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Extraction of high quality DNA from *Cola nitida* and *Cola acuminata*

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Cola spp. **are highly valued in African traditional medicine because they are rich in common and rare phytochemicals. They are important raw material in both the food and pharmaceutical industries. It is often difficult to isolate DNA from many tropical species with high polyphenolic and alkaloid contents. Many published protocols for nucleic acid isolation failed to yield sufficiently good quality amounts of DNA from leaf samples of *C. nitida* and *C. acuminata* for analysis. In this research, a simple and fast sodium dodecyl sulphate (SDS) – based extraction protocol have therefore been developed for routine isolation of high-quality nucleic acids from these *Cola* species by modifying established protocols. Genomic DNA obtained was found suitable for PCR amplifications.**

Key words: DNA extraction, polysaccharides, polyphenols, PCR, *Cola* species.

Cola species belong to the plant family Sterculiaceae (Russell, 1955). *Cola acuminata* and *Cola nitida* are the two most economically important members of the genus *Cola* due to their vast use in the food and pharmaceutical industries. They are rich in antioxidants, micronutrients, caffeine, theobromine, crude protein, carbohydrates, and other phytochemicals reported to possess several medicinal properties. *Cola* trees have been used in African traditional medicine for the treatment of various ailments including parasitic diseases (Blades, 2000; Sonibare *et al*., 2009). They are cultivated for their edible seeds known as kolanuts. The caffeine in the nuts also acts as a bronchodilator, expanding the bronchial air passages; hence their use in the treatment of whooping cough and asthma (Ebana *el al*., 1991; Jayeola, 2001). Kola nuts are used in pharmaceutical industries to produce cardiac stimulants, laxatives, sedatives and kola type beverages. Continued utilization of these medicinal plant species is however threatened by among other things habitat modification and unsustainable rates of exploitation, therefore the need for more concerted effort at their conservation (Joshi and Joshi, 2000; Tabuti *et al*., 2003).

Conservation and improvement of these species requires the use of DNA markers and availability of high quality genomic DNA. However extracting nucleic acids from many tropical species including *Cola* can be problematic due to the variations in plant cell components, including polysaccharides, cellulose, and phenols (Woodhead *et al*., 1998; Porebski *et al*., 1997). Plant materials are among the most difficult for high quality DNA extractions. Problems encountered in the isolation and purification of DNA from plant tissues could include those due to

inhibitor compounds like polyphenols which are known to oxidize and covalently link with quinines and to bind nucleic acids, giving a brown colour. The presence of polysaccharides has been shown to inhibit *Taq* polymerase activity (Fang et al., 1992). This is characterized by formation of a highly viscous solution in the sample (Do and Adams, 1991).

This study is therefore aimed at optimizing a protocol that would yield sufficient and high quality nucleic acids for downstream molecular studies involving PCR amplifications which would be the basis for genetic diversity assessment in *Cola* species making use of DNA markers.

Materials and Methods

Plant Material

Fresh, young leaves were harvested from randomly selected *Cola* seedlings for DNA extraction, using twenty accessions of each *Cola* species. The harvested leaf samples were put in well labeled polythene bags inserted into an ice pack and quickly taken to the laboratory. This is to preserve the leaf samples and the quality of DNA to be extracted. The samples were freezed at -80^{o}C for 48 hours and then lyophilized at ≤ 100 microns and condenser temperature ≤ –65^{o}C for 72 hours. Dried leaf samples were then stored in resealable plastic bags at –20^{o}C with 10 to 20g of self–indicating silica gel until use, following Chase and Hill (1991).

DNA Extraction

Extraction of genomic DNA was done using several modifications of the protocols of Dellaporta *et al*. (1983) and, Doyle and Doyle (1990). However only the following, SDS – based modifications produced reproducible results. The extraction buffer contains 100 mM Tris (hydroxymethyl) amino-methane (pH 8); 10 mM ethylene diamine tetraacetate (EDTA, pH 8); 1% (W/V) sodium dodecyl sulphate (SDS); 2% polyvinyl pyrolidone (PVP); 1 M NaCl and 1% V/V β-Mercaptoethanol.

The protocol is as follows:

Add 1g of grounded lyophilized tissue to 1 ml of the extraction buffer. Mix several times by gentle inversion (to macerate the tissue). Incubate at 65 ^{o}C for 30 minutes with occasional swirling. Add 500 µl of the extraction buffer and centrifuge at 13000 rpm for 10 minutes. Decant the supernatant into fresh tubes. Add 200 µl of 5% PEG and incubate at 4 ^{o}C for 10 minutes. Centrifuge at 13000 rpm for 10 minutes and decant supernatant into fresh tubes. Add 100 µl of 5M potassium acetate (place samples on ice) for 10 minutes and centrifuge at 15000 rpm for 10 minutes. Add 2/3 isopropanol to supernatant and mix properly. Keep the mixture in –80^{o}C for 10 minutes. Centrifuge at 13000 rpm for 10 minutes. Discard the supernatant. Wash pellets with 70% ethanol and air dry the pellets at room temperature. Suspend the pellets in 50 µl of TE and store at 4 ^{o}C.

Checking the quality and estimating the concentration of DNA

Quantification and quality of isolated DNA was measured by the use of Nanodrop® Spectrophotometer. Five µl of each DNA sample was mixed with 95 µl double distilled water, which gave a dilution factor of 100. Readings were taken at 260 nm and 280 nm. The reading at 260 nm allowed calculation of the concentration of DNA in the samples.

$$DNA\ concentration\ \left(\frac{\mu g}{\mu l}\right) = \frac{\text{A260 X 50(dilution factor) X 50 } \mu l/ml}{1000}$$

is a constant for double stranded molecules (Sambrook *et al*., 1989; Hoisington *et al*., 1994).

The quality and purity of DNA was determined by calculating the ratio of readings at 260 nm and 280 nm. Values between 1.8 and 2.0 are an indication of high quality DNA. A ratio below 1.8 is an indication of presence of protein contamination while a ratio above 2.0 is an indication of chloroform and phenol contamination. 5 μl DNA of each sample

was loaded in 1% agarose gel and the gel run at 100V for 90 minutes (Fig.1).

PCR amplification of DNA

Thirty arbitrary RAPD primers (OPB, OPH and OPT series from Operon Technologies) were screened for reproducibility and to eliminate sporadic amplification products. This was used to screen these primers for their ability to amplify the *Cola* sample's DNA. The Polymerization Chain Reaction (PCR) mixture contained 10 ng genomic DNA (in 3 µl), 0.5 *µl* of 1×PCR buffer, 0.5 *µl* of 2.5 mM dNTPs, 0.4 *µl* of MgCl2, 0.5 *µl* primer, 0.2U Taq DNA Polymerase (Bioline) and 3.72 *µl* ddH2O in a final 10*µl* volume. The PCR amplification protocol was programmed for an initial cycle of 3 min at 94°C followed by 45 cycles of 20s at 94°C, 20s at 37°C, and 40s at 72°C. After all cycles were completed, the reactions were held at 72°C for 7 min and slowly cooled down to 4°C on GeneAmp® PCR System 9700 thermo-cycler machine. The PCR products was then run on 2% agarose gel in 1 X tris borate EDTA stained with 10 mg/ml ethidium bromide for 2 ½ hours at 100V, and then visualized under UV light.

Results and Discussion

It is important to harvest plant tissues (fresh leaves in our case) fast and immediately freeze to prevent excessive degradation of the DNA. Young and succulent tissues are always preferred for DNA extraction from materials (Muge *et al*. 2009).

The highest DNA yield per volume was 270.52 ng/µl, followed by 266.03 ng/µl. The least yield in DNA was 51.37 ng/µl. The average DNA yield in all the accessions was 173.43 ng/µl. The variation in the quantity of DNA per unit volume of DNA may be due the ratio of euchromatin and hetero-chromatin present in the DNA according to Verma & Agarwal (2005). Also the absorbance of A_{260}/ A_{280} determined by spectrophotometry ranged between 1.8 and 2.00 for 87.50% of the samples. Several protocols were tried but the one that yielded the best DNA terms of quality, quantity and amplifiability was reported in this work.

Modifications of the cetyl trimethyl ammonium bromide (CTAB) – based Doyle and Doyle (1990) protocol did not produced desired result because the A_{260}/A_{280} absorbance ratio lies between 1.6 – 1.8 indicating the level of contaminating molecules. The extracts were very slimy and persistently green in some genotypes. Secondary metabolites such as polyphenolic compounds, polysaccharides and proteins poses difficulties in DNA isolation from plant tissues (Pirttila *et al*. 2001). These contaminating molecules precipitate along with DNA thus considerably affecting DNA yield quantitatively and qualitatively (Pandey *et al*., 1996).

The DNA isolation procedure is SDS – based, modified from Dellaporta *et al*. (1983). The extraction buffer contained PVP and β–mercaptoethanol to prevent oxidation of the secondary metabolites in the disrupted plant material. To remove the polyphenols quite abundant in *Cola spp,* PVP which forms complexes with polyphenolic compounds, was added and complex separated from the DNA by centrifugation. Several protocols proposed different percentages of PVP but from our results 2% worked well following Csaikl *et al*. (1998). A good isolation protocol should be simple, rapid and efficient enough, yielding appreciable levels of high quality DNA suitable for molecular studies (Sablok *et al*., 2009). As DNA quality is of crucial consequence for molecular applications (Pandey *et al*., 1996), the DNA was evaluated by performing RAPD analysis. The polymerase chain reaction (PCR) – based applications worked well with the protocol documented (Figs. 2 and 3) in this study. Distinct bands were produced by primers OPH02 and OPT04. The patterns were reproducible for duplicate DNA preparations from both *Cola* species used in this study.

Conclusions

The result of this study is an important first step in molecular studies on *Cola spp.*, an economic but highly underutilized species in Africa. The protocol developed could also be applied to other tropical trees with high levels of phytochemicals which can impair DNA isolation.

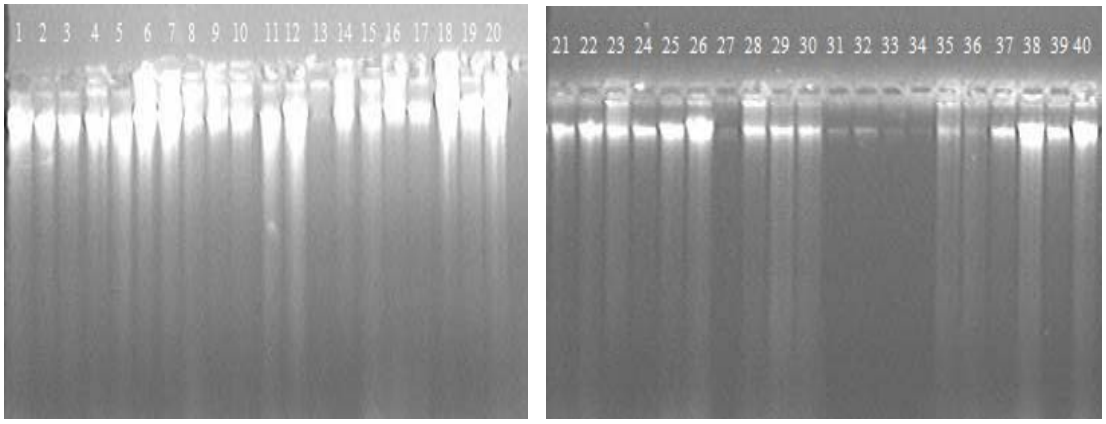

Figure 1: Genomic DNA on 1% agarose gel from both *C. acuminata* (A) and *C. nitida* (B)

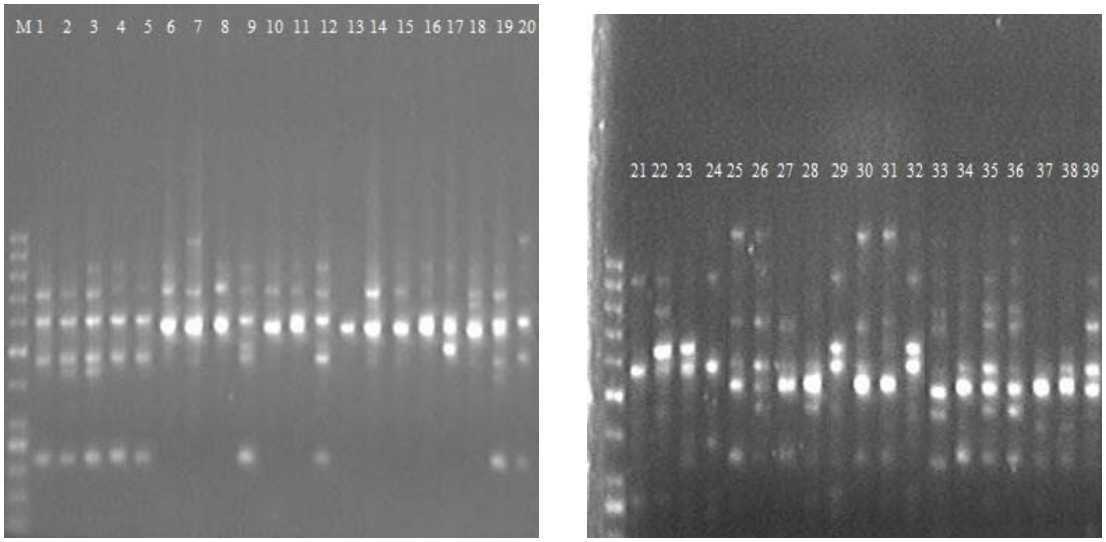

Figure 2: RAPD bands of *C. acuminata* (A) and *C. nitida* (B) amplified by primer OPH02. Lane M: Mol wt standards

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