

## Assessment of genetic fidelity of *in vitro* propagated clones of *Celastrus paniculatus* Willd by using RAPD based PCR Amplification

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**Abstract:** *Celastrus paniculatus* Willd belonging to the family *Celastraceae* is an endangered Indian medicinal plant having high pharmaceutical application. The objective of the present investigation was to assess the the clonal fidelity of *in vitro* propagated clones of *Celastrus paniculatus* with the field grown mother plant to confirm their true to type nature. Micropropagation is an alternative method for the large scale production of endangered medicinal plants. The genetic stability of *in vitro* raised clones of *celastrus paniculatus* were assessed by using RAPD analysis. Genomic DNA was isolated from healthy and fresh leaves of both mother plant and *in vitro* raised plants of *Celastrus paniculatus* by using CTAB method. Based on the reproducibility of the primers, 15 RAPD primers were selected for the present investigation. The selected primers gave rise to a total of 75 scorable bands with an average of 5.1 bands ranging from 300-2700 bp. The number of bands varied from three (OPQ-07, OPA-13) to seven (OPC-20, OPN-16). Randomly selected 10 micropropagated plants from each culture period was used. Amplification pattern was electrophoresed in 1.5% TBE, revealing that all the bands produced by micropropagated plants were monomorphic and similar to that of the field grown plant. No polymorphism was detected by RAPD analysis.

**Keywords:** *Celastrus paniculatus*, Polymorphism, Genetic fidelity, RAPD analysis

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### I. Introduction

*Celastrus paniculatus* Willd is a large, woody, climbing shrub distributed throughout India, mainly in deciduous forest. The root bark extract is a depurative, memory enhancer (Prajapati et.al.,2003) and also shows antimalarial activity ( Rastogi and Mehrotra.,1998). The leaves and leaf sap is a good antidote for opium poisoning. The seeds are effective in abdominal disorders, leprosy, various skin disorders, asthma, leucoderma, cardiac debility, inflammation and nephropathy. The seed oil is bitter, thermogenic, intellect promoting and is useful for abdominal disorders, beriberi and sores. (Warrier et.al.,1994). The phytochemical analysis revealed the presence of alkaloid such as celastrine, paniculatin, celapagine and celapanine (CSIR 1992) which possess anticancerous activity.

Plant tissue culture is regarded as one of the key areas of biotechnology and has been exploited as a valuable tool for the conservation and large-scale propagation of medicinally important and endangered plants. (Phulwaria et.al 2013; Thiagarajan and Venkatachalam 2012).The assessment of genetic stability of tissue culture raised plants is a prerequisite for their commercial application (Rai M K et.al 2012) because the influence of culture conditions, culture media, type of explant, subculture, temperature, pH etc., may lead to genomic variation in the *in vitro* raised plantlets. The genetic stability is considered as one of the most important factor while performing micropropagation. Sharma et.al 2011 revealed that DNA-based molecular markers are powerful tools for analysis of the genetic fidelity of micropropagated plantlets .

### II. Materials And Methodes

#### Plant materials

The plant material was collected from the Botanical garden, University of Calicut, Kerala for successive micropropagation by using various phytohormones. The experiments were conducted to assess the genetic fidelity among clones obtained from the high yielding *Celastrus paniculatus* with micropropagated plantlets from each culture period were used for RAPD analysis.

#### Genomic DNA isolation

Genomic DNA was isolated from healthy and fresh leaves of both mother plant and *in vitro* raised plants of *Celastrus paniculatus* by using CTAB method. (Doyle and Doyle1990). The pre-cooled samples (about 1g) were made in to powder using liquid nitrogen. Five ml of CTAB buffer (Buffer 1) was added to it, transferred to 30ml polypropylene tubes and warmed up in a water bath at 65°C for 30 minutes. It was cooled down to room temperature and centrifuged at 4°C and 8000 rpm for 10 minutes. The supernatant was collected

in to fresh tubes and mixed with equal volume of chloroform: isoamyl alcohol (24:1) and centrifuged at 8000 rpm for 10 minutes at room temperature. To the aqueous phase, equal volume of isopropanol was added and kept at  $-20^{\circ}\text{C}$  for 30 minutes. Later it was centrifuged at 12000 rpm for 10 minutes at  $4^{\circ}\text{C}$  and the pellet collected was subjected to ethanol wash using 70% ethanol. The pellet was then dissolved in  $50\mu\text{l}$  TE buffer and kept in refrigerator for overnight. After incubation,  $350\mu\text{l}$  of TE buffer was added and tapped well to dissolve all traces of DNA remained if any. It was then incubated in a water bath at  $37^{\circ}\text{C}$  for one hour after adding  $20\mu\text{l}$  of RNase A solution ( $10\mu\text{g/ml}$ ). After cooling down to room temperature,  $500\mu\text{l}$  of chloroform: isoamyl alcohol (24: 1) mixture was added, mixed well and centrifuged at 8000 rpm for 10 minutes at  $4^{\circ}\text{C}$ . To the aqueous phase,  $50\mu\text{l}$  3M sodium acetate and 1ml absolute alcohol were added, mixed well by gentle rotation and inversion and kept at  $-20^{\circ}\text{C}$  for 30 minutes. The supernatant was removed by centrifugation at 12000 rpm for 10 minutes and the pellet was air dried in laminar airflow chamber. The dried pellet was finally dissolved in  $50\mu\text{l}$  TE buffer and kept at  $-20^{\circ}\text{C}$  until use.

### **RAPD amplification**

RAPD analysis of field grown plant and in vitro raised progenies were performed to assess the clonal fidelity. Randomly selected 10 in vitro regenerated plants were assessed by using PCR based RAPD analysis. Based on the reproducibility of the primers, 15 RAPD primers were selected for the present investigation. The PCR was carried out in a volume of  $25\mu\text{L}$  of reaction mixture containing a final concentration of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1 Triton X-100, 0.2 mM of each dNTP, 3.0 mM  $\text{MgCl}_2$ , 0.4  $\mu\text{M}$  primer, 25 ng template, and 1 unit Taq DNA polymerase (BioGene, USA). Amplification was performed in a programmed thermal cycler (master cycle egradient Seppendorf, Germany) with initial denaturation at  $94^{\circ}\text{C}$  for 3min, 42cycles of denaturation at  $94^{\circ}\text{C}$  for 30s, primer annealing at  $32^{\circ}\text{C}$  for 1min, extension at  $72^{\circ}\text{C}$  for 2.5min, and final extension at  $72^{\circ}\text{C}$  for 4min.

### **Gel electrophoresis and visualization of RAPD**

All the reactions were amplified in a mastercycler gradient (Eppendorf, Germany). The PCR products were separated on 1.5% agarose gel stained with ethidium bromide in TBE buffer (pH 8.0). The size of the amplified fragments was determined using 500bp DNA ladder. The gels were documented using a gel documentation system (Alpha Innotech Corporation, USA). Two repeats were performed to confirm the results.

## **III. Results And Discussion**

### **RAPD fingerprinting**

Assessment of genetic fidelity was done with the help of RAPD analysis by using 15 RAPD primers. The selected primers gave a total of 75 scorable bands with an average of 5.1 bands ranging from 300-2700 bp. The number of bands varied from three (OPQ-07, OPA-13, OPC-04) to seven (OPC-20, OPN-16, OPA-10). Randomly selected 10 micropropagated plants from each culture period were used. RAPD amplification pattern obtained with primers OPG-15 (gel-1), OPA-04 (gel-2) and OPN-16 (gel-3) revealed that all bands produced by micropropagated plants were monomorphic and similar to that of the field grown plant.

**Table 1** RAPD primers used for testing the genetic fidelity of micropropagated plants of *Celastrus paniculatus*.

<b>RAPD primer</b>	<b>Primer sequence 5'-3' (length)</b>	<b>Total bands</b>	<b>Range of Amplification (bp)</b>
OPQ-07	CCCCGATGGT	3	400 – 1200
OPA-09	GGGTAACGCC	5	300 – 1000
OPC-02	GTGAGGCGTC	4	650 – 2000
OPA-13	CAGCACCCAC	3	400 – 1100
OPN-15	CAGCGACTGT	6	600 – 1400
OPA-04	AATCGGGCTG	6	400 – 2300
OPC-04	CCGCATCTAC	3	400 – 1000
OPH-05	AGTCGTCCCC	5	300 – 900
OPC-20	ACTTCGCCAC	7	500 – 1900
OPN-16	AAGCGACCTG	7	400 – 2700
OPG-15	ACTGGGACTC	4	300 – 1000
OPG-07	GAACCTGCGG	4	300 – 1000
OPN-02	ACCAGGGGCA	4	500 – 2300
OPA-10	GTGATCGCAG	7	300 – 2100
OPC-12	GAGAGCCAAC	7	350 – 2200

Many works reported that RAPD, have been widely used for testing the genetic fidelity among in vitro regenerated plantlets in a number of plant Species (Sharma et.al., 2011; Ramesh et.al.,2011; Ahmad and Anis 2011 Rai M K et.al.,2012).The factors like culture periods, genotype and nature of the explant and the application of various growth regulators during the in vitro studies, may disturb the internal polarity and physiology of the explants ( Lal.et.al., 2010 and Premvaranol.et.al., 2011). So assessment of the genetic stability of in vitro regenerated plantlets were necessary for further studies. Because of the simplicity, cost effectiveness and less expensive RAPD was used as better genetic stability analysis which amplify the different regions of the genome(Asthana et.al., 2011 and Martin et.al., 2004).

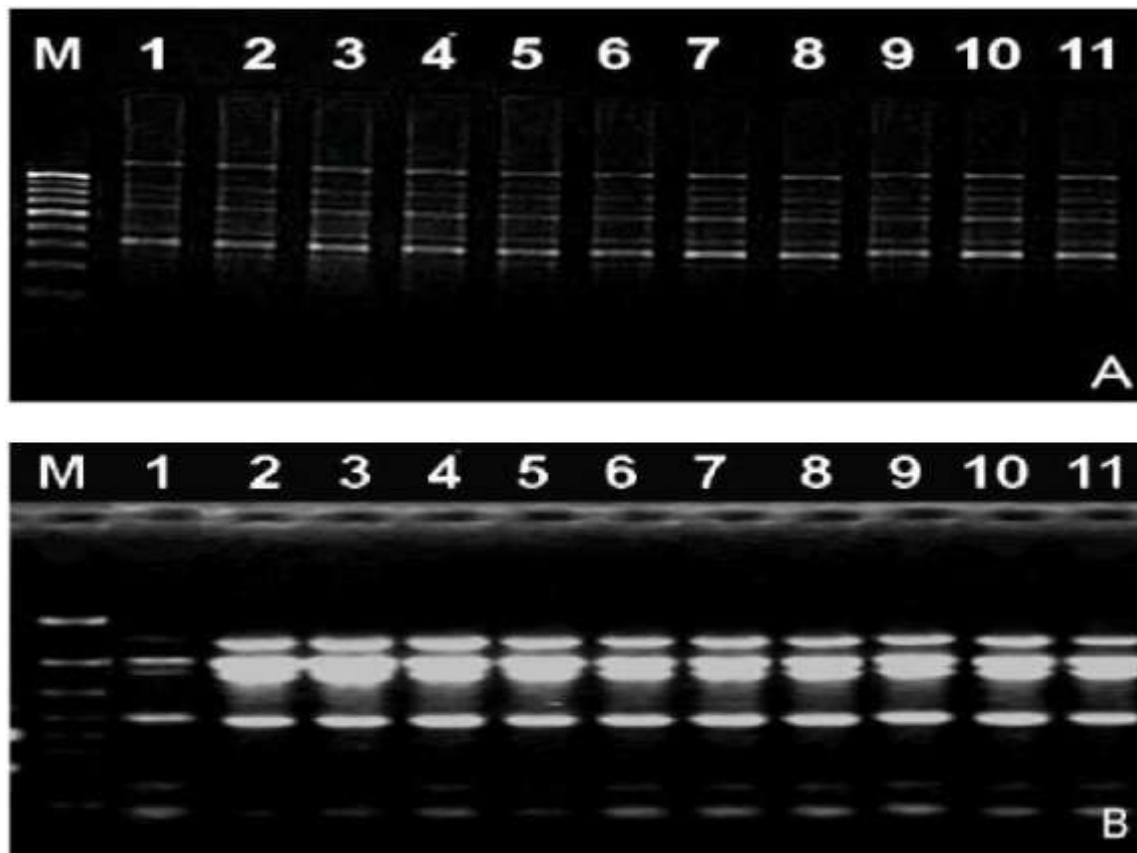


Figure 1- DNA Amplification pattern obtained with primers OPA-04 (gel. A), OPN-16 (gel.B). Lane M- DNAladder; Lane-1 DNA from mother plant; Lane 2-11 DNA from micropropagated plants.

#### IV. Conclusion

In the present investigation the true to type nature of micropropagated clones were confirmed by using PCR based RAPD analysis. No polymorphism was detected among tissue culture raised clones, hence they can successfully be employed for the commercial application without any risk of genetic instability. The RAPD fingerprinting pattern developed during the present study is very useful for molecular diagnostic purpose to detect the adulteration in herbal drug formulations.

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