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BRIEF COMMUNICATION

Micropropagation of *Tectona grandis*: assessment of genetic fidelity

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Abstract

Random amplified polymorphic DNA (RAPD) markers were used to analyze genetic fidelity of micropropagated teak (*Tectona grandis* L.) clones with respect to subcultural passage. Of the twenty primers screened, no variation in RAPD profiles was noticed in the *in vitro* clones of fifth, tenth, fifteenth and twentieth passage in comparison to the *in vivo* mother plants. Only one micropropagated plant of twenty-fifth subcultural passage, however, differed from the *in vivo* ones. It revealed the appearance of a new polymorphic DNA fragment (molecular mass 379 kb) in case of primer OPB-08. This primer, manifesting detectable variation, may be utilized as a diagnostic marker for assessing genetic fidelity of micropropagted teak plants.

Additional key words: random amplified polymorphic DNA, teak.

The long-life cycle of most tree species is a major problem in relation to forest breeding. Breeders, therefore, rely greatly on vegetative propagation as a method of maintaining elite cultivars for planting. Micropropagation is an alternative method. However, variation is known to occur as a result of this procedure (*e.g.*, Gupta and Varshney 1999). Random amplified polymorphic DNA (RAPD) markers have been used to determine the genetic stability of long-term micropropagated shoots of *Pinus thunbergii* (Goto *et al.* 1998) and *Populus deltoides* (Rani *et al.* 1995). Microsatellite DNA markers, an even more advanced generation of molecular markers have also been used to detect somaclonal variation in *Populus tremuloides* (Rahman and Rajora 2001).

Teak (*Tectona grandis* L.) is one of the most important timber yielding trees of Indian subcontinent. Successful clonal propagation of an elite teak tree was reported by Devi *et al.* (1994, 1998). Keeping the possibility of genetic variation which might occur in the clones, here we report the analysis of RAPD profiles of the micropropagated teak clones in comparison to the elite donor *in vivo* plant in relation to subcultural passages.

Apical shoots of two sixty-year old teak (*Tectona grandis* L.) trees growing in Hizuli forest of West Bengal, India were used as explants. Cultures were established in Murashige and Skoog (1962; MS) basal medium supplemented with 0.55 mM myoinositol, 0.27 mM adenine sulphate, 88 mM sucrose, 0.46 μM kinetin and 0.28 μM N^6-benzyladenine (Devi *et al.* 1994). The same culture medium was used for multiplication and maintenance but rooting was induced in liquid basal medium with coir (Gangopadhyay *et al.* 2002) supplemented with 8.29 μM indole-3-butyric acid and 0.51 μM indole-3-acetic acid. Cultures were kept under 16-h photoperiod (40 - 80 μmol m^{-2} s^{-1}) at 25 ± 1 °C and 78 % relative humidity. Each subcultural passage was of 28 d. For analysis of RAPD profiles, samples from *in vivo* donor plants and *in vitro* clones of the fifth, tenth,

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Abbreviations: CTAB - hexadecyltrimethyl ammonium bromide; MS medium - Murashige and Skoog medium; PCR - polymerase chain reaction; RAPD - random amplified polymorphic DNA.
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fifteenth, twentieth, and twenty-fifth subcultural passages were collected separately. For RAPD analysis, DNA was extracted according to the procedure (CTAB method) described by Rogers and Bendich (1988). DNA concentration in the samples was adjusted to 25 mg dm^{-3} for PCR reaction in each sample. PCR amplifications were performed according to the method of Williams *et al.* (1990) using a set of 20 oligonucleotide (decamer) primers, OPB 01 - OPB 20 (*Operon Tech.*, Alameda, USA). Amplifications were carried out in a Thermal Cycler (*Perkin Elmer System - 2400*, Norwalk, USA) and temperature profile of each cycle was : 40 s denaturation at 94 °C, 1 min annealing at 35 °C and 90 s for extension at 72 °C. Reaction continued for 45 cycles followed by 7.5 min hold at 72 °C to ensure that primer extension reaction was completed. To reduce the possibility of variation in amplification reaction, master mixing of reaction constituents was always used. PCR reaction mixture of 0.025 cm^3 consisted 1× buffer, 0.2 mM dATP, dCTP, dGTP, dTTP, 2 mM $MgCl_2$, 0.2 μM of primer, 100 ng of template DNA and 1 unit of Taq DNA polymerase. Amplified products were electrophoresed in 1.8 % agarose gel with *Φx 174 Hae III* digested DNA as size marker.

No difference in RAPD profiles between donor plant and micropropagated clones was observed after five, ten, fifteen and twenty subcultural passages in any of the primers used (data not shown). The results presented here are only those of micropropagated plants after twenty-fifth subcultural pasage in comparison to those of *in vivo* donor plants. Of the twenty random primers, only one (OPB 08) showed detectable variation between the profiles of donor and one micropropagated plant; the appearance of a new polymorphic DNA fragment (molecular mass 379 kb) was noteworthy (Fig 1*a*). The other primers either showed no variation (Fig. 1*b,c,d*) or did not show any amplification at all.

RAPD markers belong to the third generation of genetic markers (Ford-Lloyd 1996) but failure of detection of polymorphisms in almost all the primers, except the primer OPB 08, is a probable manifestation of onset of variation in and around twenty-fifth subcultural

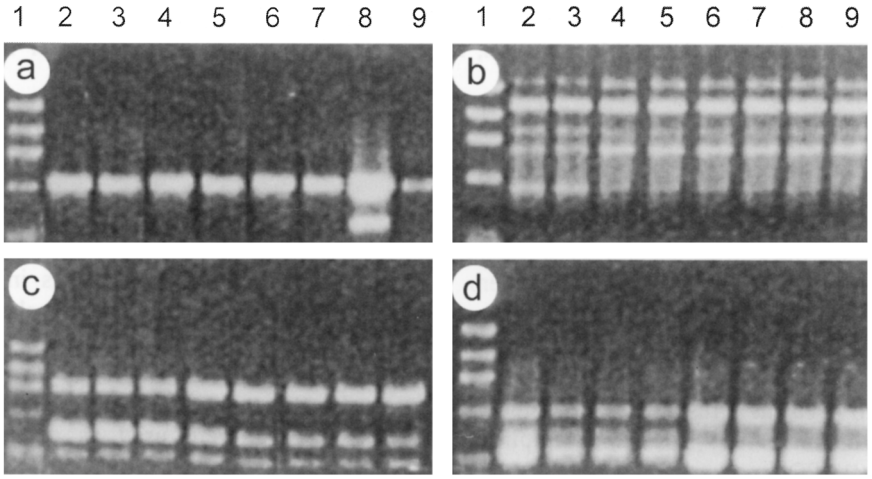

Fig. 1. RAPD profiles (*a* - OPB-08, *b* - OPB-09, *c* - OPB-10, *d* - OPB-11) of *in vivo* donor plants (*lanes* 2 - 3 from left) and randomly selected (six shown of total twenty, *lanes* 4 - 9 from left) *in vitro* clones of the 25th subcultural passage. *Lane 1* - DNA size marker (*Φx 174 Hae III* digested DNA).

passage. However, in *Pinus thunbergii* maintenance of genetic fidelity for over ten years, as assessed through RAPD, was reported (Goto *et al.* 1998).

The popular technique of micropropagation though advocated theoretically to give rise to true-to-type clones of desirable "elite" plant, occasionally leads to genetic variations due to change in either DNA sequences (point mutation, activation of transposons), in chromosome structure (duplications, translocations) or in chromosome number (leading to polyploidy) (De Klerk 1990). Furthermore, abnormalities in tissue culture, and in the plants produced from them often increase in frequency with increasing culture passages (Ramalakshmi Dutta *et al.* 2003). For most of the plants the advisable number of transfer cycles is between ten to fifteen. In case the transfer cycle is increased beyond twenty to twenty five, genetic variation starts showing up (Chatterjee and Prakash 1996). Similar trend of results has also been encountered in the present case as the micropropagted clones of teak maintained identical RAPD profiles when samples were analysed after five, ten, fifteen and twentieth subcultural passages and compared with the profiles of the *in vivo* donor plants. Appearance of a new polymorphic DNA fragment after twenty-fifth subcultural passage in one randomly selected *in vitro* clone prompted us to renew our cultural system of teak plants. Furthermore, the primer (OPB-08) that successfully manifested the variation could later be used to examine micropropagated teak plants to ascertain their genetic uniformity.

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