

## A rapid method for isolation of high quality DNA from leaves of *Gmelina arborea* (Roxb) for molecular analysis

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**Abstract-** Procuring considerable quantity of DNA of nearly fair quality from trees is difficult because of the presence of metabolites and mucilaginous substances, which is found in excess in their leaves that interfere with the isolation procedures. *Gmelina arborea*, a fast growing species of the family verbenaceae occurs naturally in tropical and sub tropical regions of Asia. Currently, though there are a number of methods for isolation of DNA like the CTAB method, SDS Method etc., no single method was suitable for obtaining fine quality DNA from the leaves of *Gmelina arborea* for further molecular studies because of the presence of polyphenols and mucilaginous substances in the leaves. Herein we describe a modified protocol based on Dellaporta et al. (1983) for isolating DNA from dried leaves of *Gmelina arborea*, which is suitable for further molecular analysis. The protocol overcomes the problem of co-precipitation with contaminating agents. The yield of the DNA was found to be 50µg from 0.2 g of starting material. The purity of the DNA was also checked by the absorbance ratios at 260nm and 280nm respectively. The DNA thus obtained was used for RAPD and ISSR analysis.

**Keywords-** DNA extraction, dried leaves, *Gmelina arborea*, molecular analysis

### Introduction

*Gmelina arborea* is a medium sized deciduous tree, which grows up to 40 m tall and 140 cm diameter. It belongs to the Family Verbenaceae and occurs naturally in 11 countries in South Asia [1]. It is a fast growing species belonging to the Family Verbenaceae. It occurs naturally from latitudes of 5° to 30° N and 70° to 110° E. It is commonly seen in countries with distinct dry seasons like Bangladesh, Cambodia, India, China, Sri Lanka, Thailand, Vietnam, Nepal, Laos, Myanmar and Pakistan. Approximately 700,000 ha of *Gmelina* have been established in Central-West and South-East Asia [2]. It is found commonly in association with *Tectona grandis*, *Melia azedarach*, *Lagestromia spp* and *Pterocarpus macrocarpus*. This species has been recognised not only for pulp and paper production but also for solid wood products [\*]. In the recent past, rapid studies in Plant molecular biology and Biotechnology has opened up interesting and challenging possibilities in characterizing and evaluating plant genetic resources. Molecular tools based on PCR have begun to contribute a lot for this cause. Procuring considerable quantity of good quality DNA from trees possess a variety of problems because of partial or complete degradation by exogenous nucleases and co-isolation with polysaccharides and poly phenols which bind irreversibly to the isolated DNA and makes the DNA nearly unfit for further molecular work [3]. PCR based methods warrants only tiny amounts of DNA where the purity of the DNA does not really matter, but the poly phenols gets oxidised hindering the reaction [4]. The first step of RAPD analysis is the preparation of the target DNA template. Intuitively, minimal DNA template is sufficient for RAPD reactions. In deed, many rapid DNA isolation protocols designed for use with PCR actually involve little isolation of DNA and employ “Grind and use” process with minimal purification steps. The quality of the DNA is influenced by the condition of the starting

material. Most protocols require freshly harvested tissues while others demand lyophilized materials. Protocols for material with high polyphenol levels are either expensive or time consuming or require CsCl ultra centrifugation steps. One method commonly used to avoid problems with poly phenols make use of PVP in the extraction buffer [5]. Currently no study has been reported at the molecular level in *Gmelina arborea*. Hence as a prelude to the molecular taxonomic studies herein, we describe an improved DNA isolation protocol based on Dellaporta et al, 1983 [6] for RAPD analysis. Further, use of dried leaf material saves time and work force during extensive field study for collecting the plant material. The present study aims to isolate genomic DNA from dried leaves of *Gmelina arborea* for PCR based processes. The protocol described here is a rapid method that yields DNA of fairly good quality and quantity from matured dried leaves, which contain high levels of polyphenols.

### Materials and Methods

#### Sample collection and Processing

Fresh leaves from different populations of *Gmelina arborea* were collected from the Seed Orchard at Panampally, Kanjikode, Kerala. The leaf samples were sorted out and shade dried on blotting paper for about 15 days for complete drying. They were then made to a fine powder by grinding in a mortar and pestle and stored at room temperature until use.

#### DNA extraction and Quantitation

##### Solutions Required

Extraction Buffer (150mM Tris-HCl pH 8.0, 50mM EDTA pH 8.0, 1.75M NaCl, 0.2% SDS, 0.14 M Beta Mercaptoethanol, 40 µg/mL Proteinase K), 5 M Potassium acetate pH 5.2, Chloroform: Iso amyl alcohol (24:1), TE buffer (10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0)

### DNA isolation

A quantity of 200 mg of dried leaf powder was taken in a clean mortar and pestle and was ground well to a fine paste thoroughly with a pinch of acid treated sea sand and 1 mL of extraction buffer. The contents were incubated at 65° C for 15 minutes and centrifuged at 14,000 g for 5 minutes at room temperature. To the supernatant equal volume of 5 M Potassium acetate was added, vortexed well and centrifuged at 14,000 g for 5 minutes. To the supernatant equal volume of 24: 1 Chloroform: Iso amyl alcohol was added, gently mixed by inversion and extracted at 14,000 g for 5 minutes at room temperature. The top aqueous layer was taken and double the volume of ice-cold Iso amyl alcohol was added and mixed by inversion. The DNA fibres were spooled to a clean tube and washed with 70% ethanol, air dried and a volume of 500 µL of TE was added and incubated at 65° C for 10 minutes. To this a concentration of 10 µg /mL RNase A was added and incubated at 37° C for 30 minutes followed by precipitation with double the volume of ethanol and the DNA was pelleted at 10,000 g for 5 minutes at room temperature. The pellet was air-dried and re dissolved in 200 µL of TE buffer.

### Quantitation of DNA concentration and purity

The yield and purity of the DNA isolated by the above protocol was checked by reading their absorbance at 260nm and the ratio of absorbance at 260 nm /280 nm respectively [7].

### Restriction Analysis

A restriction analysis was performed to check if the DNA was pure enough for digestion with restriction enzymes. A 20 µL reaction consisting of approximately 1 µg of the DNA in 1X assay buffer (33mM Tris Acetate pH 7.9, 10mM Magnesium Acetate, 66 mM Potassium acetate and 0.1mg/mL BSA) and 20 U of the restriction enzymes Bam HI, EcoR I, HindIII respectively were set up in separate reactions and incubated for 5-7 hours at 37° C and the digested samples were electrophoresed on 0.8% agarose gel.

### RAPD Reactions

Two decamer primers OPB 2 and OPB 5 obtained from Operon Technologies, USA was used to standardise the PCR conditions. Several parameters were studied and altered to get reproducible amplifications. The reaction was carried out in a 25 µL reaction cocktail consisting of 50 ng template DNA, 1X Taq polymerase Buffer (10mM Tris-Hcl pH8.0, 50 mM KCl, 0.1%w/v Triton X 100), 3.2 mM MgCl<sub>2</sub>, 0.4 µM Primer, 250 µM of each dNTP and 1U Taq DNA Polymerase (Qiagen). The PCR reaction was carried out in a MJ Research Minicycler PTC 150 programmed for an initial denaturation of 94° C for 4 minutes followed by 35 cycles of denaturation at 92° C for 1 minute, primer annealing at 39° C for 1 minute,

extension at 72° C for 1 minute and final extension at 72° C for 10 minutes. The amplified products were electrophoresed on 1.5% agarose gel in 1X TAE buffer at constant current of 50 V and documented using Alpha Innotech Gel Documentation system.

### ISSR Reactions

Two ISSR primers UBC#854 and UBC#851 obtained from University of British Columbia were used to standardise the conditions. The reaction was carried out in a 25 µL reaction cocktail consisting of 50 ng template DNA, 1X Taq polymerase Buffer (10mM Tris-HCl pH 8.0, 50 mM KCl, 0.1%W/V Triton X 100), 4.0 mM MgCl<sub>2</sub>, 0.8µM Primer, 250 µM of each dNTP and 1U Taq DNA Polymerase (Qiagen). The PCR reaction was carried out in a MJ Research Minicycler PTC 150 programmed for an initial denaturation of 94° C for 4 minutes followed by 35 cycles of denaturation at 92 °C for 1 minute, primer annealing at 55° C (for UBC#851) and at 59° C (for UBC#854) for 40 seconds, extension at 72° C for 1 minute and a final extension was given at 72° C for 10 minutes. The amplified products were analysed by separating on a 1.5% agarose gel in 1X TAE buffer at constant current of 50 V and documented using Alpha Innotech Gel Documentation system.

### Results and Discussion

Forest tree species has not been explored much. *Gmelina arborea* is one such species. Forest tree species produce secondary metabolites such as polyphenols, carbohydrates, mucilages etc. Presence of secondary metabolites hinders effective isolation and the quality of the isolated DNA. Therefore, this new protocol based on modification of Dellaporta et al., 1983 has been standardised for isolation of high quality DNA from leaves of *Gmelina arborea* amenable for further molecular studies. The yield and purity of the DNA thus obtained was compared with the DNA isolated by CTAB method [8] and SDS method of Dellaporta et al. Though all the protocols yielded DNA, the modified protocol gave DNA of fine quality and quantity "Fig. (01)". The purity and the quantity of the DNA isolated by this method showed that the quality was the finest with the current standardised protocol yielding 5 mg DNA/g of leaf powder. There was a profuse shearing of DNA isolated by Dellaporta and CTAB methods "Fig. (01)" with an absorbance ratio being 1.8 and 1.5 respectively indicates the DNA being contaminated with polyphenols and other secondary metabolites [9]. Such a DNA is not suitable for further molecular studies. The DNA isolated by the currently standardised protocol was used for restriction analysis and the pattern of restriction was observed "Fig. (02)". RAPD and ISSR PCR reactions were done with the DNA isolated by the mentioned protocol using primers OPB2, OPB5 and UBC 854 and 851 respectively. The conditions for obtaining effective reproducible amplifications were

standardised "Fig. 03, 04". The key steps in our protocol was the homogenisation of the tissue sample with acid treated sea sand which assisted in partial removal of mucilaginous substances. Such contaminating substances were found to co-precipitate and coat the DNA isolated by the earlier reported CTAB and Dellaporta et al. method making the DNA nearly unfit for molecular work [10]. The incorporation of Proteinase K in the extraction buffer and modifications of the components of the buffer favoured an increase in the yield of the DNA. In conclusion, this method is rapid, simple, inexpensive and takes hardly an hour and a half for completion. This favours handling of large sample volumes simultaneously and overcomes the problem of sample collection and storage at cold conditions during extensive field visits. We state that, this modified protocol is suitable for extracting DNA from dried leaves of *Gmelina arborea* and the DNA yields were sufficiently good enough for RAPD and ISSR analysis. The protocol can be further extended to related tree species with relatively minor modifications.

#### Abbreviations

BSA- Bovine Serum Albumin  
 CsCl- Caesium chloride  
 CTAB- hexadecyl trimethyl ammonium bromide  
 PCR- Polymerase Chain Reaction  
 ISSR- Inter Simple Sequence Repeats  
 RAPD- Random Amplified Polymorphic DNA  
 SDS- Sodium dodecyl Sulphate

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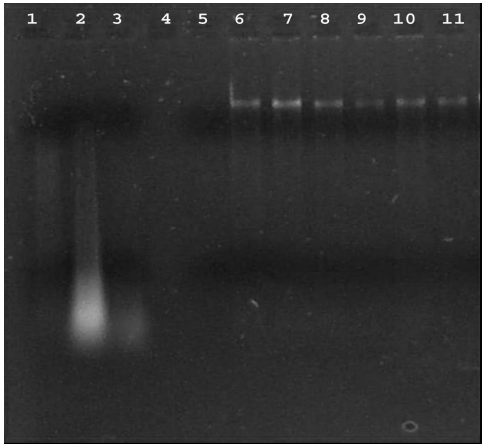


Fig 1: A 0.8% agarose gel electrophoresis of DNA isolated from dried leaves of *Gmelina arborea* by various methods. Lane 1 and 3 represents bands obtained by Dellaporta method, Lane 2 represents bands obtained by CTAB method. Lanes 6- 11 represents the intact DNA bands obtained by the standardised protocol based on Dellaporta et al.

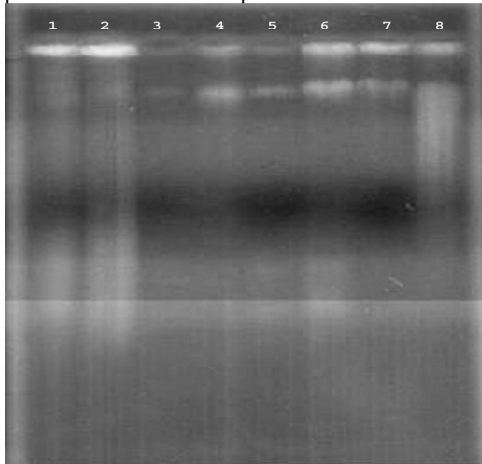


Fig 2: A 1.2% agarose gel electrophoresis of the restricted *Gmelina arborea* DNA. Lane 1 represents DNA digested with Hind III, Lane 2 represents DNA digested with Bam HI, Lanes 3-7 represents uncut DNA and Lane 8 represents DNA digested with EcoRI.

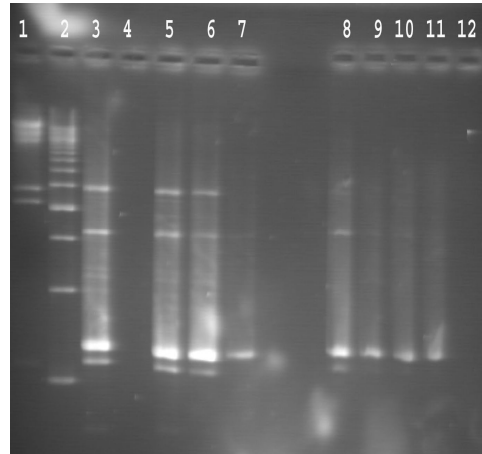


Fig 3: RAPD banding pattern obtained for the DNA isolated by the standardized protocol. Lane 1 and 2 Marker DNA, Lane 3-7 represents amplified product of OPB2 primer and Lane 8-12 represent amplified product of OPB5.

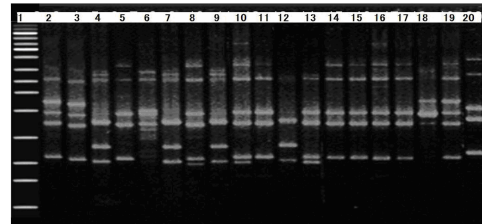


Fig 4 (A): ISSR banding pattern obtained for the DNA isolated by standardized protocol. Lane 1 represents marker DNA, Lane 2-20 represents amplified product of UBC 851.

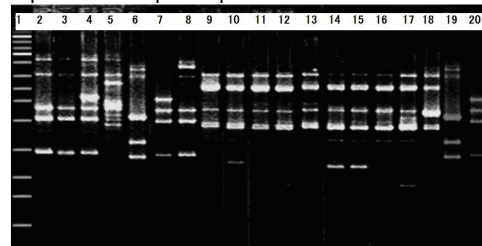


Fig 4 (B): ISSR banding pattern obtained for the DNA isolated by standardized protocol. Lane 1 represents marker DNA, Lane 2-20 represents amplified product of UBC 854.