Genetic fidelity studies in tissue culture raised plantlets of Himalayan poplar (*Populus ciliata* Wall.)

Genomic assessment of the *in vitro* raised variations is necessary to protect the useful characteristics of the elite cultivars[1–3]. *Populus ciliata* has significant potential among the commercially propagated tree species such as teak, *Eucalyptus*, red wood and radiata pine[3]. Himalayan poplar occupies a unique and important position in the rural economy of India as it is a rare forest species ideal for successful intercultivation with agronomically important crops. *Populus ciliata* is a deciduous tree with sexually differentiated male and female plants, grown at an altitude of 1200–3500 m and largely distributed in temperate and subtemperate regions of the Himalaya[3]. With an increase demand for pulp and paper, phytoremediation of contaminated soils and reforestation of lowlands, poplars are found to be of great importance at commercial level[1,3]. The limitations which are associated with the use of poplar at commercial level include increased lignin content that affects the cost of paper production, as it is difficult to lessen the lignin content in *Populus*[3,4]. Also poplars are vulnerable to a large number of pests. Thus for the better genomic advancement of a species, the major prerequisite is to develop highly efficient and reliable plant regeneration system without affecting genetic integrity of the species[3,4]. *In vitro* plant regeneration in *Populus* has been carried out earlier[3,4].

In order to grow poplar on a large scale, an efficient and reliable regeneration protocol is required along with the testing techniques to maintain genetic integrity of originally existing germplasm. RAPD markers are among the widely used techniques for genetic fidelity studies of tissue culture-grown plants. In the present communication, we have studied the genetic stability of tissue culture-raised plantlets of Himalayan poplar using RAPD-PCR studies.

An efficient plant regeneration protocol has been standardized from leaf and petiole explants of Himalayan poplar in our lab[4]. Genomic DNA was isolated using CTAB method with some modifications[5], from healthy, fresh young leaves of mother plant and from tissue culture-raised plants (randomly selected) of Himalayan poplar (*Populus ciliata* Wall.). Purification and quantification of DNA samples were carried out, followed by RAPD-PCR studies. Amplification of purified genomic DNA was carried out in BIORAD MJ Mini DNA amplification system using 25 random decamer oligonucleotide primers (Sigma Alderich). Each 25 µl of PCR reaction mixture contained *Taq* DNA polymerase buffer (1×), random primer (100 µM), dNTPs mixture (6 mM), autoclaved double distilled water (15.75 µl), *Taq* DNA polymerase (0.75 units) along with 100 ng genomic DNA. A total of 40 cycles was performed in thermocycler, each cycle consisted of inital denaturation step at 94°C for 1 min then annealing at 35°C for 1 min and final extension at 72°C for 2 min. Each PCR samples were given pre-PCR amplification at 92°C for three and half minutes and post-amplification at 72°C for 10 min. Amplified product was electrophoresed in 1.20% agarose gel. Genetic stability data analysis was carried out only for informative primers

Table 1. Total number of amplified fragments, monomorphic and polymorphic fragments generated by RAPD-PCR using 20 random decamer oligonucleotide primers

Primer	Primer sequence 5′ → 3′	Size range of amplified bands in base pair	Total number of amplified bands	Total number of monomorphic amplified bands	Total number of polymorphic amplified bands	Monomorphism (%)
OPB-03	CATCCCCCTG	1410 to 326 bp	4	4	–	100
OPB-04	CGACTGGAGT	1387 to 251 bp	4	4	–	100
OPB-05	TGCGCCCTTC	1409 to 293 bp	6	6	–	100
OPB-06	TGCTCTGCCC	1117 to 365 bp	5	5	–	100
OPB-08	GTCCACACGG	1444 to 181 bp	4	4	–	100
OPB-10	CTGCTGGGAC	1467 to 245 bp	8	7	1	87.5
OPB-11	GTAGACCCGT	3076 to 233 bp	7	5	2	71.4
OPB-12	CCTTGACGCA	2148 to 229 bp	6	4	2	66.67
OPB-14	TCCGCTCTGG	1276 bp	1	1	–	100
OPB-15	GGAGGGTGTT	530 to 156 bp	3	3	–	100
OPB-16	TTTGCCCGGA	2267 to 902 bp	4	4	–	100
OPB-17	AGGGAACGAG	1293 to 220 bp	7	2	5	28.57
OPB-18	CCACAGCAGT	1484 to 178 bp	8	6	2	75.00
OPC-05	GATGACCGCC	2662 to 340 bp	6	6	–	100
OPC-06	GAACGGACTC	788 to 648 bp	2	2	–	100
OPC-07	GTCCCGACGA	1393 to 792 bp	3	3	–	100
OPC-16	CACACTCCAG	1560 to 291 bp	5	3	2	60.00
OPC-18	TGAGTGGGTG	737 to 610 bp	2	2	–	100
OPC-19	GTTGCCAGCC	962 to 528 bp	4	4	–	100
OPC-20	ACTTCGCCAC	2375 to 917 bp	5	5	–	100
Total			94	80	14	89.46

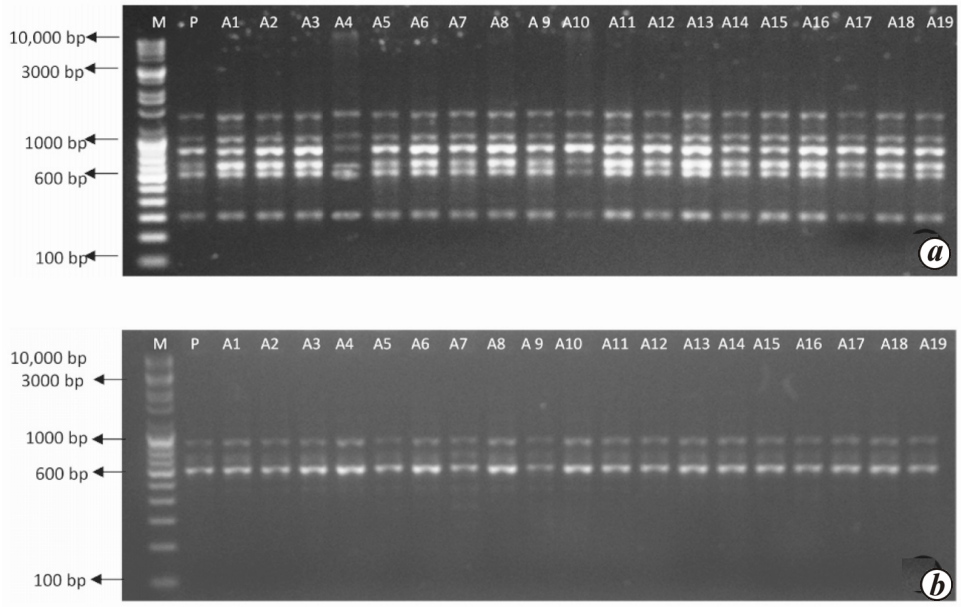

Figure 1 ***a***, ***b***. Genetic fidelity studies in tissue culture-raised plantlets of Himalayan poplar (*Populus ciliata* Wall.). ***a***, RAPD profile generated by the amplification of DNA of mother plant (P) along with 19 *in vitro* raised plantlets (A1–A19) of Himalayan poplar with primer OPB-O5. ***b***, RAPD profile generated by the amplification of DNA of mother plant (P) along with 19 *in vitro* regenerated plantlets of Himalayan poplar with primer OPC-07. M, Marker/ladder (100–10,000 bp).

The *in vitro* differentiated plantlets from leaf and petiole explants protocol standardized in our lab were used[4]. Genetic integrity of *in vitro* raised plants of Himalayan poplar was checked by using randomly amplified polymorphic DNA. For RAPD studies, 25 random decamer primers were screened but only 20 gave scorable banding patterns in all the regenerated plantlets. A total of 94 amplicons were amplified with 4.23 average number of bands per primer. Out of 94 amplicons, 80 were monomorphic and revealed 89.46% monomorphism among randomly selected 19 tissue culture grown plants with mother plant (Table 1, Figure 1). A little variation was observed in tissue culture-grown plantlets of the Himalayan poplar which might due to *in vitro* differentiation or cell division[3,6,7]. Rani and Raina[8] observed 26% variation in *Populus deltoids*, whereas 23.3% and 7% polymorphism were reported among the micropropagated plantlets of apple rootstocks[9,10]. In the present study, the variation to the extent of approximately 10% was observed.

In conclusion, molecular analysis of genetic integrity of tissue culture-grown plants with RAPD markers proved as fast and cost effective technique to ensure the true to type nature of regenerants and for carrying out genomic manipulation for quality and quantity improvement.

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