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Research Article

Genetic Stability Assessment of In-Vitro Propagated *Celastrus Paniculatus* Using DNA Based Markers

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Abstract

Tissue cultured plantlets of *Celastrus paniculatus,* an endangered medicinal plant, subjected to assess the genetic fidelity of the genome. We have used a DNA based molecular markers to investigate the genetic stability of *in-vitro* regenerated *C. paniculatus* through nodal explant. The nodal explant responded satisfactory in terms of growth related traits like high shoot length (2.8cm ± 0.17), high number of shoots (2.6 ± 0.92) as well as high number of leaves per explant (5.6 ± 1.49) when inoculated in the MS medium supplemented with BA(0.886)+2ip(0.24). It produced clear reproducible and scorable bands(72 RAPD bands) when screened with 12 random amplified polymorphic DNA (RAPD) primers. The number of bands resolved per amplification were primer dependent and varied from 3 (OPC-16) to 11 (OPD-20) with an average of 6 bands per primer. All banding profiles from micropropagated plants were monomorphic and similar to the mother plant. This study is of high significance as these could be commercially utilized for large scale production of true-to-type plantlets in *C. paniculatus*.

Keywords: *Celastrus paniculatus,* endangered, *in-vitro* propagation, genetic fidelity, RAPD marker

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Introduction

Celastrus paniculatus (Family: Celastraceae) is an unarmed woody climbing shrub commonly known as Malkangni, Kangani, Jyotishmati, Sphutabandhani, Svarnalota, Black-Oil tree, Intellect tree, Climbing-staff plant. It is a rare and endangered medicinal plant distributed throughout India mostly in tropical forests and subtropical Himalayas. The species is vulnerable in Western Ghats of south India [1]. The seeds are used to heal indolent ulcers and sores, as well as infectious skin conditions such as scabies in the form of a poultice [2]. The oil being a powerful stimulant is also used as an ointment for relieving rheumatic pains inflicted by malaria [3]. Moreover, it has Anti-fertility[4], Wound healing activity [5], Anti-bacterial activity[6], Anti-fungal activity[7], Analgesic and Anti-inflammatory [8] properties.

With such a wide range of uses, *C. paniculatus* is being commercially exploited. Hence, the population of this plant has decreased exponentially in the last few years in their natural habitat. Due to several medicinal uses of *C. paniculatus*, the conservation of this plant is very essential. *In vitro* conservation of micropropagated plants is a safe method to protect the species from risk of natural disasters as well as increase their population[9]. If steps are not taken for their, mass propagation, cultivation and conservation, they may be lost from the natural habitat forever. Genetic fidelity is one of the most important pre-requisites in the micropropagated plant species. The occurrence of genetic instability arising due to somaclonal variation in the regenerates can seriously limit the utility of the micropropagation system[10]. Hence, it is important to establish genetic homogeneity of micropropagated plants to confirm the quality of the plantlets for its commercial utility. In order to help us with such an assessment, now a days molecular markers such as RAPD are used to analyse any somaclonal variations in the *in-vitro* propagated plants. RAPD markers are universal, ten base pair length primers, that are cost effective and easy to use.

Materials and Methods

The experiment was conducted in the Division of Plant Genetic Resources (PGR), Indian Institute of Horticultural Research (IIHR), Bangalore.

Plant material and cultural conditions

The nodal explants collected from disease-free plants of *C. paniculatus* raised in glasshouse were thoroughly washed in running tap water with a neutral liquid detergent for 3 to 5 minutes. These explants were cut into convenient sizes after removal of the leaf sheaths. The cut pieces were surface sterilized with 0.1% mercuric chloride for 4 to 6 minutes in a laminar flow cabinet and rinsed 3 to 4 times in sterile distilled water to remove the traces of sterilants prior to inoculation. Murashige and Skoog's (1962) medium was used as basal medium. Surface disinfected explants were inoculated on basal Murashige and Skoog (MS) medium supplemented with combination of BA, 2ip and NAA. The pH of the medium was adjusted to 5.7 before adding agar and was autoclaved at 121^0 C and 105 kg/cm2 of pressure for 20 minutes. All the cultures were incubated at 25 ± 10^0 C under white fluorescent light with 50µ mole m^{-2} s^{-2} light intensity during a photoperiod of 16:8 h light and dark cycles.

DNA extraction and PCR amplification conditions

The genomic DNA of *C. paniculatus* was isolated by following the protocol of [11] from both *in-vitro* and *ex-vitro* grown mother plants. Genetic fidelity of in-vitro raised plantlets was tested using RAPD marker. For this purpose, 15 *in-vitro* raised hardened plants were chosen randomly from the population and compared with the mother plant. 12 RAPD primers used for screening. PCR amplifications were carried out in a total volume of 25µl containing 25ng of genomic DNA as template, 2.5ml of 10x assay buffer (100 mM Tris Hcl, pH 8.3, 500mM KCl and 0.1% gelatin), 1.5 mM $MgCl_2$, 200µM dNTPs, 0.5 unit (U) of Taq polymerase and 15ng of primer. The primers showing polymorphic band were used to analyse the genetic fidelity of *in-vitro* raised plants. PCR amplification was performed in a DNA thermal cycler (Gene Amp PCR system 9700, Applied Biosystems, CA, USA), which was programmed for initial DNA denaturation at 94^0C for 5 min, followed by 30 cycles of 45 seconds denaturation at 94^0C, 45 seconds annealing (temperature specific to the primer) at 37^0C and 45 second extension at 72^0C, with a final extension at 72^0C for 8 min. Amplified products were resolved by electrophoresis on 1.8% agarose gel in TAE buffer (40mM Tris base, 20mMsodium acetate, glacial acetic acid to pH 7.2) stained with ethidium bromide (0.5µg µl-1) for 3h at 60 volts and photographs were taken by using the Gel Documenting system (Bio-Rad, USA).

Results and Discussion

In vitro *shoot multiplication*

Nodal segments from glasshouse plants of *C. paniculatus* were used as explant and inoculated to MS media containing varying combinations and concentrations of BA, 2ip and NAA. Effect of different plant growth regulators on growth related traits like shoot length, number of shoots and number of leaves were observed. Estimation of growth parameters was done after 8 weeks of inoculation. Data were statistically analysed by analysis of variance (ANOVA) and significance was calculated. MS medium supplemented with BA (0.886 mg/l)+2ip(0.24 mg/l) shown significantly high shoot length (2.8 ± 0.17) in cm. The same hormonal combination showed significantly high number of shoots (2.6 ± 0.92) as well as high number of leaves per explant (5.6 ± 1.49) (**Table 1** and **Figure 1**). This result was not found to be consistent to the results of [12] and [13]. [14] found that average shoot induction from shoot tip explant was higher in kinetin supplemented medium (65 %) followed by BAP supplemented medium (62 %) and TDZ supplemented medium (54.3%).

Assessment of genetic stability

RAPD analysis was done to ascertain the genetic stability of *in-vitro* raised plants of *C. paniculatus.* RAPD gel profile amplified by the primers OPB-08, OPC-06, OPC-11, OPC-16, OPD-08, OPD-20, OPE-01, OPF-10, OPG-02, OPG-06, OPG-14 and OPG-18 (**Table 2**). All the tried 12 primers gave amplification and total of 72 RAPD bands were generated, which were monomorphic indicating that micropropagated plants were similar to mother plant (**Figure 2**). The number of bands resolved per amplification were primer dependent and varied from 3 (OPC-16) to 11 (OPD-20) with an average of 6 bands per primer. The size range of amplification products also varied with selected primers as well as the plant from which DNA was isolated and ranged from 250bp to 10000bp. Number of monomorphic bands was highest (11) in case of primer OPD-20 (ranging from 250-2300bp in size) and lowest (3) in case of primer OPC-16 (ranging from 300-1000bp in size). No RAPD polymorphism was observed in the micropropagated plants indicating no genetic variation occurred in micropropagated plants of *C. paniculatus.* They showed a similar profile to

that of its mother plant and no somaclonal variations. Somaclonal variation is one aspect of biotechnology, a novel variability for crop improvement [15]. [16] also found similar results after genetic fidelity analysis of *in-vitro* raised plants of *Chlorophytum arundinaceum* which is an endangered medicinal plant and found no genomic changes in the *in-vitro* developed plants after RAPD analysis.[17] also noticed similar results after the assessment of genetic fidelity of micropropagated plants of *Simmondsia chinensis.* [18] also noticed similar results after evaluating the genetic fidelity of *in-vitro* raised plant of greater galangal (*Alpinia galanga* L.).

Table 1 *In vitro* shoot multiplication in *C. paniculatus* using different plant growth regulators

Serial No.	MS Media + Growth regulators (mg/l)	Shoot length(cm) (Mean ± SD)	No. of shoots/ explant (Mean ± SD)	No. of leaves/explant (Mean ± SD)
1	BA(0.443)	2.1 ± 0.13	1.2 ± 0.50	2.5 ± 0.65
2	BA(0.443)+2ip(0.24)	2.4 ± 0.14	1.4 ± 0.70	3.1 ± 0.76
3	BA(0.443)+NAA(0.27)	2.6 ± 0.18	1.3 ± 0.49	3.6 ± 1.26
4	BA(0.886)	2.4 ± 0.19	1.3 ± 0.67	3.5 ± 0.72
5	BA(0.886)+2ip(0.24)	2.8 ± 0.17	2.6 ± 0.92	5.6 ± 1.49
6	BA(0.886)+NAA(0.27)	2.7 ± 0.07	1.4 ± 0.51	4.2 ± 1.10
7	BA(2.22)	2.5 ± 0.17	1.3 ± 0.48	4.2 ± 1.13
8	BA(2.22)+2ip(0.24)	2.8 ± 0.13	2 ± 0.94	5.2 ± 1.80
9	BA(2.22)+NAA(0.27)	2.5 ± 0.19	1.8 ± 0.86	4 ± 1.15
10	BA(5.37)	2.3 ± 0.17	1.7 ± 1.06	4.2 ± 1.03
11	BA(5.37)+2ip(0.24)	2.4 ± 0.16	1.7 ± 1.06	4.2 ± 1.22
12	BA(5.37)+NAA(0.27)	2.4 ± 0.19	1.5 ± 0.93	3.5 ± 0.92
13	BA(10.74)	2.2 ± 0.11	1.8 ± 1.03	4.7 ± 1.33
14	BA(10.74)+2ip(0.24)	2.4 ± 0.13	1.7 ± 0.89	4.1 ± 1.09
15	BA(10.74)+NAA(0.27)	2.3 ± 0.18	1.6 ± 0.64	4.2 ± 1.01

Figure 1 *In-vitro* raised plantlets of *C. paniculatus* using nodal explants

Table 2 List of primers, their sequences, total number of bands and size of amplified fragments generated by RAPD primers in both micropropagated and glasshouse grown mother plants of *C. paniculatus*

Sl. No.	Primers	No. of bands produced	Range of amplicons [bp]
1.	OPB-08	6	900-10000
2.	OPC-06	5	450- 3000
3.	OPC-11	6	400- 1500
4.	OPC-16	3	300-1000
5.	OPD-08	7	300-10000
6.	OPD-20	11	250-2300
7.	OPE-01	5	300-10000
8.	OPF-10	5	650-2300
9.	OPG-02	6	650-10000
10.	OPG-06	6	300-650
11.	OPG-14	7	300-10000
12.	OPG-18	5	550-1450
Mean		**6**	
Total		**72**	

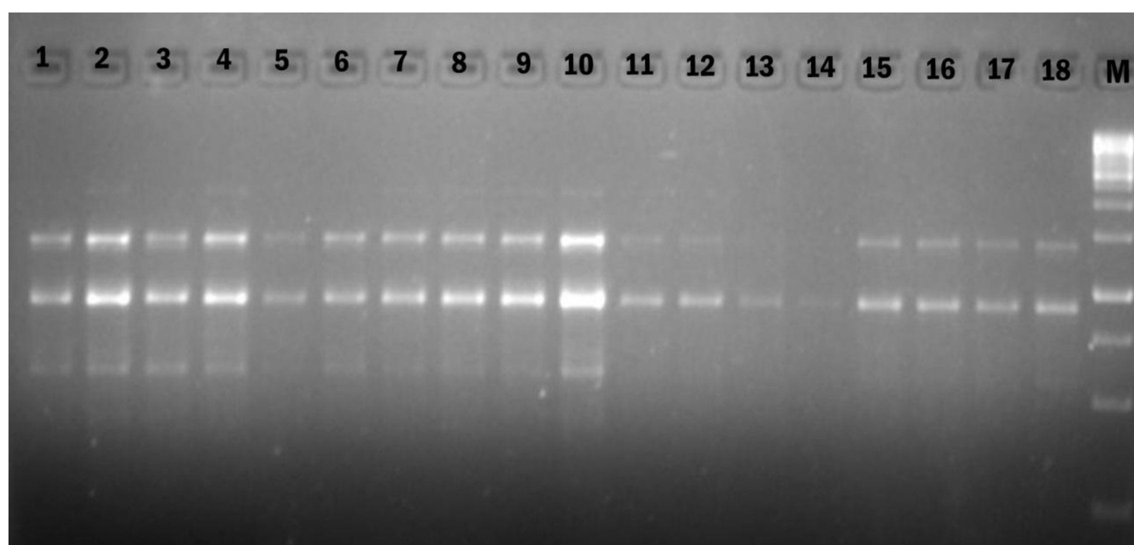

Figure 2 RAPD banding pattern in both micropropagated and glasshouse grown mother plants of *C. paniculatus* (Lane 1-3: Mother plant, Lane 4-18: Micropropagated plants and M: Marker)

Randomly amplified polymorphic DNA markers are used to estimate the genetic makeup of micropropagated plants because of its simplicity and cost effectiveness. The present study provides the first report on the genetic fidelity of micropropagated *C. paniculatus* obtained from nodal explants using RAPD marker. A total of 12 RAPD markers were employed to assess the genetic stability. All banding profiles from micropropagated plants were monomorphic and similar to those of the mother plant indicating no genetic variation in *in-vitro* raised plants. The result obtained in our experiment suggest that *in-vitro* shoot multiplication using nodal segment as explant may be used for rapid clonal propagation and conservation with a low risk of generating somaclonal variation.

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