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Full Length Research Paper

Regeneration of *Andrographis paniculata* Nees: Analysis of genetic fidelity and andrographolide content in micropropagated plants

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Andrographis paniculata **Nees is a valuable medicinal plant which yields the therapeutic compound andrographolide. The objective of the present study was to develop reliable *in vitro* propagation techniques in this plant species. The efficiency of shoot regeneration in *A. paniculata* was tested on the Murashige and Skoog (MS) medium supplemented with 6-benzylaminopurine (BAP), thidiazuron (TDZ), kinetin (Kn) and 2-isopentenyl adenine (2-iP) at concentrations of 0.5, 1.0, 2.0, 5.0 and 10.0 µM and BAP (1.0 µM) in combination with other cytokinins like TDZ, Kn and 2-iP (0.5, 1.0, 2.0, 5.0 and 10.0 µM) by using nodal explants. Maximum number of 39 shoots per explant was recorded on MS medium supplemented with BAP (1.0 µM) and Kn (5.0 µM). An anatomical study confirmed shoot regeneration via direct organogenesis. Regenerated shoots were cultured on MS medium supplemented with 1-naphthaleneacetic acid (NAA), indole-3- acetic acid (IAA) and indole-3-butyric acid (IBA) at concentrations of 0.5, 1.0, 2.0 and 5.0 µM for the induction of roots. Cent percent shoots developed roots after transfer to half strength MS medium supplemented with IBA (2.0 µM). The rooted plantlets were successfully acclimatized and established in soil. Randomly amplified polymorphic deoxyribonucleic acid (DNA) (RAPD) analysis was carried out to check for possible genetic alterations in regenerated plants and the results revealed that the recovered plants did not exhibit any type of polymorphism. The andrographolide content was determined in regenerated plants using high performance liquid chromatography (HPLC) and regenerated plants had considerable amount of andrographolide, so regenerated plants could be used as raw material for andrographolide extraction.**

Key words: *Andrographis paniculata*, andrographolide, nodal culture, micropropagation.

INTRODUCTION

Andrographis paniculata Nees, commonly known as "King of Bitters" belongs to the family Acanthaceae and is a valuable medicinal plant distributed in India and South Asian counties. The leaves and aerial parts of this plant have been used in traditional systems of medicine for the treatment of hepatitis, bronchitis, colitis, cough, fever, mouth ulcers, sores, tuberculosis, bacillary dysentery, venomous snake bites, common cold, urinary tract infections and acute diarrhoea (Panossian et al., 2002). The plant is also reported to have anticancer and immunostimulatory (Kumar et al., 2004; Trivedi and Rawal, 2001), hypoglycaemic (Borhnuddin et al., 1994), hepatoprotective (Nagalekshmi et al., 2011), cardioprotective (Ojha et al., 2012), antihypertensive (Huang, 1987), antiviral (Holt and Linda, 1998), antifungal (Sule et al., 2012), antioxidant (Trivedi and Rawal, 2001), immune enhancement and anti-HIV activities (Calabrese et al.,

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Abbreviations: BAP, 6-Benzylaminopurine; **TDZ,** thidiazuron; **Kn,** kinetin; **2iP,** 2-isopentenyl adenine; **CTAB,** cetyltrimethyl ammonium bromide; **RAPD,** randomly amplified polymorphic DNA; **PCR,** polymerase chain reaction; **HPLC,** high performance liquid chromatography; **NAA,** 1-naphthaleneacetic acid; **IAA,** indole-3- acetic acid; **IBA,** indole-3-butyric acid.

2000).

The active ingredients of *A. paniculata* are diterpene lactones, the most prominent being andrographolide (Saxena et al., 1998). Conventional propagation of *A. paniculata* using seeds and by vegetative means is having limitations and it is not possible to produce large number of clones. *In vitro* propagation methods are the alternatives for the propagation of medicinal crops where there are limitations of conventional propagation. The modern tools of biotechnology can be exploited to improve quality and content of bioactive compounds of medicinal crops once a protocol for its *in vitro* regeneration is available.

Except for the preliminary study of micropropagation by Martin (2004), Purkayastha et al. (2008), Kataky and Handique (2010, 2011) and Basu and Yogananth (2011), no comprehensive *in vitro* studies have been reported on *A. paniculata*. Behera et al. (2010) reported similar composition of trace elements among *in vivo* and *in vitro* regenerated roots of *A. paniculata*. Here we describe an efficient protocol for direct plant regeneration from nodal explants of *A. paniculata* along with the randomly amplified polymorphic deoxyribonucleic acid (DNA) (RAPD) analysis to determine the genetic fidelity of the regenerated plants and analysis of andrographolide content in regenerated plants.

MATERIALS AND METHODS

Culture medium

Nodal explants (1 cm long) of *A. paniculata* Nees were obtained from 2 years old plant growing in the botanical garden, Karnatak University, Dharwad, India. Plants were identified by Dr. M. Jayraj and voucher specimens of the plants used are deposited in the Herbarium, Department of Botany, Karnatak University, Dharwad, India. Explants were thoroughly washed under running tap water and with detergent (Laboline, 0.1%, v/v, Hi Media, Mumbai) for 5 min, then surface sterilized with 2% sodium hypochlorite (Hi Media, Mumbai) for 3 min, followed by 0.1% mercuric chloride (Hi Media, Mumbai) wash for 2 min and then four washes of sterile distilled water in the sterile condition. The cut edges of the explants were trimmed and cultured on Murashige and Skoog (1962) medium containing 3% sucrose, 0.8% agar supplemented with 6-benzylaminopurine (BAP), thidiazuron (TDZ), kinetin (Kn) and 2-isopentenyl adenine (2iP) individually at 0.5, 1.0, 2.0, 5.0 µM and 10.0 µM concentrations and BAP (1.0 µM) in combination with KN, 2-iP, TDZ (0.5, 1.0, 2.0, 5.0 µM and 10.0 µM).

The pH of the medium was adjusted to 5.8 prior to the addition of agar. 20 ml of medium was dispensed into each of culture tubes and plugged with non-absorbent cotton wrapped with one layer of cheesecloth. Culture medium was autoclaved at 120°C for 20 min and cultures were incubated at 25 ± 2°C under 16 h photoperiod (cool, white fluorescent tubes, irradiance of 40 µmol m^{-2} s^{-1}). Explants were subcultured once in four weeks to fresh medium. Shoots attained 3 to 4 cm in height were transferred to full and half strength MS medium containing 3% sucrose and with 1-naphthaleneacetic acid (NAA), indole-3- acetic acid (IAA) and indole-3-butyric acid (IBA) at concentrations of 0.5, 1.0, 2.0 and 5.0 µM for the induction of roots.

After 4 weeks, the rooted plantlets were transferred to plastic cups containing sterilized soil and vermiculite (1:1) under controlled growth chamber conditions (25 ± 2°C, 16 h photoperiod, 80% relative humidity and irradiance of 50 µmol m^{-2} s^{-1}). After 2 weeks, the plantlets were kept under shade for 2 weeks and then placed outdoors under full sun. There were 12 replicates for each treatment and the experiment was repeated twice. The cultures were observed periodically and the morphological changes were recorded at weekly intervals. Results were subjected to analysis of variance (ANOVA) and the mean values were separated according to Duncan's multiple range test at P=0.05. All tissue culture chemicals used in the studies were analytical grade and obtained from Hi media Laboratories, Mumbai, India.

Histological analysis

For histological studies, cultured explants were fixed in FAA (10 ml of formalin: 85 ml of 70% ethyl alcohol: 5 ml of glacial acetic acid) for 12 h at room temperature and dehydrated by ethanol-butyl alcohol series and embedded in paraffin as recommended by Johansen (1940). The material was sectioned (thickness of 6 µm) and double stained with safranin (Hi media, Mumbai) and fast green and examined under compound microscope (Nikon, Tokyo, Japan).

Genetic fidelity analysis

Genomic deoxyribonucleic acid (DNA) was isolated from mother and *in vitro* regenerated plants from fresh young leaves using cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). The DNA samples were screened with 60 randomly amplified polymorphic DNA (RAPD) (OPAZ $_{1-20}$ $OPBE_{1-20}$ and $OPBF_{1-20}$ series Operon, USA) primers for amplification products. The polymerase chain reaction (PCR) was performed with 20 ng template DNA; *Taq* buffer with 1.5 mM $MgCl_2$; dNTPs (1 mM/µl); *Taq* polymerase (2 units) and primer (5 pm) reaction volume of 20 µl. Amplifications were performed on an Eppendorf master cycler for 45 cycles. Amplification products were run on 1.5% agarose gel prepared in 0.5 × TAE buffer, stained with ethidium bromide and visualized under UV light (UVitec gel documentation). Molecular biology chemicals were obtained from Genei Laboratories, Bangalore, India.

Extraction and analysis of andrographolide in regenerated plants

Extraction and quantification of andrographolide in the regenerated plants was carried following the method of Praveen et al. (2009). The air dried powdered plant samples (500 mg) were extracted by mixture of dichloromethane and methanol (1:1) by cold maceration 10 min. The extract was filtered and solvent was removed under vacuum. The extract was washed 2 to 3 times with toluene and then dissolved in methanol. The andrographolide fractions were analyzed using high performance liquid chromatography (HPLC) (Waters 996 photodiode array detector; Waters millennium 2010 chromatography manager; Waters, Milford, Mass) with XTerra RP18 column (injection volume 10 µl; 150 mm × 3 mm, 5 µm). The mobile phase was acetonitrile:water (70:30, v/v; Spectrochem, Mumbai) flow rate was 1 ml/min, column temperature was 26°C, the detector wavelength was 230 nm. Andrographolide standard (98% purity) was obtained from ChromaDex (Laguna Hills, CA, USA). The total andrographolide content was an average of three replicates.

RESULTS AND DISCUSSION

To study the effect of different growth regulators on bud

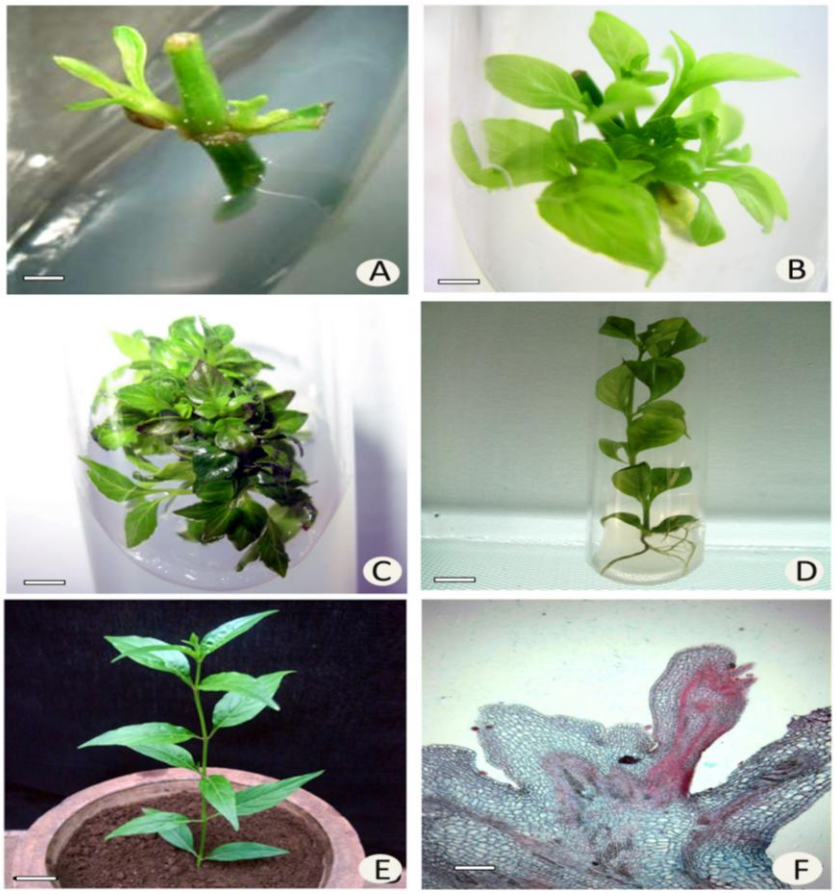

Figure 1. *In vitro* regeneration of *A. paniculata* via induction of axillary shoots. A. Two weeks old culture showing sprouting of axillary bud from nodal explants on MS medium supplemented with 1.0 µM BAP (bar = 0.22 cm); B. four weeks old culture showing multiple shoots on MS medium supplemented with 1.0 µM BAP (bar = 0.29cm), C. six weeks old culture showing multiple shoots on MS medium supplemented with 1.0 µM BAP and 5.0 µM Kn (bar = 0.40 cm); D. rooted shoot on half strength MS medium supplemented with 2.0 µM IBA (bar = 0.96 cm); E. regenerated plant, 6 weeks after transplantation (bar = 2.50 cm); F. longitudinal section of the nodal explant consisting of many dome shaped shoot primordia with vascular tissue. (bar = 0.14 mm).

break, nodal explants were incubated on Murashige and Skoog (MS) medium supplemented with various cytokinins individually. Nodal explants became swollen within a week of culture, developed axillary shoots at the nodal region in another one week (Figure 1A) and shoots grew further in another two weeks of culture (Figure 1B). The percentage of response varied with the type of cytokinin used and its concentration. Among the various cytokinins tested, BAP alone resulted in maximum number of explants initiating the shoots. The response was best on MS medium supplemented with 1.0 µM BAP by producing 9.25 shoots (Table 1). The stimulatory effect of BAP on multiple shoot formation has been reported earlier for several medicinal plant species including *Ocimum basilicum* (Begum et al., 2002), *Feronia limonia* (Hiregoudar et al., 2003), *Sesbania drummondii* (Cheepala et al., 2004) and *Vitex trifolia* (Hiregoudar et al., 2006).

Effect of BAP (1.0 µM) in combination with other cytokinins (0.5-10.0 µM Kn, 2iP and TDZ) was tested and results showed synergetic effect of cytokinins and induced number axillary shoots. The results were best at BAP (1.0 µM) in combination with Kn (5.0 µM) and 39.08 shoots developed after six weeks of culture (Table 2, Figure 1C). However, combination of BAP + 2iP and BAP + TDZ was not much beneficial in triggering the percentage of responding explants (Table 2) and provoking multiple shoot formation indicating that multiple shoot formation depends on the optimum balance of growth regulator combination and concentration in the medium. Similar differential response was observed when combinations of different cytokinins were tested in *Garcinia*

Table 1. Effect of cytokinins supplemented to MS medium on nodal explants of *A. paniculata* Nees.

Growth regulator (µM)				Explant cultured	Percentage of response	Mean number of shoot*	Mean shoot length (cm)*
BAP	Kn	2iP	TDZ				
0.0	0.0	0.0	0.0	12	0.00	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}
0.5	0.0	0.0	0.0	12	75.00	2.16 ± 0.32^{de}	1.54 ± 0.20^{f}
1.0	0.0	0.0	0.0	12	91.66	9.25 ±1.12^{a}	1.83 ± 0.16e^{f}
2.0	0.0	0.0	0.0	12	83.33	1.66 ± 0.22^{de}	1.33 ± 0.14^{f}
5.0	0.0	0.0	0.0	12	83.33	1.83 ± 0.16^{de}	1.87 ± 0.13^{ef}
10.0	0.0	0.0	0.0	12	75.00	4.85 ± 0.20^{c}	1.50 ± 0.15^{f}
0.0	0.5	0.0	0.0	12	66.66	0.91 ± 0.19^{e}	2.08 ± 0.37^{ef}
0.0	1.0	0.0	0.0	12	75.00	1.41 ± 0.15^{de}	3.50 ± 0.37^{bc}
0.0	2.0	0.0	0.0	12	83.33	1.33 ± 0.14^{e}	3.91 ± 0.22^{b}
0.0	5.0	0.0	0.0	12	91.66	1.83 ± 0.20^{de}	5.00 ± 0.33^{a}
0.0	10.0	0.0	0.0	12	91.66	2.66 ± 0.41^{d}	1.58 ± 0.15^{f}
0.0	0.0	0.5	0.0	12	75.00	1.5 ± 0.15d^{e}	2.00 ± 0.21^{ef}
0.0	0.0	1.0	0.0	12	75.00	1.83 ± 0.16^{de}	2.50 ± 0.33^{de}
0.0	0.0	2.0	0.0	12	91.66	1.58 ± 0.15^{de}	3.08 ± 0.22^{cd}
0.0	0.0	5.0	0.0	12	91.66	1.91 ± 0.19^{de}	5.16 ± 0.47^{a}
0.0	0.0	10.0	0.0	12	66.66	1.83 ± 0.16^{de}	1.58 ± 0.15^{f}
0.0	0.0	0.0	0.5	12	66.66	1.0 ± 0.00^{e}	2.0 ± 0.34^{ef}
0.0	0.0	0.0	1.0	12	75.00	2.66 ± 0.41^{d}	1.58 ± 0.15^{f}
0.0	0.0	0.0	2.0	12	75.00	4.41 ± 0.54^{c}	1.51 ± 0.15^{f}
0.0	0.0	0.0	5.0	12	83.33	7.16 ± 0.44^{b}	1.33 ± 0.14^{f}
0.0	0.0	0.0	10.0	12	83.33	4.41 ± 0.54^{c}	1.54 ± 0.20^{f}

*Mean values followed by the same letter are not significantly different according to DMRT at p=0.05.

Table 2. Effect of BAP in combination with other cytokinins supplemented to MS medium on nodal explants of *A. paniculata* Nees.

Growth regulator (µM)				Explant cultured	Percentage of response	Mean shoot number*	Mean shoot length (cm)*
BAP	Kn	2iP	TDZ				
0.0	0.0	0.0	0.0	12	0.00	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}
1.0	0.5	0.0	0.0	12	75.00	9.25 ± 1.99^{d}	2.33 ± 0.43^{bc}
1.0	1.0	0.0	0.0	12	83.33	13.16 ± 1.10^{c}	2.41 ± 0.33^{b}
1.0	2.0	0.0	0.0	12	83.33	20.66 ± 1.39^{b}	2.50 ± 0.31^{b}
1.0	5.0	0.0	0.0	12	91.66	39.08 ± 1.13^{a}	3.58 ± 0.19^{a}
1.0	10.0	0.0	0.0	12	91.66	25.12 ± 0.23^{b}	2.08 ± 0.31^{bc}
1.0	0.0	0.5	0.0	12	0.00	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}
1.0	0.0	1.0	0.0	12	75.00	3.25 ± 0.64^{ef}	1.41 ± 0.31^{bc}
1.0	0.0	2.0	0.0	12	66.66	2.91 ± 0.62^{ef}	1.41 ± 0.28^{bc}
1.0	0.0	5.0	0.0	12	66.66	2.33 ± 0.49^{ef}	1.25 ± 0.25^{c}
1.0	0.0	10.0	0.0	12	50.00	1.12 ± 0.21^{ef}	1.20 ± 0.20^{c}
1.0	0.0	0.0	0.5	12	66.66	1.08 ± 0.28^{ef}	1.83 ± 0.50^{bc}
1.0	0.0	0.0	1.0	12	83.33	8.66 ± 1.42^{d}	2.08 ± 0.31^{bc}
1.0	0.0	0.0	2.0	12	50.00	1.75 ± 0.49^{ef}	1.75 ± 0.47^{bc}
1.0	0.0	0.0	5.0	12	66.66	2.00 ± 0.47^{ef}	1.75 ± 0.41^{bc}
1.0	0.0	0.0	10.0	12	66.66	2.37 ± 0.29^{ef}	2.03 ± 0.13^{bc}

*Mean values followed by the same letter are not significantly different according to DMRT at p=0.05.

indica (Malik et al., 2005).

Histological studies indicated direct shoot regeneration from the nodal explants without intervention of callus phase (Figure 1F). The meristematic cells at the outer protoderm layer of the axillary bud divided at various planes to produce several meristemoids on the surface

Table 3. Effect of auxins supplemented to MS medium on rooting of the shoots of *A. paniculata* Nees.

Medium	Growth regulator (µM)			Percentage of response	Mean number of root*
	IAA	IBA	NAA		
Control	0.0	0.0	0.0	0.0	0.0 ± 0.0^{o}
MS full strength	0.5	0.0	0.0	83.33	5.25 ± 0.80^{jk}
	1.0	0.0	0.0	91.66	7.33 ± 0.94^{gh}
	2.0	0.0	0.0	91.66	9.50 ± 1.09^{def}
	5.0	0.0	0.0	100.0	10.33 ± 0.58^{cd}
	0.0	0.5	0.0	75.00	5.16 ± 0.19^{jk}
	0.0	1.0	0.0	83.33	6.16 ± 0.89^{hij}
	0.0	2.0	0.0	91.66	9.91 ± 1.00^{cde}
	0.0	5.0	0.0	100.0	11.25 ± 0.41^{bc}
	0.0	0.0	0.5	66.60	2.41 ± 0.57^{mn}
	0.0	0.0	1.0	83.33	4.66 ± 0.71^{kl}
	0.0	0.0	2.0	91.66	8.75 ± 0.60^{ef}
	0.0	0.0	5.0	100.0	11.08 ± 0.7^{bc}
MS half strength	0.5	0.0	0.0	75.00	2.41 ± 0.25^{mn}
	1.0	0.0	0.0	66.66	$3.66 \pm 0.33l^{m}$
	2.0	0.0	0.0	100.0	11.08 ± 0.28^{bc}
	5.0	0.0	0.0	83.33	5.75 ± 0.62^{ijk}
	0.0	0.5	0.0	50.00	1.66 ± 0.22^{n}
	0.0	1.0	0.0	66.66	6.75 ± 0.46^{hi}
	0.0	2.0	0.0	100.00	18.33 ± 0.76^{a}
	0.0	5.0	0.0	83.33	4.58 ± 0.48^{kl}
	0.0	0.0	0.5	66.66	1.25 ± 0.17^{n}
	0.0	0.0	1.0	66.66	2.00 ± 0.21^{n}
	0.0	0.0	2.0	50.00	8.33 ± 0.35^{fg}
	0.0	0.0	5.0	91.66	11.75 ± 0.46^{b}

*Mean values followed by the same letter are not significantly different according to DMRT at p=0.05.

layer indicating the early stages of differentiation of the shoots. These meristemoids, by further cell division gave rise to small protrusions of tissue, which gradually became green and organized into a growing point. After six weeks of culture, the shoot bud development and its vascular connection with the explant tissue was observed. The shoot cultures were multiplied by repeatedly subculturing the original nodal explants on shoot multiplication medium (MS medium supplemented with 1.0 µM BAP and 5.0 µM Kn) after harvesting newly formed shoots. Similar findings on subculturing the *in vitro* generated nodal explants to fresh shoot multiplication medium was reported in *Leptadenia reticulata* (Arya et al., 2003), *Sophora flavescens* (Zhao et al., 2004), and *Vitex trifolia* (Hiregoudar et al., 2006), *Gardenia jaminoides* (George et al., 1993) and *Crossandra infundibuliformis* (Girija et al., 1999).

For root induction, shoots of 3 to 5 cm were excised from the shoots of the primary cultures and cultured on the half and full strength MS medium supplemented with IAA, IBA and NAA, at various 0.5, 1.0, 2.0, 5.0 µM concentrations. The roots were induced from the shoots maintained on full strength MS medium without auxins but the roots were very thin. The rooting was best on half strength MS medium supplemented with 2.0 µM IBA (Table 3, Figure 1D). Similar results were also reported in *A. paniculata* by Purkayastha et al. (2008), Kataky and Handique (2010, 2011), Basu and Yogananth (2011). The plantlets were transplanted to pots containing sterile soil and vermiculite (1:1) initially and reared in controlled conditions for two weeks. Later they were transferred to bigger pots containing potting mix (Figure 1E) and survival percentage was 95%.

Clonal fidelity of *in vitro*-raised *A. paniculata* plants was tested using RAPD markers. Figure 2 demonstrates the RAPD profiles obtained with few of the best primer (OPAZ-03, 07 and OPBE-10) and the number of bright bands varied from four to six. Primer OPAZ-03 and

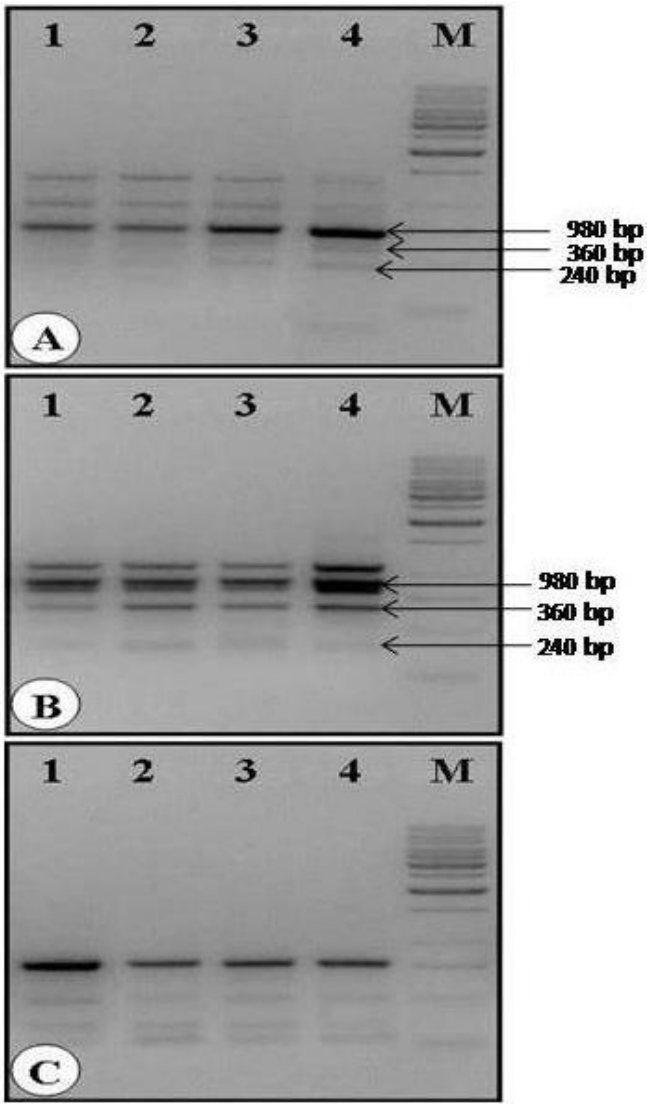

Figure 2. RAPD profile of *in-vitro* regenerated and mother plants of *A. paniculata* obtained with RAPD primers. [Lane M - DNA Marker (1 kilo base pair ladder); Lane 1, 2 & 3 - regenerated shoot of *A. paniculata*; Lane 4 - mother plant of *A. paniculata* used for explants]. A. RAPD profile of *in-vitro* regenerated and mother plants of *A. paniculata* obtained with primers OPAZ-03; B. RAPD profile of *in-vitro* regenerated and mother plants of *A. paniculata* obtained with primers OPBF-10; C. RAPD profile of *in-vitro* regenerated and mother plants of *A. paniculata* obtained with primers OPAZ-07.

OPBF-10 produced six monomorphic bright bands, while OPAZ-07 produced four monomorphic bands in both the *in vitro* grown plantlets and the mother plant of *A. paniculata*. Results revealed the genetic stability of regenerants, and our results support the views of Cassells and Curry (2002), and Susek et al. (2002) that direct regeneration from nodal explants did not include any somaclonal variation.

HPLC fingerprints of both mother (*in vivo*) and *in vitro* regenerated plants are presented in Figure 3. Variation in the andrographolide content among various plant material tested was observed (Figure 4). The amount of andrographolide in the stems of *in vivo* mother plant was 16.76 mg g^{-1} DW and it was 30.51 mg g^{-1} DW in the leaves. The content of the compound in the stems of regenerated plants was 50.62 mg g^{-1} DW, whereas, it was 34.51 mg g^{-1} DW in the leaves of regenerated plants (Figure 4). *In vitro* plant material tested for andrographolide showed a maximum quantity of andrographolide compared to *in vivo* plant parts. The

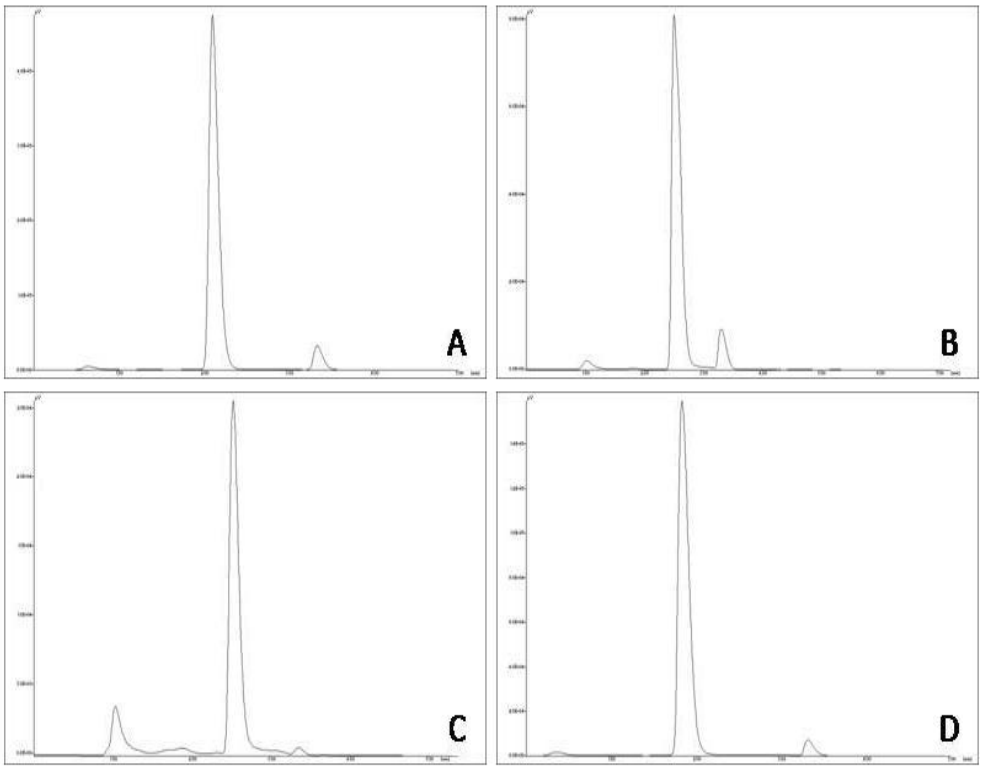

Figure 3. HPLC chromatogram of andrographolide from *A. paniculata.* A. HPLC profile of andrographolide isolated from the stem of mother plant. B. HPLC profile of andrographolide isolated from the leaf of mother plant. C. HPLC profile of andrographolide isolated from the stem of *in vitro* regenerated plant. D. HPLC profile of andrographolide isolated from the leaf of *in vitro* regenerated plant.

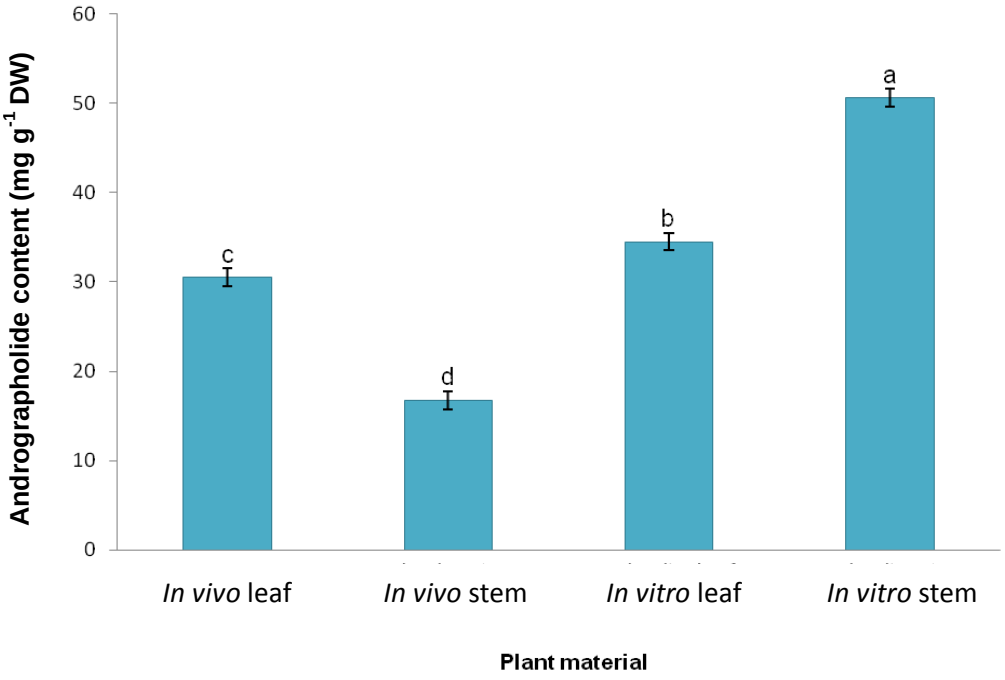

Figure 4. Andrographolide content of mother and *in vitro* plants. *Bars* represent mean ± SE. Mean values marked with different letter are significantly different ($P \leq 0.05$).

plantlets produced through *in vitro* culture could be used as raw material for continuous production of andrographolide without affecting the quality of the chemical and is useful for maintaining homogeneity in the chemical makeup of the plants for pharmaceutical use.

Conclusion

The present study demonstrates a simple and efficient method for high frequency direct shoot regeneration from nodal explants of *A. paniculata*. The system is rapid and maximum 39 shoots per explant were regenerated on MS medium supplemented with BAP (1.0 µM) and Kn (5.0 µM). Proliferating shoot cultures were established by repeatedly sub-culturing the nodes on the same medium. Maximum rooting (100%) occurred in shoots cultured on half-strength MS medium supplemented with 2.0 µM IBA. RAPD analysis showed genetic stability of regenerants. HPLC fingerprints revealed higher amount of andrographolide in the regenerants than mother plants.

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