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Short Communications

Standardization of DNA isolation and PCR parameters in *Garcinia* spp. for RAPD analysis

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Doyle and Doyle protocol with a little modification was used for the isolation of DNA from leaves and fruit rind of *Garcinia* spp. In case of leaves and dry fruit rind, the best DNA isolation was possible with 4% CTAB (100 m*M* Tris, 30 m*M* EDTA & 1.4 *M* NaCl), followed by 1.5% PVP and 0.3% mercaptoethanol. In case of fresh fruit rind, the DNA was best isolated with 2% CTAB (100 m*M* Tris, 30 m*M* EDTA & 1.4 *M* NaCl). PCR parameters were optimized using random RAPD primers. Other parameters included the DNA concentration at 50 ng/reaction, annealing temperature of 43°C, 2.5 m*M* dNTP in presence of 1 U Taq DNA polymerase and 2.0 m*M* $MgCl_2$. The protocol will be of great help to study the genetic diversity of *Garcinia* germplasms.

Keywords: DNA extraction, *Garcinia,* fresh fruit rind, dry fruit rind, RAPD-PCR

Garcinia is a large genus of polygamous trees or shrubs belonging to the family Clusiaceae. There are about 200 species in Asia and Africa. Of 35 species reported in India, 7 are endemic to Western Ghats, 6 in Andaman and Nicobar islands and 6 in North-eastern India. There are distinct morphological variations among the species. *G. mangostana* is a delicious fruit, while the Western Ghats species, namely, *G. gummigutta, G. indica, G. tinctoria* and *G. cowa*, are not much exploited. In recent times, these underexploited tree spice species are gaining much commercial, industrial and medicinal importance due to the presence of (-) hydroxyl citric acid (HCA), which is an anti-inflammatory and antioxidant compound[1].

Genetic studies give a direct knowledge of the gene variations with in a species or between the different species. To study this variation, DNA isolation protocol needs to be optimized. It has been frequently recommended that the optimal concentration for a PCR reaction component should be determined empirically by performing a series of reactions at different Mg^{2+} and EDTA concentrations[2]. The aim of the present study was to standardize the DNA extraction and RAPD protocol of the above mentioned species of Western Ghats. In this study, various % of CTAB buffer with various concentrations of EDTA and Mg^{2+} in PCR amplification were tried to find an optimum condition for DNA extraction and PCR reaction of *Garcinia* tissue.

Fruit rind and leaves of *Garcinia* species, viz., *G. gummigutta, G. indica, G. tinctoria* and *G. cowa* were collected from the Western Ghat regions. Wherever fresh fruit was not available, dried rind from the market was collected. Fresh leaves and rind tissue were stored in iceboxes until reaching the laboratory. DNA extraction from fresh leaves, fresh fruits and dry fruits were carried out with different percentages of the CTAB buffer. Leaves were pulverized in liquid nitrogen and the DNA was isolated with 4% CTAB in addition to 1.5% PVP and 0.3% mercaptoethanol. In the case of fresh rind, the tissues were crushed with 2% CTAB (100 m*M* Tris, 30 m*M* EDTA & 1.4 *M* NaCl) adding up with 1.5% PVP and 0.3% mercaptoethanol. In the case of dried rinds the modification of the original protocol was done by soaking the sample in the extraction buffer for 2 d and then extracted with 4% CTAB buffer. In the present study, 30 m*M* EDTA was used in place of 20 m*M* EDTA of Doyle and Doyle method[3]. After isolation, purification of the DNA sample was done with RNase and phenol:chloroform:isoamyl alcohol (25:24:1). Purity of the isolated DNA samples were quantified by measuring absorbance at A260 nm in a UV spectrophotometer and A260/A280 ratio was evaluated. Mol wt and concentration of the DNA were calculated using gel electrophoresis with 1% agarose and (10 mg/mL) ethidium bromide staining.

RAPD was performed as per Williams[4] and the amplification involved an initial denaturation at 93°C for 3 min, 40 cycles of denaturation at 93°C for 30 sec, annealing at 43°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 min. Amplification products were resolved on a 2% agarose gel and stained with ethidium bromide

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(10 mg/mL). Electrophoresis was performed at 70 V for 2.5 h. The gels were photographed and visualized in a Gel documentation system (Kodak MI-Gel Logic 200). 3 µL DNA (50 ng) sample with 0.35 µL of 50 m*M* $MgCl_2$, 0.2 µL of 5U Taq DNA polymerase and 1 µL of 10 m*M* dNTP were used for 25 µL PCR reactions. Here 2 µL (10 pico mole) primer was used for the amplification. About 30 random RAPD primers were studied and 13 primers were screened for good amplification.

Yield of DNA from young leaf tissue of *Garcinia* species were high compared to fresh fruit rind (Table 1 & Fig. 1). The DNA yield from the dry fruit rinds was much lesser. The quality ratio (260/280) of the samples showed values between 1.62 to 1.75. The quality and quantity of DNA is dependent on the type of tissue. 13-25 ng DNA was isolated from fresh leaves, while the fruit rinds yielded 9.2-18.2 ng DNA from 1 g tissue (Table 1 & Fig. 2). When 4% CTAB buffer was used for fresh fruit rinds, the quality of DNA was low because of more protein precipitation. The colour and purity of DNA was found to be better with 2% CTAB but yield was very poor because CTAB helps in disintegrating the cell membrane[5,6]. Better yield was noticed with 30 m*M* EDTA than with 10 and 20 m*M* EDTA. The optimum concentration of EDTA was found to be 30 m*M*. Dried rind extraction was tried with 1, 2, 3 and 4% CTAB buffer with varying concentration of EDTA and PVP but the yield was very less. Dry rind was socked in CATB for 2 d and then crushed in 4% buffer provided good results. The extraction with chloroform:isoamyl alcohol (24:1) was repeated twice to get better precipitation. The isolated DNA sample could be stored at −20°C upto 1 yr and could be amplified by PCR for the study of genetic variability.

RAPD analysis with 50 ng of DNA sample for 25 µL reaction sample was found to be the best to get distinct bands with high polymorphism (Figs 3 & 4). Varying concentrations (0.5-5 m*M*) of $MgCl_2$ were studied for amplification, where 2.0 m*M* $MgCl_2$ proved better. Low levels of $MgCl_2$ resulted in incomplete amplification[7]. Since Mg^{2+} makes complex with the single nucleotide in PCR reaction

Table 1—Yield and purity of DNA from the leaf and rind tissue of *Garcinia* spp.

Species	Leaf		Fresh rind		Dried rind	
	OD value (A260/280)	Yield (µg/g)	OD value (A260/280)	Yield (µg/g)	OD value (A260/280)	Yield (µg/g)
G.gummigutta	1.74	25.2	1.75	18.2	1.62	5.1
G. indica	1.65	22.2	1.72	19.5	1.63	8.5
G. cowa	1.68	28.2	1.69	16.2	NA	NA
G. tinctoria	1.62	13.5	1.62	9.2	NA	NA

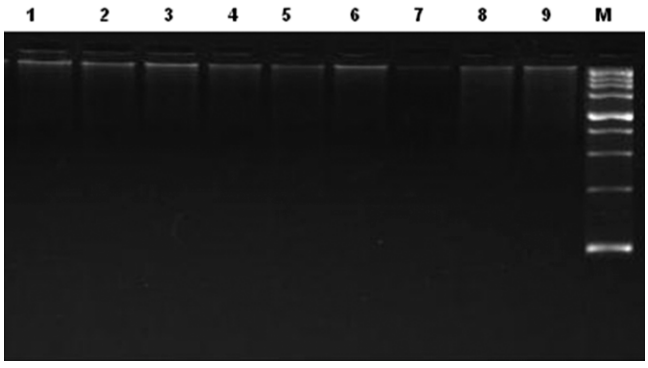

Fig. 1—Agarose gel of total genomic DNA isolated from the leaf and dry rind of *Garcinia* spp.: 1 & 2, *G. gummigutta*; 3 & 4, *G. indica*; 5, *G. cowa*, 6 & 7, *G. tinctoria*; 8, *G. gummigutta* (dry rind); 9, *G. indica* (dry rind); & M, 1 Kb ladder.

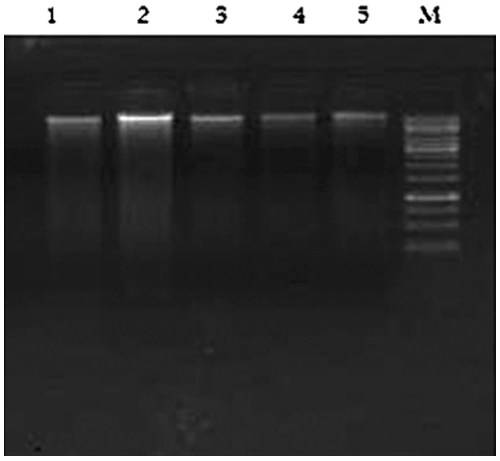

Fig. 2—Agarose gel of total genomic DNA isolated from the fresh rind of *Garcinia* spp.: 1, *G. gummigutta*; 2, *G. indica*; 3, *G. cowa*; 4 & 5, *G. tinctoria*; & M, 1 Kb ladder.

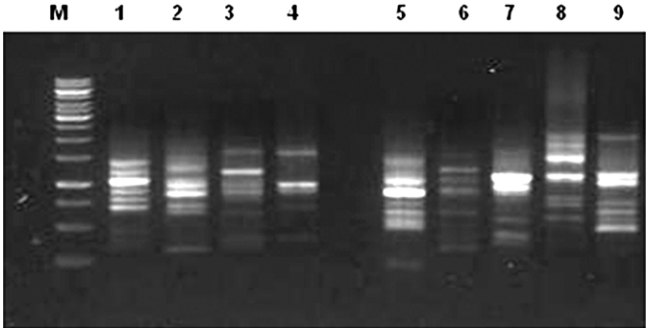

Fig. 3—RAPD profiling of of *Garcinia* leaf and dry rind sample: 1 & 2, *G. gummigutta*; 3 & 4, *G. indica*; 5, *G. cowa*, 6 & 7, *G. tinctoria*; 8, *G. gummigutta* (dry rind); 9, *G. indica* (dry rind); & M, 1 Kb ladder (annealing at 43°C for 1 min, primer AV-03, sequences-TTTCGGGGAG).

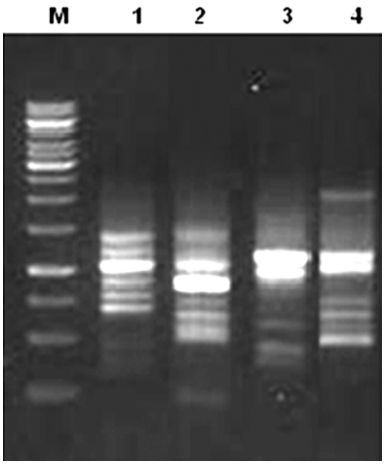

Fig. 4—RAPD profiling of *Garcinia* fresh fruit sample: 1, *G. gummigutta*; 2, *G. indica*; 3, *G. cowa*; 4, *G. tinctoria*; & M, 1 Kb ladder (annealing at 43°C for 1 minute, primer AV-03, sequences-TTTCGGGGAG).

and Mg-nucleotide complex are the substrate for DNA polymerase, the concentration of Mg^{2+} influences the productivity and fidelity of polymerase reaction[8,9]. Below 1 m*M* of $MgCl_2$ no bands were observed. 2.5 m*M* of dNTPs along with 1U Taq polymerase with annealing at 43°C for 1 min was better compared to at 37°C (Figs 3 & 4). At 37°C, less polymorphism with diffused bands were obtained.

The extraction of DNA from dry *Garcinia* rind was done for the first time and it is very useful for biodiversity study as transporting the fresh rind and leaves is difficult when collected from remote areas.

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