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A simple and efficient genomic DNA extraction protocol for large scale genetic analyses of plant biological systems

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ABSTRACT

Extraction of high quality genomic DNA from higher plants is hindered by the presence of secondary metabolites, which reduce the yield and quality of the DNA. We describe an alternative protocol for genomic DNA extraction from fresh and dry plant leaves that is amenable to PCR-based genetic analysis. Existing methods were either very lengthy, expensive or not suitable for extraction of genomic DNA from dry leaves. Our method used SDS and high salt concentrations to extract DNA and does not require use of hazardous materials or special laboratory equipment. Genomic DNA extracted using our method was used for PCR-based genetic characterization of different varieties of cashew trees, *Anacardium occidentale*, via SSR markers as well as *Zea mays* varieties. This protocol improves existing methods in that it has the advantage of being adaptable to studies with a large number of samples and limited resources. The method is rapid, cost efficient and uses non-hazardous reagents. Genomic DNA extracted using this method has sufficient quality for downstream PCR-based genetic analysis.

1. Introduction

Plants produce secondary metabolites that interfere not only with extraction of high quality genomic DNA but also with the subsequent reactions such as PCR and related genetic analyses (Kotchoni and Gachomo, 2009; Kotchoni et al., 2011). The widely used genomic DNA extraction procedures rely on lengthy protocols that use hazardous chemicals or expensive commercially available kits. Examples include the CTAB method and its modifications (Murray and Thompson, 1980; Allen et al., 2006), which use reagents like liquid nitrogen, hydrochloric acid, sodium hydroxide, 2-mercaptoethanol, phenol and chloroform that are either toxic or caustic and therefore require use of a fume hood. These procedures are lengthy with a minimum of 5–6 h per extraction (Allen et al., 2006) and are also expensive. Such methods are therefore not suitable for large scale DNA extractions in laboratories with minimum resources (Kotchoni and Gachomo, 2009; Margam et al., 2010).

The aim of our study was to develop a rapid and cost efficient method for extraction of genomic DNA from fresh leaves of *Zea mays* and dry leaves of *Anacardium occidentale*. The quality of DNA produced from this method needed to be high enough for downstream PCR-based genetic analysis.

2. Materials and methods

2.1. Plant material

All plant material used in this study was from different cultivars of *A. occidentale*, collected from several geographical locations in Benin (West Africa) (Table 1). Young healthy leaves were collected from each cultivar and air dried at room temperature and used in this study to validate the protocol.

2.2. DNA extraction

2.2.1. Solution and solvent

(i) Extraction buffer: 1% SDS, 0.5 M NaCl (no EDTA, Tris–HCl or pH adjustment needed); (ii) isopropanol; (iii) 70% (v/v) ethanol.

Abbreviations: DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; NaCl, chloride sodium; M, molar; EDTA, ethylenediaminetetraacetic acid; HCl, hydrochloric acid; pH, hydrogen potential; rpm, revolutions per minute; RT, room temperature; V, volume; ddH_2O, deionized distilled water; TM, thermal cycler; SSR, simple sequence repeat; CTAB, cyltrimethylammonium bromide; Tris–HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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Table 1
List of accessions numbers of *Anacardium occidentale* (cashew) trees from different geographical locations of Benin, Africa tested in this study.

No	ID	Site of collection	Age of plant (years)
1	O32	Bassila	12
2	O27	Bassila	15
3	O11	Founga	15
4	E35	Serekali	15
5	E26	Serekali	12
6	E5	Konmi	15
7	E25	Serekali	12
8	E11	Bembèrèkè	12
9	E4	Konmi	15
10	O24	Founga	12
11	E13	Bembèrèkè	12

2.2.2. Extraction method

- Take half of a young dry leaf and cut it into small pieces (see Fig. 1 for illustration) then grind it using a porcelain mortar and pestle in 400 μl of the extraction buffer. Add more buffer until it reaches a final volume of 1200 μl in order to have enough homogenate to place into microfuge tube. Harvest the homogenate into 1.7 ml microfuge tubes.
- Spin (13,500 rpm, 4 min, RT) using a microcentrifuge.
- Transfer the supernatant into a new microfuge tube and add an equal volume of isopropanol (500 μl in our study) and mix gently by inversion. Place the mixture on ice for 5 min.
- Spin (13,500 rpm, 4 min, RT) using a microcentrifuge.
- Discard the supernatant and wash the DNA pellet with 500 μl 70% (v/v) ethanol.
- Spin (13,500 rpm, 2 min, RT) using a table microcentrifuge.
- Discard the ethanol. Blot away the excess ethanol from the pellet by inverting/placing it on a clean paper-towel. Let the pellet air-dry.
- Dissolve the DNA in 50 μl ddH_2O and store it at 4 °C for immediate use or −20 °C for long-term storage. If the lab is equipped with a −80 °C freezer, we recommend storing at −80 °C for long-term storage in order to preserve the quality.

2.3. PCR amplification and gel electrophoresis

The PCR was carried out in accordance to the Quanta Biosciences kit recommendations. The total volume of PCR mixture was 20 μl and comprised of 10 μl of master mix [AccuStart II PCR ToughMix (2×)], 4 μl template DNA, 1 μl of each primer (forward and reverse) and 4 μl water.

The reaction PCR reaction was performed in a thermal cycler (BIO-RAD; T100™) using an initial 94 °C denaturing step for 3 min followed by 34 cycles at 94 °C for 30 s, annealing for 30 s at the primer's annealing temperature, extension at 72 °C for 1 min 20 s and a final extension at 72 °C for 5 min.

The PCR product was run on a 1% agarose gel stained with ethidium bromide.

PCR primers for 3 SSR markers of *A. occidentale* were used to amplify different regions of the plant genome, as described by Croxford et al. (2006) (Table 2).

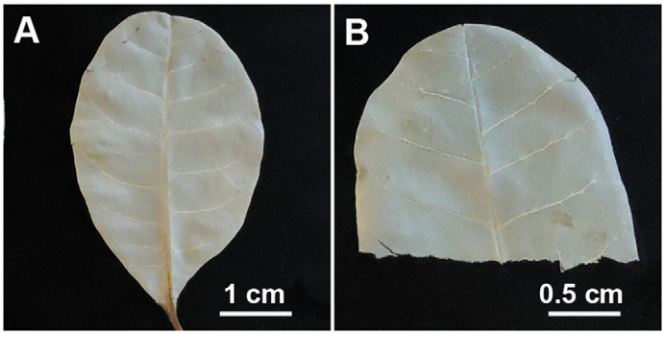

Fig. 1. Air-dried leaf of *Anacardium occidentale* L. for long term storage in absence of liquid nitrogen and −80 °C freezer for storage of fresh materials. The overall dried leaf is depicted (A), and the preferable upper half part of the leaf used in the DNA extraction protocol is depicted here (B).

3. Results and discussion

A simple, fast and reliable protocol for extraction of genomic DNA from dry leaves of *A. occidentale* was established in this study. Other available DNA extraction protocols were either very lengthy, very expensive or not suitable for extracting DNA from dry leaves of *A. occidentale* (Doyle and Doyle, 1987; Edwards et al., 1991; Kotchoni and Gachomo, 2009; Margam et al., 2010). For example, the use of other rapid DNA extraction protocols such as those described by Wang et al. (2011); Kotchoni and Gachomo (2009); and Azmat et al. (2012) did not give DNA of good quality from dried leaves of *A. occidentale* probably due to the presence of secondary metabolites. Although the CTAB method and commercially available kits gave high quality DNA they were either very lengthy or expensive respectively and therefore not suited to our study.

In the method described here the plant cell wall was broken using mechanical force in the presence of the extraction buffer. SDS in the buffer liberated DNA by lysing cell and nuclei (Manak, 1993). Subsequent centrifugation co-precipitated cell debris with polysaccharides and protein complexes that interfere with the quality of the DNA.

Our study required extraction of DNA from many samples and the DNA quality had to be high enough to allow for PCR-based analysis such as SSR marker based genetic characterization (Table 3). We established a method that is simple, safe and fast compared to the CTAB method which uses hazardous chemicals, is more time consuming and significantly expensive (Doyle and Doyle, 1987). When using the CTAB method it takes at least 6 h to complete a DNA extraction but our method takes 10 min. In comparison to the CTAB method, our protocol is safe enough to be performed on the lab bench in any laboratory without requiring the use of a chemical hood. Commercially available kits are expensive and therefore not an alternative for laboratories with limited resources.

To check the quality of the genomic DNA extracted from dry leaves of different cultivars of *A. occidentale* using this method, PCR amplifications of SSR markers were done. DNA fragments were clearly obtained from PCR following agarose gel electrophoresis from fresh leaves of different maize varieties (Fig. 2). This was an indication that the DNA extracted using this method was free from plant secondary metabolites e.g. flavonoids, terpenes, and phenolic compounds, which interfere with the yield and quality of the DNA (Porebski et al., 1997). These secondary metabolites were successfully removed during the extraction process. In addition, we successfully obtained a nice cartography of selected

Table 2
Microsatellites markers of *Anacardium occidentale* L. used for PCR-based amplification in this study.

Locus	Primer sequence (5′–3′)	Repeat motif	Ta °C	Allelic size Range (bp)
mAoR6	F: CAAAACTAGCCGGAATCTAGC R: CCCCATCAAACCCTTATGAC	$(AT)_5\,(GT)_{12}$	58.2	143–157
mAoR7	F: AACCTTCACTCCTCTGAAGC R: GTGAATCCAAAGCGTGTG	$(AT)_2\,(GT)_5$ $AT(GT)_5$	58.2	178–181
mAoR48	F: CAGCGAGTGGCTTACGAAAT R: GACCATGGGCTTGATACGTC	$(GAA)_6\,(GA)_3$	58.2	172–178

Table 3
Representation of DNA migration profile of the selected *Anacardium occidentale* cultivars based on microsatellite marker gel analysis.

Loci	1	2	3	4	5	6	7	8	9	10	11
mAoR3					—		—	—	—	—	
mAoR6		—		—				—	—	—	
mAoR7	—			—	—	—	—	—	—	—	—
mAoR11											
mAoR17							—			—	
mAoR42					—			—	—	—	
mAoR48					—			—		—	
mAoR52					—				—		

A. occidentale plant varieties using a wide range of SSR markers on DNA extracts from dried *A. occidentale* leaves (Fig. 3). The speed of extraction, minimal costs, absence of toxic chemicals and high quality of DNA obtained make this method ideal for extraction of DNA from dry plant material in any laboratory that has limited equipment and resources. The proposed protocol can be adopted for any PCR-based genetic analysis of dried plant materials, especially for large number of plant samples in lab settings with limited resources.

4. Conclusion

In summary, we established a rapid, environmentally friendly and cost efficient method for genomic DNA extraction from dry leaves. The quality of DNA obtained using this method was sufficient for downstream PCR-based genomic analysis such as genetic characterization of different varieties of *A. occidentale* using SSR markers.

Existing genomic DNA extraction protocols may need modifications to accommodate for differences in types and concentrations of secondary metabolites in various plant species.

Authors' contributions

SOK conceived the study. EWG, SOK, and KCS wrote the paper. KCS, TK, and LJ performed the study. SOK and EWG analyzed, discussed and assessed the data. HA-S, LA, AS, and LB-M contributed reagents/materials/analysis tools.

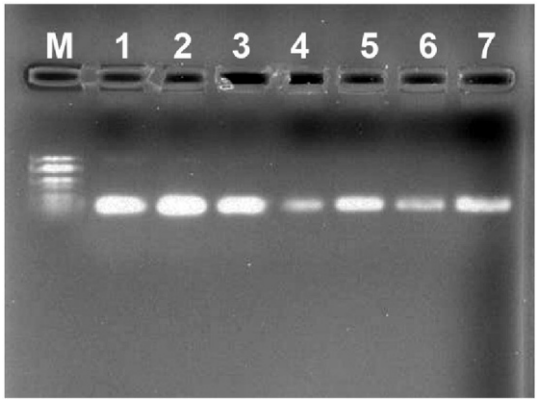

Fig. 2. The DNA extraction from fresh plant materials is amenable to PCR-based DNA fragment amplifications. We depicted here that the 16S-ribosomal subunit gene fragments from seven different varieties of fresh maize leaves are clearly amplified using this extraction protocol. M = DNA maker (DNA ladder).

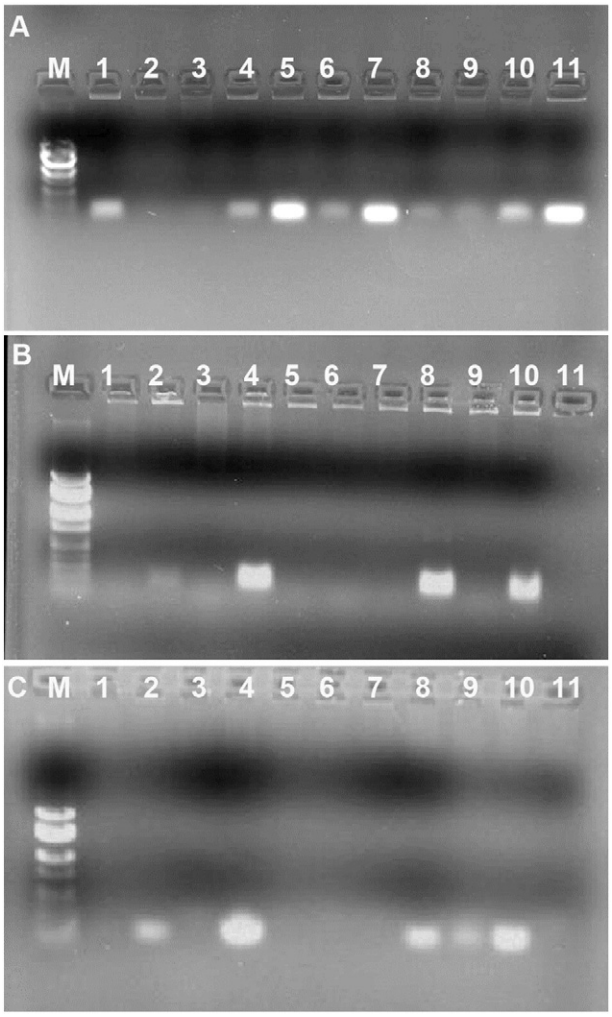

Fig. 3. The DNA extraction from dried *Anacardium occidentale* L. leaf materials is amenable to PCR-based genetic analysis. The amplification profiles of cashew varieties by three SSR markers are depicted here. The following SSR, mAoR7 (A), mAoR48 (B) and mAoR6 (C) markers are used. M = DNA maker (DNA ladder).

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