

Full Length Research Paper

Random amplified polymorphic DNA analysis detects variation in a micro propagated clone of *Trichodesma indicum* (L.) R. Br.

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RAPD profile revealed variation among the individuals of a single clone of micro propagated plants of *Trichodesma indicum*. Callus was developed from a single zygotic embryo differentiated into multiple micro shoots on Murashige and Skoog's medium supplemented with 2.32 μ M kinetin. A single node excised from a micro shoot produced multiple shoots after three sub cultures on MS medium containing 0.23 μ M kinetin. Morphologically, all the *in vitro* raised plantlets appeared alike except size variation. Random amplified polymorphic DNA (RAPD) profile of a clone of 10 plantlets prepared with MAP 1 - 20 primers amplified 119 bands: 117 monomorphic and 2 polymorphic. Perhaps, variation occurred during subculture of nodal segments rather than differentiation of shoots from callus.

Key words: *Trichodesma indicum*, RAPD, MAP-primers, somaclonal variation, micro propagation.

INTRODUCTION

The available DNA markers facilitate assessment of genetic variability in plants. Random amplified polymorphic DNA (RAPD) fragments are amplified products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus do not require prior knowledge of a DNA sequence (Welsh and McClelland, 1990). They are easy to be used and have been applied in determination of genetic diversity in somaclonal variation of *Lycopersicon esculentum* (Soniya et al., 2001), *Gossypium hirsutum* (Sheidai et al., 2008) and *Chlorophytum arundinaceum* (Lattoo et al., 2006). Adhapushpi (*Trichodesma indicum*) is a perennial medicinal herb distributed in tropical and sub tropical Asia, Africa and Australia (Samvastar, 1996). It is a multi drug plant used to reduce or cure inflammation, pain, osteoarthritis and conjunctivitis (Kulkarni and Ansari, 2004). The plant is used for expulsion of dead fetus

(CSIR, 1986), abortion (Tarafer, 1983), inhibition of diarrhea (Perianayagam et al., 2005), reduction of sulfur dioxide-induced cough reflex in mice (Srikanth et al., 2002), as brain tonic (Jain, 1991) and to treat breast cancer (Tirky, 2002). Sandhu and Singh (2005) reported pyrrolizidine alkaloids (PAs) as main constituent in *T. indicum* in "A dictionary of medicinal plants". *T. indicum* is a cross-pollinated species, in which buzz-pollination is carried out by two species of *Anthophora* (Ahmed et al., 1995).

Verma et al. (2009) observed large variation (71%) in DNA fingerprints in plants micro propagated from zygotic embryos of a small population of *T. indicum*. Since the plants were developed from different embryos of a small population through callus, it was difficult to assign contribution of different developmental stages of micro propagation in causing variation. Therefore, present experiment was designed to determine probable stage for variation occurrence.

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Abbreviations: RAPD, Random amplified polymorphic DNA; PCR, polymerase chain reaction; CTAB, cetyl trimethyl ammonium bromide.

MATERIALS AND METHODS

Generation of samples

The procedure for micro propagation of *T. indicum* was as described by Verma et al. (2008). It includes a two-step method of

Table 1. Primer sequence and number of bands generated by MAP 1- 20 primers in *T. indicum*.

Sample no.	Primer	Sequence of primer (5'→3')	Amplified bands (No) M-monomorphic-P-polymorphic
1	MAP 1	AAATCGGAGC	3 M
2	MAP 2	GTCCTACTCG	5 M
3	MAP 3	GTCCTTAGCG	10 M
4	MAP 4	TGCGCGATCG	4 M
5	MAP 5	AACGTACGCG	4 M
6	MAP 6	GCACGCCGGA	6 M
7	MAP 7	CACCCTGCGC	8 M
8	MAP 8	CTATCGCCGC	7 M
9	MAP 9	CGGGATCCGC	6 M
10	MAP 10	GCGAATTCCG	6 M
11	MAP 11	CCCTGCAGGC	6 M
12	MAP 12	CCAAGCTTGC	4 M
13	MAP 13	GTGCAATGAG	4 M
14	MAP 14	AGGATACGTG	10 M
15	MAP 15	AAGATAGCGG	7 M
16	MAP 16	GGATCTGAAC	7 M + 2 P
17	MAP 17	TTGTCTCAGG	6 M
18	MAP 18	CATCCCGAAC	7 M
19	MAP 19	GGACTCCACG	4 M
20	MAP 20	AGCCTGACGC	3 M

in vitro shoot proliferation, in which immature fruits of *T. indicum* collected from a plant grown in Botanical Garden of School of Life Sciences of Pandit Ravishankar Shukla University, Raipur, were used as explants source. The zygotic embryos, peeled out from sterilized fruit, when placed on the MS medium supplemented with 2.32 μ M kinetin, produced callus that differentiated and produced multiple shoots. The nodal segments of a healthy shoot were sub cultured thrice on MS medium with 0.23 μ M kinetin. All the shoots derived from a single node were rooted on half-MS medium containing 2.46 μ M IBA for 72 h pulse period before their transfer to hormone free medium.

DNA extraction and amplification

Genomic DNA was extracted from fresh tender leaves of 10 *in vitro* regenerated plants using CTAB (Cetyl trimethylammonium bromide) method described by Khanuja et al. (1999). Polymerase chain reaction (PCR) for amplifying the DNA preparations was carried out in a 25 μ l volume of reaction mixture. A PCR tube contained 20 ng DNA, *Taq* DNA polymerase (0.6 unit), 100 mM dNTPs, 2.5 μ l 10x polymerase buffer and 5 pmol decanucleotide MAP (1 - 20) primers. Amplification was carried out in a thermocycler (MJ Research U. S. A.) using the following stages: denaturation at 94°C for 2 min, annealing at 35°C for 1 min and extension at 72°C for 2 min for 44 cycles and final extension at 72°C for 5 min. The PCR products were subjected to separation on 1.2% agarose gel containing 0.5 μ l¹ ethidium bromide along with Hind III double digest (Bangalore Genie) marker. The RAPD profiles were compared by visual examination of bands of ten regenerated plants.

In vitro propagation

Callus and shoot differentiation occurred in embryo explants of *T.*

indicum cultured on MS medium supplemented with kinetin. The explants on MS medium with 2.32 μ M kinetin showed 9.59 \pm 0.09 shoots per callus and 98.79 \pm 0.09 mm shoot length. Nodes first subcultured on MS medium with 0.23 μ M kinetin produced 7.58 \pm 0.09 shoots per node and 86.48 \pm 6.51 mm shoot length. These observations were consistent with Verma et al. (2009), in which shoot differentiation and shoot proliferation in *T. indicum* exhibited similar trend. However, the shoots derived from only one node of a single differentiated shoots were used for rooting.

RESULTS AND DISCUSSION

The RAPD analysis of DNA samples on ten members of a clone micro propagated from a single node revealed 1.74% somaclonal variation. The MAP primers 1 - 20 amplified 119 bands: 117 monomorphic and 2 polymorphic bands (Table 1). Primers MAP 3 and MAP 14 produced maximum 10 fragments, while MAP 1 and MAP 20 primers amplified only 3 fragments. Extension of the MAP 2 and MAP 16 primers produced a fragment with maximum size 4268 base pairs whereas MAP 12 amplified a minimum-sized product with 831 base pairs. Only MAP 16 produced polymorphic bands. MAP 16 amplified polymorphic bands 6 and 8 (Figure 1). The band number 6 (approximate size 3530 bp) was absent in samples 3 and 5. Similarly, the band number 8 (approximate size 1904 bp) was absent in samples 2, 9 and 10. Other primers produced monomorphic bands, e.g. primer MAP 12 amplified 5 bands in the same 10 plants DNA samples (Figure 2). The observations suggest that mutation occurred

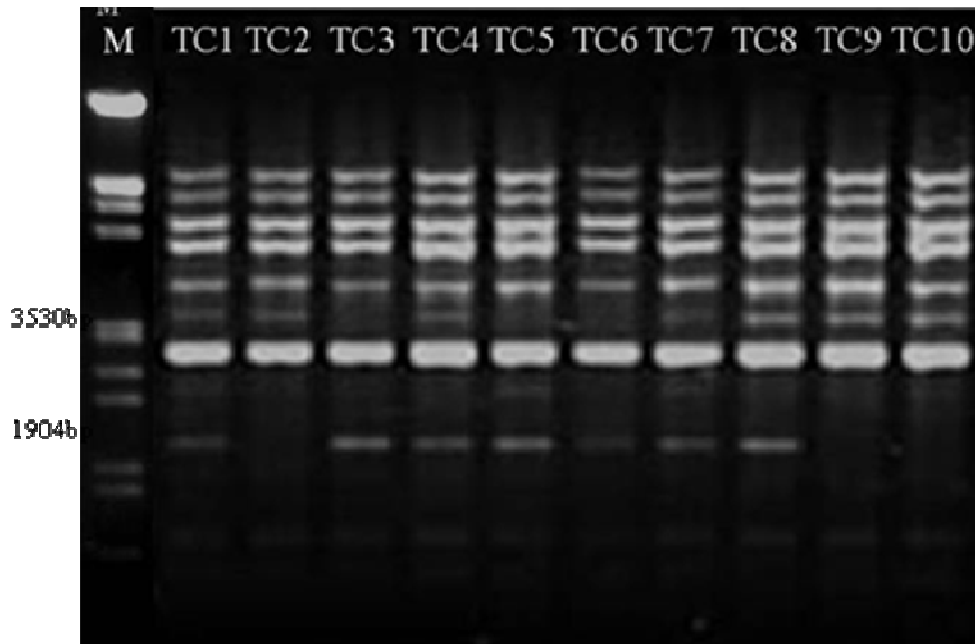


Figure 1. Primer MAP 16 DNA amplification profile of TC-1 to TC-10 shows band 6 (approximately 3530 bp) missing in samples 3, 5 and 6; and band 8 (approximately 1904 bp) missing in samples 2, 9 and 10.

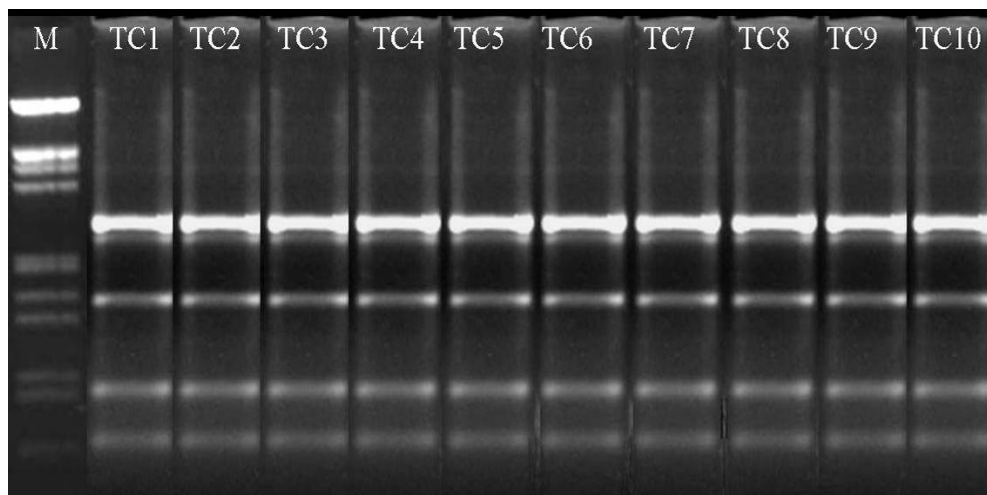


Figure 2. Primer MAP 12 DNA amplification profile shows 4 - 5 bands ranged from 4268 to 831 bp in samples TC-1 to TC-10.

at primer annealing sites during the stage of shoot proliferation from nodal segments and not during differentiation of shoots from callus. Popescu et al. (1997) observed variation in DNA fingerprinting in strawberry plants regenerated from leaf and petiole-derived callus. They reported that the frequency of somaclonal variation was generally low but increased in subcultures of intervals up to 12 weeks. Hu et al. (2008) obtained 20.8% polymorphic bands in the regenerated plants developed from petiole explants of *Amorphophallus albus*. Saker et al.

(2000) detected somaclonal variation in date palm by using isozymes and RAPD analysis. The frequency of somaclonal variations was found to be age dependent. RAPD analysis showed genetic variation in 4% of the analyzed plants. Al-Zahim et al. (1999) detected somaclonal variation in *Allium sativum* (L.) using RAPD and cytological analysis. They reported that changes in some bands in regenerants of different cultivars may be due to the existence of a mutation sensitive part in the garlic genome. In the present case, occurrence of somaclonal

variation in three subcultures of short intervals of 4 weeks is perhaps due to instability of *T. indicum* genome.

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