagos	FHI 56562	MWC 39902
Pancovia sp.	19-Sep-09	Cameroon: Bimbia forest reserve	LUH 1188	MWC 39972
Pancovia sp.	10-Sep-09	Cameroon: Bimbia forest reserve	LUH 1186	MWC 39973
Paullinia pinnata	19-Sep-09	Cameroon: Bimbia forest reserve	LUH 1193	MWC 39960
Placodiscus bacoensis	6-Apr-76	Ghana: Yakossi	GCH 3193	MWC 39933
Placodiscus leptostachys	19-Sep-09	Cameroon: Bimbia forest reserve	LUH 3454	MWC 39929
Placodiscus oblongifolius	23-Feb-59	Ivory coast: Beberi	GCH 2796	MWC 39940
Placodiscus sp.1	19-Sep-09	Cameroon: Bimbia forest reserve	LUH 3455	MWC 39930
Radlkofera calodendron	19-Sep-09	Cameroon: Bimbia forest reserve	LUH 3457	MWC 39975
Radlkofera sp.2	14-Dec-09	Nigeria: Owena forest	LUH 3459	MWC 39977
Radlkofera sp.3	22-Sep-09	Cameroon: Buea mountain	LUH 3460	MWC 39978
Sapindus trifoliatus	31-Aug-68	Nigeria: Abeokuta	FHI 61564	MWC 39905
Zanha golungensis	08-Jul-08	Nigeria: FRIN, Ibadan	LUH 3462	MWC 39961

(Table 1). The quality of extracted DNA samples was determined using agarose gel electrophoresis and this revealed DNA of high quality (Fig. 1). The DNA samples were also quantified using spectrophotometry and this revealed that the concentration of the DNA samples ranges from 20-3716 ng μL^{-1} (Fig. 2). Also, purity of the DNA samples were measured at 260 and 280 nm and the absorbance ratio ($A_{260/280}$) ranged from 1.41-2.01 (Fig. 3). Further PCR amplification of the samples yielded good quality of DNA (Fig. 4).

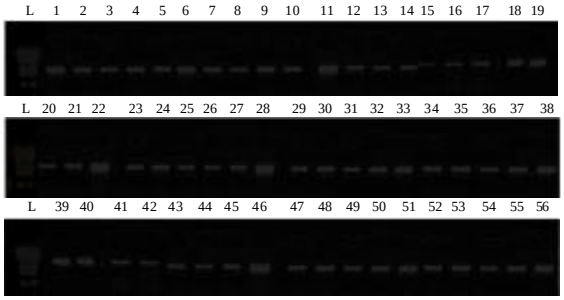

Fig. 1: Electropherogram of extracted DNA samples on 1% agarose gel

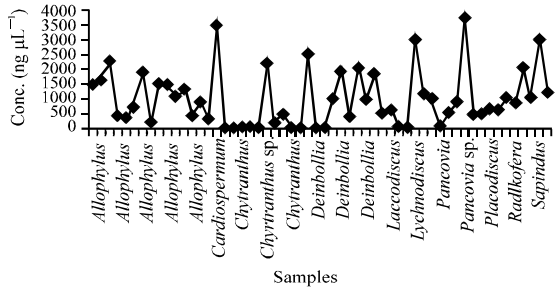

Fig. 2: Concentration of extracted DNA samples

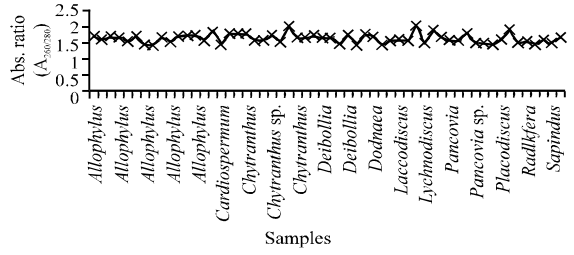

Fig. 3: Relative absorbance ratio of extracted DNA samples

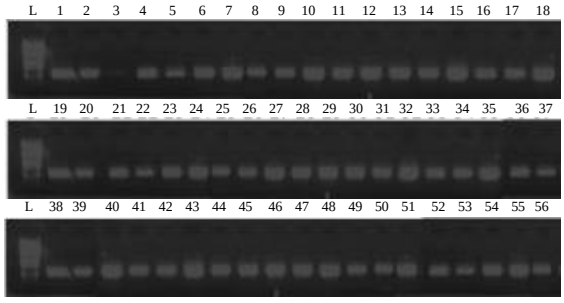

Fig. 4: Electropherogram of ITS amplification

DISCUSSION

Despite, the fact that several protocols exist for the extraction of plant DNA, a major but limiting step in genetic research is poor extraction of plant DNA (Attitalla, 2011) hence, the focal point of this research. After the CTAB protocol was given by Doyle and Doyle (1987), several attempts have been made to modify the protocol in order to obtain genomic DNA of higher quality and quantity from various types of plants. For example, Dehestani and Tabar (2007) worked with plants containing high levels of secondary metabolites and they obtained 100-250 µg g^{-1} of plant tissue using a modification involving additional PVP, increased concentrations of EDTA and mercaptoethanol. This is in conformity with modifications made in this study as increased amount of PVP (2%) and mercaptoethanol (0.4%) yielded DNA of good quality and enhanced proteins degradation. Shankar *et al.* (2011) reported a DNA yield of 712-808 µg g^{-1} when they employed the modified CTAB protocol in isolating DNA from four *in vitro* banana cultivars; this is in agreement with the result presented in this study. More recently, research involving modification of the CTAB protocol by Tiwari *et al.* (2012) enhanced extraction and purification of DNA from plants. Their modification included increased water bathing time and extraction temperature, increased concentrations of NaCl, EDTA and mercaptoethanol. In this present study, the amount of reagents used as well as incubation temperature of samples were modified however, the results from the agarose gel and biophotometer revealed that the cell constituents were properly released into the buffer and DNA subsequently isolated with high molecular weight bands. These modifications enabled easy extraction and less degradation of DNA as well as proper denaturation of proteins. 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 5-20 min and it was not a continuous span. Despite these modifications and the variations in quantities of reagents used, genomic DNA was successfully extracted from all the collected samples and the quality of the genomic DNA when tested on 1% agarose gel showed high molecular weight bands. The absorbance of the DNA samples at 260 nm ranged from 0.008-0.872 while at 280 nm the values ranged from 0.005-0.510. The purity of DNA for most samples as measured by the ratio of absorbance at 260 and 280 nm gave a range of 1.41-2.01 indicating good quality DNA with minimal contamination. The concentration of DNA 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. Although about 20% of the samples yielded DNA of lower concentration (<200 ng μL^{-1}). Amplification of the DNA samples using ITS primers yielded good DNA bands. The CTAB 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 utilizes environment friendly reagents in the isolation of genomic DNA from fresh young leaves. It is a very useful technique in third world countries where access to sophisticated equipment is limited since DNA of good quality and quantity was obtained.

CONCLUSION

Although, the methodology employed is not completely new, this study is the probably the first record of its large-scale application in the study of representative members of the family Sapindaceae in Africa. Therefore, this study has contributed to the genomic conservation of African Sapindaceae and to the production of quality genomic DNA from members of the family Sapindaceae for PCR based analysis.

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