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Full Length Research Paper

A simple and efficient method for extraction of genomic DNA from tropical tuber crops

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DNA extraction in many plants is difficult because of metabolites that interfere with DNA isolation procedures and subsequent applications, such as DNA restriction, amplification and cloning. We have developed a reliable and efficient method for isolating genomic DNA free from polysaccharide, polyphenols and protein contaminants from tropical tuber crops (Elephant foot yam, Cassava, Sweet potato Taro and Tannia). The method involves inactivation of contaminant proteins by using CTAB/Proteinase K and precipitation of polysaccharides in the presence of high concentration of salt. The purity of genomic DNA was confirmed by $A_{260/280}$ and $A_{260/230}$ ratios calculated from the spectrophotometric readings and further by restriction analysis of the isolated DNA using restriction enzymes *Eco*RI and *Hind* III. The described protocol also resulted in the isolation of sufficiently higher yield of DNA from leaf sample of tropical tuber crops. The new protocol can be successfully used for both small and large scale preparation of genomic DNA from leaf tissues of tuber crops which is highly suitable for further down stream processes like PCR amplification and restriction digestion analysis.

Key words: DNA isolation, RAPD, restriction enzyme digestion, tuber crops.

INTRODUCTION

Tropical tuber crops form the means of sustenance for millions of people in the tropical and sub-tropical world. They have the history of saving mankind in times of famine. The development of molecular markers as a tool for tuber crops germplasm characterization and early progeny selection is highly desirable for developing an efficient breeding program to speed the integration of new genetic material into elite germplasm. In addition, germplasm characterization of tropical tuber crops using molecular markers will contribute to knowledge of genetic relationships between accessions of the wild and cultivated gene pool and hence facilitate the breeding of improved genotypes in tuber crops to satisfy market needs and also helps develop genotypes possessing tolerance against diverse biotic and abiotic challenges.

A prerequisite for successful implementation of these crops improvement strategies is the ability to isolate good amount of high quality genomic DNA which can be used for Southern blot analysis, polymerase chain reaction (PCR) amplification, restriction fragment length polymorphisms (RFLPs), arbitrary primed DNA amplification (RAPD, AP-PCR, DAF) and genomic library construction (Clark, 1997). To fulfill this criterion a rapid, simple and reliable DNA isolation method is highly solicited. The isolation of good quality DNA from leaves of tropical tuber crops is complicated due to presence of phenolic compounds, highly viscous polysaccharides and DNA degrading endonucleases. During tissue homogenization, phenolics become oxidized and irreversibly bind to the protein and nucleic acid (Loomis, 1974; Aljanabi et al., 1999).

This irreversible binding produces a gelatinous material, which is hard to separate from organelles and the DNA becomes unsuitable for PCR amplification and restriction enzyme digestion analyses (Porebski et al., 1997). Polysaccharides are also problematic (Scott and

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Abbreviations: CTAB, Cetyltrimethylammonium bromide; EDTA, hexadecyltri-methylammoniumbromide; PVPP, polyvinylpolypyrrolidone; PEG, polyethylene glycol; RAPD, randomly amplification of polymorphic DNA.

Playford, 1996) as acidic polysaccharides inhibit the digestion of lambda DNA by certain endonucleases like *Hind* III (Do and Adams. 1991) and classical 2-primer PCR amplification (Demke and Adams, 1992) by inhibiting *Taq* DNA ploymerase activity (Fang et al., 1992).

In addition, polysaccharides can cause anomalous reassociation kinetics of DNA sample (Merlo and Kemp, 1976). They co precipitate with DNA during alcohol precipitation to form highly viscous solution (Do and Adams, 1991) making DNA unsuitable for digestion by restriction enzymes and southern blot hybridization. The DNA tends to stick to the wells of the gel during electrophoretic analysis. NaCl at concentration of more than 0.5 M together with CTAB is known to remove polysaccharides (Murray and Thompson 1980, Paterson et al., 1993).

The problem of polyphenols is exacerbated if green, over matured tissue is taken rather than etiolated leaves (Sharma et al., 2000). The method we developed for extracting DNA is specially designed to isolate genomic DNA from leaves of tropical tuber crops within a short period of time using small amounts of plant tissues and yielding a high quality of purified DNA suitable for restriction digestion and PCR-based analysis.

MATERIALS AND METHODS

Plant materials

Young leaf tissues of Elephant foot yam (*Amorphophallus paeoniifolius*, var. Gajendra), Cassava (*Manihot esculenta*, var. M4), Sweet potato (*Ipomoea batatas*, var. Gouri), Taro (*Colocasia esculenta*, var. Telia) and Tannia (*Xanthosoma sagittifolium*) were collected during early hours in the morning from Central Tuber Crops Research Institute, Thiruvananthapuram (India). These were kept between moist tissue paper in plastic bag and kept away from sunlight. The leaves were de-starched by covering them for 12 to 18 h before use.

Reagents and chemicals

Extraction buffer consisting of 100 mM tris-HCl (pH 8), 20 mM EDTA (pH 8), 2 M NaCl, 2% CTAB (w/v), 2% PVP (Mr. 40,000), 2% β-mercaptoethanol (v/v) was prepared. In addition, phenol : chloroform : isoamyl alcohol (25:24: 1), 4% PEG solution, Wash solution [15 mM ammonium acetate in 75% (v/v) of ethanol] and a TE buffer consisting of 10 mM tris-HCl (pH 8) and 1 mM EDTA (pH 8) also prepared and used.

DNA extraction protocol

Dry leaf tissues (300 mg) were ground to a fine powder using liquid nitrogen. Prewarmed extraction buffer (1 ml) was added to the samples and it was ground once more in the buffer. All the samples were transferred to 2.0 ml eppendorf tubes, 5 ∝L Proteinase K (10 mg/ml) was added. The tube was incubated in 37ºC for 30 min and then at 65ºC for another 30 min with frequent swirling. Samples were centrifuged at 8,000 g for 10 min at RT and supernatant was transferred to fresh eppendorf tube. Equal volume of phenol : chloroform : isoamyl alcohol (25:24:1) were added and mixed by gentle inversion for 30 - 40 times. The samples were centrifuged at 8,000 g for 10 min at RT and supernatant was transferred to a fresh tube. 200 ∝L of 2 M NaCl solutions containing 4% PEG was added. It was observed that addition of this solution and incubation of the samples for at least 15 min at 4ºC increased the recovery of DNA yield. The samples were centrifuged at 8,000 g for 10 min at RT. The supernatant was transferred to a fresh tube and precipitated with 2/3 volume of isopropanol. The precipitated nucleic acids were collected and washed twice with wash solution. The obtained nucleic acid pellet was air-dried until the ethanol was removed and dissolved in appropriate amount of TE buffer (50 - 70 ∝L). The nucleic acid dissolved in TE buffer were treated with ribonuclease (RNase, 10 mg/ml), incubated at 37ºC for 30 min and stored at –20ºC until use.

DNA analysis

The quality of extracted DNA was analyzed by means of agarose gel electrophoresis (0.8%), followed by ethidium bromide staining. The purity of the DNA was estimated by spectrophotometry by calculating $A_{260/280}$ and $A_{260/230}$ ratio, and the yield was estimated by measuring absorbance at 260 nm. DNA purity was further confirm-ed by restriction digestion with two restriction enzymes, *Eco*RI and *Hind* III followed by gel electrophoresis.

To check the suitability of extracted DNA for downstream analysis, RAPD analysis was done with OPA-14 (5'-TCTGTGCTGG-3') primer. Each PCR reaction mixture of 25 ∝L consisted of 200 ng genomic DNA, 2.5 ∝L of 10 x reaction buffer, 4 ∝L of 25 mM $MgCl_2$, 2 ∝L of 2.5 mM dNTPs, 200 ng primer and 1U *Taq* DNA ploymerase (Promega Corporation, USA). PCR amplification was performed in Techne progene thermal cycler (Techne Cambridge Ltd.). The PCR reaction mixtures were heated at an initial step of 94ºC for 2 min and then subjected to 35 cycles of following programme: 94ºC for 30 s, 37ºC for 1 min, 72ºC for 1 min 45 s. After the last cycle temperature was maintained at 72ºC for 8 min. Amplified DNA was electrophorised in a 1.2% agarose gel containing 0.5 mg/ml ethidium bromide and photographed on UV light.

For comparison, the genomic DNA of same leaf sample was extracted using the existing CTAB protocol (Doyle and Doyle, 1987) and further DNA analysis was performed using same the conditions and reagents as described.

RESULTS

DNA yield and purity

DNA extraction using the existing CTAB protocol (Doyle and Doyle, 1987) gave a low DNA yield from leaf samples of tropical tuber crops (Elephant foot yam, Cas-sava, Sweet potato Taro and Tannia). In addition, heavy contamination of polysaccharides, phenolic and protein was noticed which was revealed by spectrophotometer analysis. The samples were viscous and upon electrophoresis fire type bands were visualized. Moreover, DNA was found to be stacked to the wells with protein smear, which confirmed the presence of polysaccharide and protein contamination (Table 1, Figure 1).

The modified CTAB protocol designed by us increased the DNA yield up to 3 - 4 fold compared to existing CTAB method (Table 2, Figure 2). The $A_{260/280}$ and $A_{260/230}$ ratio indicated the absence of protein and polysaccharides contamination.

Table 1. DNA yield and purity isolated from different genus of tuber crops by using Doyle and Doyle (1987) method.

Tuber crops	DNA yield (∝g/g of sample)	$A_{260/280}$	$A_{260/230}$
Elephant foot yam(var. Gajendra)	132	1.60	1.22
Cassava (var. M4)	111	1.58	1.18
Sweet potato (var. Gouri)	128	1.63	1.28
Taro (var. Telia)	94	1.48	1.02
Tannia	162	1.55	1.12

Table 2. DNA yield and purity, isolated from different genus of tuber crops by modified protocol.

Tuber crops	DNA yield (∝g/g of sample)	$A_{260/280}$	$A_{260/230}$
Elephant foot yam (var. Gajendra)	348	1.91	1.82
Cassava (var. M4)	354	1.90	1.81
Sweet potato (var. Gouri)	392	1.92	1.86
Taro (var. Telia)	364	1.92	1.80
Tannia	360	1.96	1.83

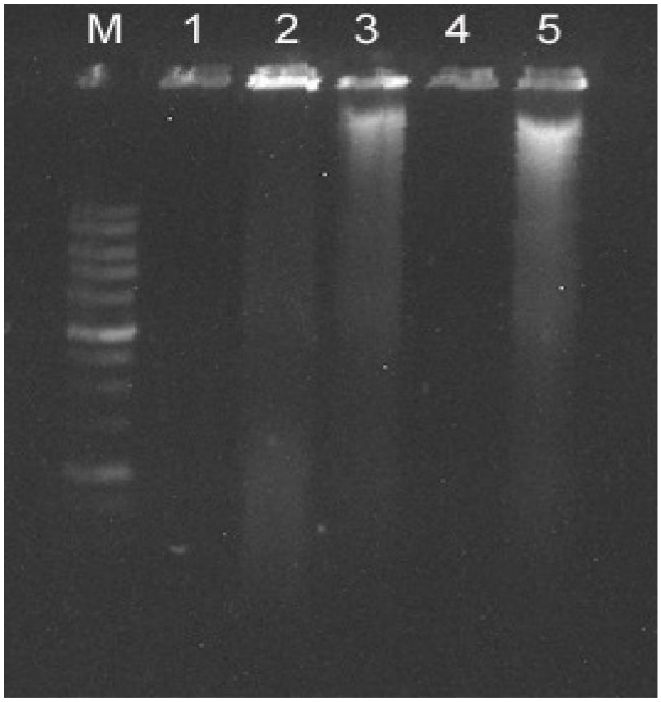

Figure 1. DNA isolated by using Doyle and Doyle protocol. Lane 1 belongs to Taro; lane 2, Tannia; lane 3, Elephant foot yam; lane 4, Cassava; lane 5, Sweet potato.

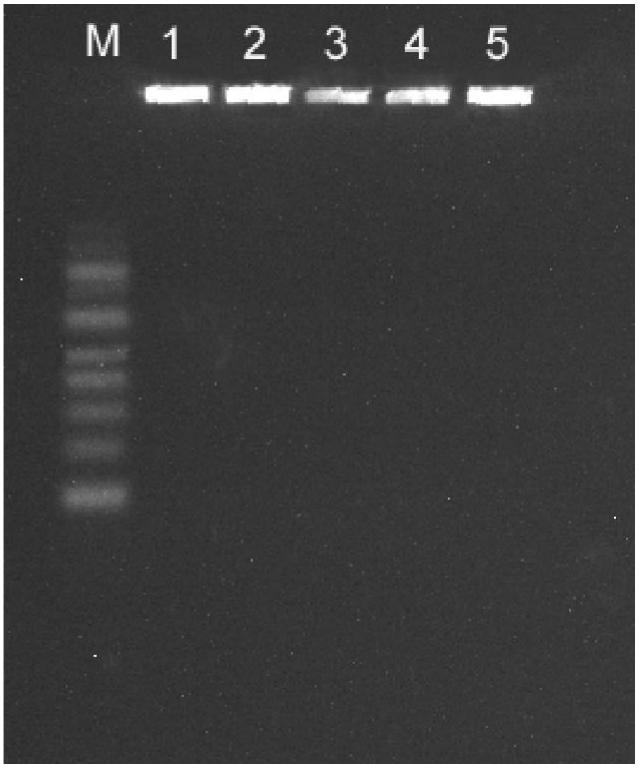

Figure 2. DNA isolated by using present protocol. Lane 1 belongs to Taro; lane 2, Tannia; lane 3, Elephant foot yam; lane 4, Cassava; lane 5, Sweet potato.

The purity of DNA was further confirmed by means of restriction digestion analysis using the enzymes *Eco*RI and *Hind* III and monitoring the banding profile of the digested DNA after incubating the reaction mixture at 37ºC for 3 h (Figure 5). The results indicated that isolated DNA was amenable for further downstream applications.

PCR analysis

Successful and reproducible amplifications were obtained by using OPA-14 (5'-TCTGTGCTGG-3') primer in the

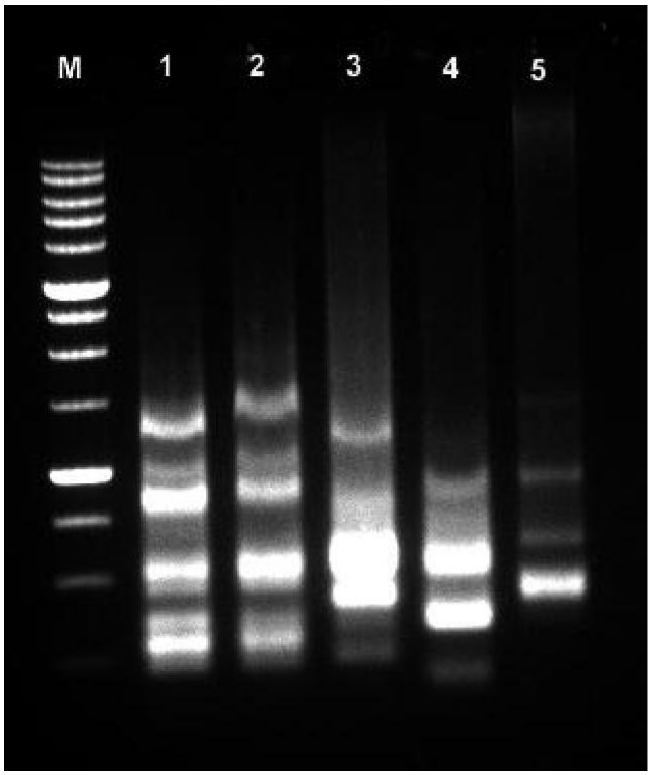

Figure 3. RAPD assay conducted with the DNA isolated by means of improved protocol given in present study by primer OPA-14 (Lanes 1 - 5). Lane 1 shows the amplification product belonging to Taro; lane 2, Tannia; Lane 3, Elephant foot yam; Lane 4, Cassava; Lane 5, Sweet potato.

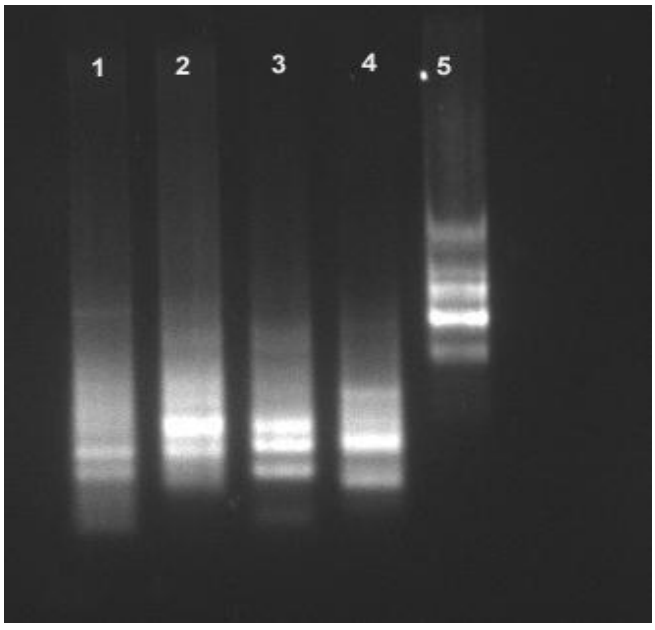

Figure 4. RAPD assay conducted with the DNA isolated by means of Doyle and Doyle by primer OPA-14 (Lanes 1 - 5). Lane 1 shows the amplification product belonging to Taro; lane 2, Tannia; Lane 3, Elephant foot yam; Lane 4, Cassava; Lane 5, Sweet potato.

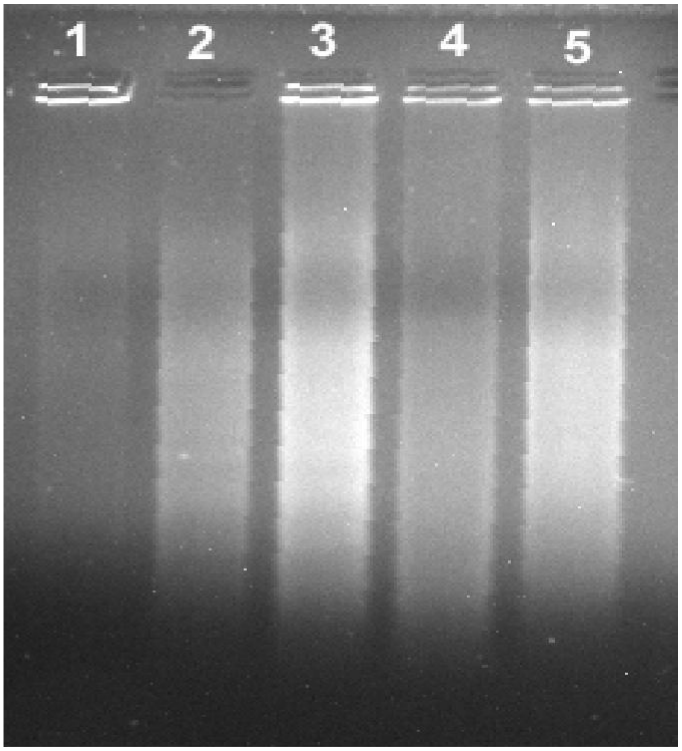

Figure 5. Restriction enzyme digestion with *Eco*RI and *Hind* III Lane 1 Sweet potato; Lane 2 Tannia; Lane 3, Elephant foot yam; Lane 4, Cassava; Lane 5, Taro.

DNA samples isolated from different genera of tropical tuber crops. The results demonstrated that DNA extracted with this modified protocol is suitable for PCR-based analysis (Figure 3). When same primer and same conditions were applied for RAPD amplification of DNA isolated by Doyle and Doyle (1987) method, reproducibility of bands was not observed (Figure 4).

DISCUSSION

The basic purpose of this study was to improve and simplify the DNA extraction protocol that can be applied to most of the tropical tuber crops which can yield large amount of pure, intact DNA amenable for restriction digestion, PCR and Southern hybridization analysis compared to the DNA extracted using conventional method (Doyle and Doyle, 1987) without any ultra centrifugation or column purification steps. The DNA extraction protocol described here is rapid and technically easy for preparing nucleic acid that is useful as an amplification target. The key steps of modified protocol are (1) extraction with high salt CTAB buffer to remove polysaccharides, (2) use of β-mercaptoethanol (2%) and PVP (2%) to remove polyphenolic compounds (3) use of PEG and proteinase K to remove protein completely.

Other important features of this protocol are that (1) the procedure works well with most of the genera of tropical

tuber crops without any modification of step, (2) large numbers of samples can be processed simultaneously and (3) the method is simple and not labor intensive. In addition, as the present extraction protocol is carried out in Eppendorf tubes, the chances of contamination and loss of DNA are minimized. This method can also be used for PCR based marker studies and screening of transgenic. The same protocol can be scaled-up if we need to go for large-scale extractions and this method can also be attempted to other crops where extraction of pure genomic DNA is a constraint.

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