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Evaluation of genetic stability of micropropagated plants of three species of *Garcinia* using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers

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Random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers were used to analyze the genetic stability of micropropagated as well as mother plants of three species of *Garcinia*, viz., *G. gummigutta*, *G. indica*, and *G. tinctoria*. Twelve RAPD primers and four ISSR primers generated clear, distinct and reproducible bands. Total genomic DNA extracted from fresh leaves of *in vitro* regenerated as well as mother plants was used for developing RAPD and ISSR profiles. All RAPD and ISSR profiles from micropropagated plants were monomorphic and similar to those of the field grown mother plants. No genetic variation was detected within the micropropagated plants in comparison to their mother plants. The results indicate that the *Garcinia* plants regenerated using micropropagation system standardized at our laboratory were genetically stable.

Keywords: *Garcinia*, genetic stability, ISSR, micropropagation, RAPD

Garcinia (Family: Clusiaceae) has assumed great significance in the biological research arena today due to its medicinal properties and its wide use in culinary preparations. Fruit rind of *Garcinia* is a rich natural source of (-) hydroxy citric acid (HCA), which is a potent metabolic regulator of obesity and also lowers the blood lipids, such as, cholesterol and triglycerides. As a result its demand is increasing day by day. However *Garcinia* cannot be fully exploited as it is included under the list of endangered species of medicinal plants of southern India¹. Hence, it is important to find efficient methods of propagation for conservation, multiplication and utilization of this pharmaceutically important species.

Garcinia is traditionally propagated through seeds and soft wood grafting but factors like limited fruiting

season, lesser seed number and seed viability as well as slow growth of the seedlings are some of the problems which hamper its multiplication. To overcome these problems, *in vitro* culture of this medicinal plant is the only alternative. In *Garcinia*, seeds are apomictically developed and, therefore, plants are homogenous. This implies that the mother tree and its progenies are of same genetic composition². However, micropropagation can bring in somaclonal variations. On the other hand, for commercial planting, the micropropagated plants should be true to the type of mother plant. Hence, it is important to ensure the genetic fidelity of the micropropagated plants. Several strategies like morphological, biochemical and molecular analysis were used to assess the genetic fidelity of *in vitro* derived clones, but most of them have limitations³. Random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) marker analyses were used to study the genetic fidelity of micropropagated plants. RAPD has several advantages over other conventional methods⁴, as it is simple, quick to perform, requires very little plant material, yields true genetic markers and quick DNA extraction protocols are suitable^{5,6}. ISSR polymorphism has also been successfully employed to reveal genetic variation in many crop plants⁷. As a dominant marker, ISSR targets simple sequence repeats (microsatellites) that are abundant throughout the eukaryotic genome and evolve rapidly. The technique does not require prior knowledge of DNA sequence for primer design and has advantages similar to those of RAPDs⁸. In the present study, the genetic stability of micropropagated plants of three species of *Garcinia*, viz., *G. gummigutta*, *G. indica*, and *G. tinctoria*, was monitored using the RAPD and ISSR markers.

A protocol for micropropagation of *Garcinia* from whole seeds and seed fragments was developed at Indian Institute of Spices Research (IISR), Calicut, India. Seeds from the fruits were collected washed, cleaned with soap water and separated, and surface sterilized as per standard protocol. These seeds were inoculated into MS medium supplemented with 2.5 mg L⁻¹ 6-benzyl amino purine (BAP) and 2 mg L⁻¹ α -naphthalene acetic acid (NAA) for multiplication

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Table 1—Primers used in RAPD analysis and number of scorable bands for each primer

Primer	Sequence	No. of scorable bands in species of <i>Garcinia</i>		
		<i>G. indica</i>	<i>G. tinctoria</i>	<i>G. gummigutta</i>
OP AB-11	5'GTGCGCAATG3'	7	5	5
OP AB-01	5'CCGTCGGTAG 3'	5	4	6
AF-11	5'AAGACCGGGA3'	6	4	5
AP-20	5'CCCGGATACA3'	4	7	3
PO-05	5'CCCGGTAAC 3'	7	7	7
W-15	5'ACACCGGAAC3'	5	5	6
AV-03	5'TTTCGGGGAG 3'	4	1	4
AB-16	5'CCCGGATGGT 3'	6	6	5
BB-18	5'CAACCGGTCT 3'	7	6	6
AO-12	5'TCCCGGTCTC 3'	5	6	5
OP AA-11	5'ACCCGACCTG 3'	4	6	3
OP AA-01	5'AGACGGCTCC3'	4	7	2

and growth. Fully grown healthy plantlets were obtained after 2 months of culture. Leaves from these *in vitro* derived plantlets were used for DNA extraction to conduct RAPD and ISSR profiling.

A protocol for DNA isolation and molecular profiling was also developed at IISR⁹ and it was used for assessment of genetic stability in the present study. DNA was extracted from fresh leaves of micropropagated and field grown plants by the CTAB method based on Doyle and Doyle¹⁰ with some modifications⁹. Twelve arbitrary 10-base RAPD primers (Table 1) were used for PCR amplification, following the protocol of Williams *et al*⁴ with minor modifications¹⁰. Amplification reaction was performed with 25 µL volume of 2.5 µL 10× assay buffer, 1 µL of 10 mM dNTP, 2 µL of 10 picomole primer, 1 unit of Taq DNA polymerase (Bangalore Genei, India), 0.35 µL 50 mM MgCl₂, and 50 ng of genomic DNA. For DNA amplification, the reaction was performed using a PCR profile consisting of 1 cycle of 3 min at 94°C, 39 cycles of 30 sec at 94°C, 60 sec at 37°C, 60 sec at 72°C and 1 cycle of 15 min at 72°C. After amplification, the products were separated on 2.0% agarose gel by electrophoresis and stained with ethidium bromide. The gels were documented with Alpha imager under ultraviolet light.

For ISSR analysis, four ISSR primers (Table 2) were used for PCR amplification. The reaction volume used for amplification reaction was similar to that of RAPD reaction. The PCR profile consisted of 1 cycle of 5 min at 94°C; 36 cycles of 1 min at 94°C, 1 min at 51°C, 1 min 30 sec at 72°C and 1 cycle of 10 min at 72°C. Amplification products were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide, and gel documented with Alpha

Table 2—Primers used in ISSR analysis and number of scorable bands for each primer

Primer	Sequence	No. of scorable bands in species of <i>Garcinia</i>		
		<i>G. indica</i>	<i>G. tinctoria</i>	<i>G. gummigutta</i>
857a	(AC) ₈ CG	8	7	5
840b	(GA) ₈ TT	7	6	5
835b	(AG) ₈ TC	9	8	8
835a	(AG) ₈ CC	7	7	6

imager, in ultraviolet light¹⁰. The profiles of the micropropagated plants were compared with those of their mother plants for genetic stability analysis.

Out of thirty different decamers initially tested, twelve primers produced clear and distinct amplification products. These twelve arbitrary primers (Table 1), were used to develop RAPD profiles (Fig. 1). Similarly, four ISSR primers (Table 2), which gave clear PCR products, were used to obtain ISSR profiles (Fig. 2). The size of the DNA fragments produced by RAPD primers ranged from 228 to 1670 bp (Fig. 1). Twelve primers produced 64 bands in *G. indica*, 63 in *G. tinctoria* and 57 in *G. gummigutta*. Primer OP AB-11, PO-05 and BB-18 gave the highest number of bands in *G. indica*, while primer AP-20, PO-05 and OP AA-01 in *G. tinctoria* and PO-05 in *G. gummigutta* gave the highest number of bands.

ISSR profiles showed DNA fragments ranging from 375 to 2687 bp (Fig. 2). Four primers produced 31 bands in *G. indica*, 28 in *G. tinctoria* and 24 in *G. gummigutta*. In both the cases, *G. indica* showed the highest number of bands. A common band of size 750 bp was noticed in the RAPD profile of the three species (Fig 1). With further study, this band could be taken as generic band specific for *Garcinia* spp.

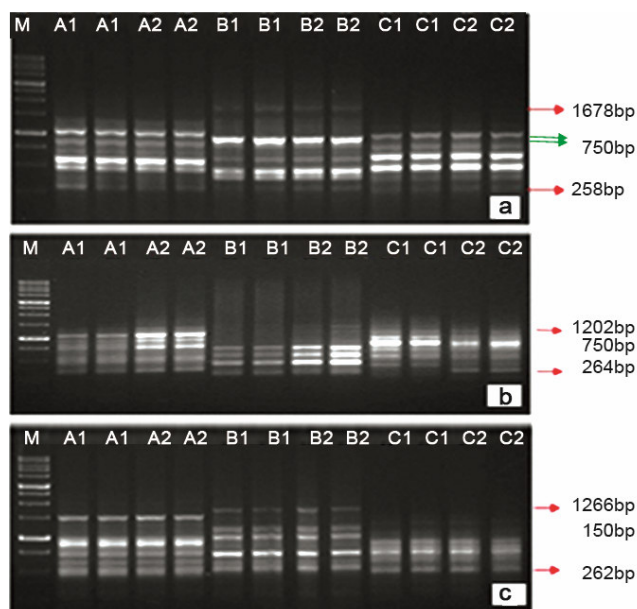


Fig. 1 (a-c)—RAPD profiles generated by primer BB-18 (a), OP AB-01 (b) and AO-12 (c): A1, *G. indica* mother plant; A2, *G. indica* micropropagated plant; B1, *G. tinctoria* mother plant; B2, *G. tinctoria* micropropagated plant; C1, *G. gummigutta* mother plant; C2, *G. gummigutta* micropropagated plant; & M, 1 kb ladder. [Red arrow indicates the size of the fragments (lowest and highest base pair value) and green arrow indicates the 750 bp band, which could be considered as the generic band]

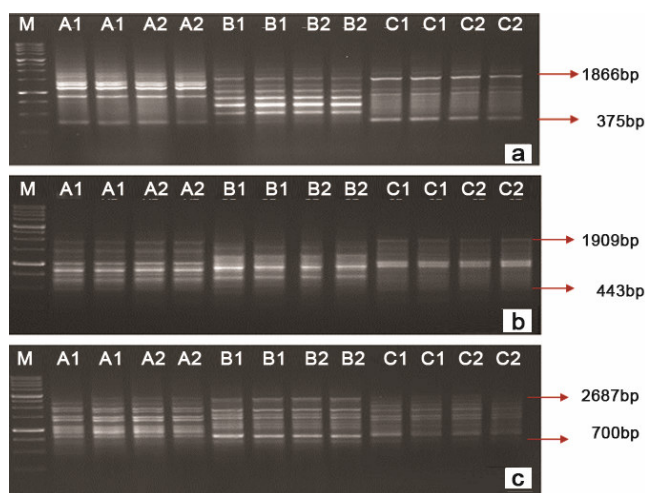


Fig. 2 (a-c)—ISSR profiles generated by primers 857a (a), 840b (b) and 835b (c): A1, *G. indica* mother plant; A2, *G. indica* micropropagated plant; B1, *G. tinctoria* mother plant; B2, *G. tinctoria* micropropagated plant; C1, *G. gummigutta* mother plant; C2, *G. gummigutta* micropropagated plant; & M, 1 kb ladder. [Arrow indicates the size of the fragments (lowest and highest base pair value)]

In the present study, RAPD profiles generated by twelve primers and ISSR profiles produced by four primers indicated a uniform pattern among the

micropropagated plants of a species in comparison to its mother plants (Figs 1 & 2). This uniformity was seen in all the three species studied. All the bands generated were monomorphic and were similar with the bands observed in mother plants. Similar observations were also made in earlier studies where molecular markers (RAPD & ISSR) were used for estimating genetic stability of micropropagated plants of Norway spruce¹¹ and meadow fescue¹². This further supports the view that micropagation is a viable technology for multiplication and conservation of *Garcinia* genetic resources and this technology can be effectively used for multiplication of planting materials of 'elite' high yielding trees of *Garcinia* spp.

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