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RESEARCH ARTICLE

Efficient micropropagation and assessment of genetic fidelity of *Boerhaavia diffusa* L- High trade medicinal plant

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Abstract *Boerhaavia diffusa* L is a medicinal herb with immense pharmaceutical significance. The plant is used by many herbalist, Ayurvedic and pharmaceutical industries for production biopharmaceuticals. It is among the 46 medicinal plant species in high trade sourced mainly from wastelands and generally found in temperate regions of the world. However, the commercial bulk of this plant shows genetic variations which are the main constraint to use this plant as medicinal ingredient and to obtain high value products of pharmaceutical interest from this plant. In this study, we have regenerated the plant of *Boerhaavia diffusa* L through nodal explants and evaluated genetic fidelity of the micropropagated plants of *Boerhaavia diffusa* L with the help of random amplified polymorphic DNA (RAPD) markers. The results obtained using RAPD showed monomorphic banding pattern revealing genetic stability among the mother plant and in vitro regenerated plants of *Boerhaavia diffusa* L.

Keywords *Boerhaavia diffusa* L · Medicinal ingredient · RAPD · Genetic fidelity · Regenerated

Introduction

Boerhaavia diffusa L commonly called *rakt punarnava* is herbaceous member of the Nyctaginaceae family (Mayank and Irchhaiyar 2012). There are two varieties of these plants, one with white flower known as *shweta punarnava* (*Boerhaavia erecta*) while other with red flower called as *rakta punarnava* (*Boerhaavia diffusa*) out of which *rakt punarnava* (*B. diffusa*) is mainly used for medicinal purposes (Vijayakrishna et al. 2014; Chaudhary and Dantu 2011). Out of the 40 species of *Boerhaavia* genus, five are found in India – *B. diffusa, B. chinensis, B. erecta, B.rependa,* and *B. rubicund* (Chopra 1969; Dev 2006). The stem of *B. diffusa* is prostrate, woody or succulent, cylindrical, often purplish, hairy, and thickened at its nodes (Rajpoot and Mishra 2011). In Ayurvedic medicine, root and aerial parts of this plant were reported for the treatment of diabetes (Murti et al. 2010). It is also used in the treatment of stomachache, anemia, cough and a potent antidote for snake and rat bites (Roy 2008). The plant possesses pharmaceutically important chemical constituents such as punarnavine, eupalitin, boeravinones A-H, β-sitosterol and β-sitosterol-β-D-glucoside (Chaudhary and Dantu 2011; Bharali et al. 2003).

B. diffusa has a great demand by the local herbalist and Ayurvedic companies. It is among the 46 medicinal plant species in high trade sourced mainly from wastelands (Ved and Goraya 2007). *B. diffusa* is an exclusive or important constituent of several Ayurvedic preparations such as Abana (HeartCare), Bonnisan, Diabecon (GlucoCare), Evecare (MenstriCare), Geriforte(GeriCare / StressCare), Lukol, V-Gel (FemCare Gel), Digyton, Geriforte Aqua, Geriforte Vet Immunol, NefrotecVet, Punarnava, Chyavanaprash (Chaudhary and Dantu 2011).

The commercial bulk of *B. diffusa* represents a heterogeneous population. Consequently, it quite often results in poor quality roots and biomass (Saini et al. 2011). *B. diffusa* is propagated by seeds, but the seed viability is poor and has very low germination percentage (Kanfade et al. 2011).

For the development of improved varieties in terms of superior yield and quality of herb/root of *B. diffusa*, mutation breeding by RAPD was attempted by Shukla et al. (2004).

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Few attempts were made on in vitro regeneration of *B. diffusa* using leaves and stem (nodes and shoot tip) as explants (Sudarshana et al. 2008; Wesely et al. 2010; Saini et al. 2011,) but the protocol for genetic integrity has not been established of the micropropagated plants of *B. diffusa* L. DNA-based molecular markers have been proposed as an excellent tool for identifying geographical variation, genetic diversity, phylogenetic relationship, authentication of plant species, pharmacognostic characterization, species characterization and genetic mapping in medicinal plants (Joshi et al. 2004). Molecular marker techniques, such as random amplified polymorphic DNA (RAPD) analysis, have been used to assess variability or similarity within or between the plants derived from plant tissue culture and the donor plant (Jeong et al. 2009; Jain et al. 2011). RAPD is a simple, largely automatable technique which requires only a small amount of DNA and can be performed without the use of radioactivity (Williams et al. 1990). This technique which is an efficient and inexpensive method of generating molecular data has been employed in many taxonomic and phylogenetic studies (Khan et al. 2000).

With the constraints of conventional propagation methods and to use this plant commercially as medicinal ingredient, present study was undertaken to develop efficient protocol for rapid regeneration of *B. diffusa* using nodal explants and to evaluate the genetic fidelity of the regenerated plantlets with mother plant.

Materials and methods

Plant material, explants preparation and surface sterilization

Tender shoots were collected and defoliated from established plant of *B. diffusa* from campus of botanical garden at North Maharashtra University, Jalgaon and used as a source of explants for experiment. The plant specimen was authenticated and deposited at the Botanical Survey of India Regional Office, Western Circle, Pune, India. These shoots were then thoroughly washed under running tap water for 15 min accompanied with 2–3 drops of Tween 20 (Himedia Laboratories), treated with 0.4 g/L antifungal Bavistin (BASF Pvt. Ltd., India) for 1 hr and surface sterilized with 0.1 % $HgCl_2$ for 2 min. After this treatment, the shoots were washed four times with sterilized distilled water to remove $HgCl_2$ completely. Surface-sterilized shoots were blotted dry with sterile paper towels. These shoots were then aseptically cut into approximately 1 cm nodal explant and inoculated on medium. All the surface sterilization steps were carried out in laminar air flow hood (LabHosp^{TM}).

Media preparation, in vitro shoots induction and maintenance of culture conditions

For all experiments, MS medium (Murashige and Skoog 1962) was used with 3 % (*w/v*) sucrose as carbon source and 0.8 % (*w/v*) agar. The pH was adjusted to 5.6±0.2 using 1 N HCl or 1 N NaOH before adding 0.8 % (*w/v*) of agar. The medium was then autoclaved under 105 kPa at a temperature of 121 °C for 15 min. All growth regulators were filter-sterilized (Millipore 0.22 μm) and added after autoclaving. For shoot induction, surface sterilized nodal segments of *B. diffusa* L were inoculated aseptically on MS medium provided with different concentrations of phytohormones viz., 6-benzylaminopurine (BAP; 1 μM-12 μM), Kinetin (Kn;1 μM -12 μM), either alone or in combination with α-Napthalene acetic acid (NAA;1-5 μM), Indole-3-butyric acid (IBA; 1–5 μM). Cultures were incubated at 25±2 °C in 16/8 hrs photoperiod at a photon flux of 50–70 mmol m^{-2} s^{-1} provided by cool and white fluorescent tubes (Philips™, India) and 55±5 % relative humidity.

Shoot elongation and multiplication

The in vitro raised shoots were individually subcultured thrice on fresh MS medium supplemented with 0.1–2.0 μM of BAP alone, 3 % (*w/v*) sucrose and 0.8 % (*w/v*) agar. The MNS/E and MLS/E of shoots were recorded after 3 weeks of each subculture.

In vitro root induction and acclimatization of cloned plantlets

The in vitro raised shootlets (about 5–6 cm in length) were excised and transferred on half strength MS medium gelled with 0.8 % (*w/v*) agar and 3 % (*w/v*) sucrose and augmented with various concentrations of IAA (1 μM -10 μM) and IBA (1 μM - 10 μM) for 4 weeks. In vitro raised plantlets were hardened in polycups containing a mixture of sterile garden soil: peat moss (Peat Pot India, Rajasthan): sand (1: 2: 1; *w/w/w*) irrigated with 10 times diluted MS basal salts. These plants were acclimatized in a culture room at 25±2 °C in 16/8 hrs photoperiod with light intensity of 2000 lux provided by white, florescent tubes (Philips™) and 55±5 % relative humidity for two weeks. These plantlets were then kept in green house at 80–90 % relative humidity 28±2 °C before subsequent transfer to field.

Statistical analysis

Each experiment was carried out in completely randomized design with at least ten replications for each treatment. Data from all experiments were subject to analysis of variance (ANOVA) using SPSS statistical software version 16.0 and means were compared using Duncan's Multiple Range Tests ($P \leq 0.05$).

Assessment of genetic stability using RAPD analysis

Total genomic DNA was extracted from the fresh young leaves of the mother plant and in vitro-regenerated plants using genomic DNA isolation kit (RKN09, Chromous Biotech, Bangalore, India) method. The concentration of DNA was determined by spectrophotometer and quality of genomic DNA was checked following electrophoresis on 0.8 % agarose gel. In the present study, clonal fidelity of in vitro-raised clones was assessed by the use of PCR-based RAPD (random amplified polymorphic DNA) markers because of their simplicity, cost-effectiveness, quickness in operation and small quantity of DNA required.

The genetic fidelity of tissue culture-raised plants was assessed using 14 RAPD primers (Chromous Biotech Pvt. Ltd., Bangalore, India). For generating RAPD profile, in vitro raised, field grown plants were randomly selected from the population and compared with the mother plant from which the explants were taken. PCR amplifications were carried out in total reaction volume of 50 μl containing 1 μl (25–30 ng) of genomic DNA, 5 μl of 10X PCR buffer, 1.5 μl $MgCl_2$ (2 mM), 2 μl of dNTPs (10 mM each of dATP, dGTP, dTTP and dCTP), 2 μl RAPD primer, 0.5 μl of Taq DNA polymerase and 39.5 μl water. The primers showing polymorphic bands were then used for analyzing the clonal fidelity of micropropagated plants. PCR amplification was performed in a DNA thermal cycler (Applied Biosystems, India) which was programmed for initial DNA denaturation at 94 °C for 5 min, followed by 40 cycles of 1 min denaturation at 94 °C, 1 min annealing temperature (temperature specific to the primer) 45 °C and 2 min extension at 72 °C, with a final extension at 72 °C for 10 min. Amplified products were resolved by electrophoresis on 2 % agarose gel containing 1 μg/ml ethidium bromide, at a constant voltage (60 V) and the

Table 1 Effect of different concentrations of phytohormones on shoot induction from nodal explants of *Boerhaavia diffusa* (zeros removed and added '–' in place of zeros)

Phytohormones (μM)				% shoot induction	Mean number of shoots/explant (MNS/E) (Mean±SE*)	Mean length of the shootlet/explant (cm) (MLS/E) (Mean±SE*)
BAP	Kinetin	NAA	IBA			
1.0	–			77.5±1.3^{k}	3.3±0.0^{cde}	3.2±0.6^{ef}
2.0	–			95.7±1.1^{m}	6.9±0.4^{l}	4.3±0.4^{g}
4.0	–			86.2±1.4^{l}	5.3±0.1^{gh}	3.4±0.7^{fg}
6.0	–			71.1±1.2^{j}	4.2±0.0^{gh}	2.5±0.3^{bcdef}
8.0	–			65.4±1.7^{hi}	3.2±0.0^{cde}	2.0±0.1^{abcd}
10	–			58.2±0.6^{ef}	3.0±0.1^{cd}	1.7±0.2^{abcd}
12	–			41.9±1.0^{c}	2.2±0.1^{a}	1.4±0.1^{abc}
	1.0	–		63.9±1.3^{ghi}	3.2±0.0^{cde}	2.6±0.3^{cdef}
	2.0	–		84.8±1.3^{l}	5.9±0.1^{k}	3.2±0.6^{ef}
	4.0	–		67.8±1.3^{ij}	4.6±0.2^{i}	2.7±0.2^{def}
	6.0	–		59.4±1.4^{ef}	3.5±0.1^{def}	2.5±0.3^{cdef}
	8.0	–		51.2±1.6^{d}	2.8±0.1^{bc}	1.8±0.2^{abcd}
	10	–		39.4±0.8^{c}	2.1±0.0^{a}	1.6±0.1^{abcd}
	12	–		67.7±1.3^{hi}	1.9±0.0^{a}	1.1±0.0^{a}
2.0		1.0	–	59.8±1.0^{efg}	4.6±0.2^{i}	2.8±0.3^{def}
2.0		3.0	–	41.2±1.0^{c}	3.2±0.0^{cde}	2.2±0.2^{abcde}
2.0		5.0	–	61.9±1.7^{fgh}	2.2±0.0^{a}	1.9±0.1^{abcd}
2.0	–		1.0	50.2±1.0^{d}	4.4±0.2^{gh}	1.9±0.4^{abcd}
2.0	–		3.0	40.2±1.0^{c}	3.1±0.1^{cde}	1.8±0.3^{abcd}
2.0	–		5.0	56.9±1.4^{e}	2.4±0.3^{ab}	1.5±0.3^{abc}
	2.0	1.0		48.6±1.9^{d}	4.1±0.1^{h}	1.8±0.3^{abcd}
	2.0	3.0		38.7±0.7^{c}	3.6±0.1^{ef}	1.4±0.1^{ab}
	2.0	5.0		56.7±1.2^{e}	2.9±0.2^{cd}	1.2±0.0^{a}
	2.0		1.0	42.9±0.9^{c}	3.9±0.0^{fg}	1.4±0.0^{abc}
	2.0		3.0	34.3±1.5^{b}	3.2±0.0^{cde}	1.2±0.0^{a}
	2.0		5.0	29.6±2.4^{a}	2.1±0.3^{a}	1.1±0.0^{a}

*Values correspond to means±standard error (SE) of three independent experiments with at least 10 replicates. Means in each column followed by same letters are not significantly different according to DMRT (Duncan's Multiple Range Test) at $P<0.05$

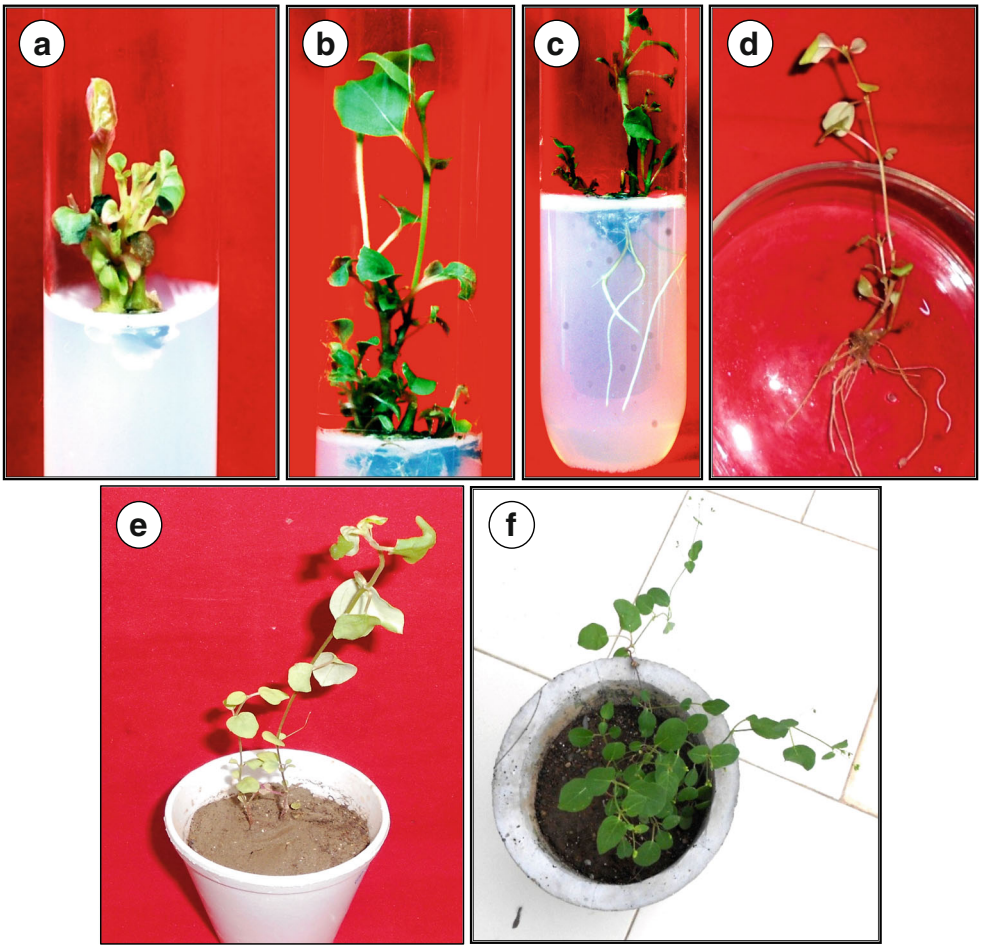

Fig. 1 Plant regeneration of *Boerhaavia diffusa* L using nodal explants **a**) Multiple shoot induction on MS medium supplemented with 2 μM of BAP after 15–16 days of culture. **b**) The shoot multiplication and elongation on MS medium supplemented with 0.5 μM of BAP after 3 weeks of subculture. **c**) Rooting of microshoots which was cultured on half strength MS medium supplemented with 4 μM of IBA alone after 4 weeks of culture. **d**) in vitro rooted plant showing multiple rootlets **e**) primary hardening in polycups containing a mixture of sterile garden soil: peat moss : sand (1: 2: 1; *w/w/w*) for 2 weeks. F) Plantlet acclimatized and established in earthen pot containing natural soil (for 25 days)

number of bands were recorded using a gel documentation system (Applied Biosystems, India). The size of the amplification products was estimated using a 100 bp and 500 bp DNA ladders (Chromous Biotech Pvt. Ltd., Bangalore, India). RAPD profile showed banding pattern from the PCR amplified products in the 2 % agarose gel.

Data scoring and analysis

For molecular studies, consistently reproducible and well-resolved bands, ranging from 300 to 2,000 bp, were manually scored and the scoring of bands was recorded in the form of their presence ("1") or absence ("0") in the gel.

Results and discussion

Explant preparation and surface sterilization

The Explants especially from older branches collected in months of June-July were more prone to fungal contamination than other period of harvesting. This contamination

Table 2 Effect of different concentrations of BAP on shoot elongation in in vitro raised shoots from nodal explants of *Boerhaavia diffusa* L

Sr. No.	Phytohormone (μM) BAP	Mean Number of Shoots/ Explant (MNS/E) (Mean±SE*)	Mean shoot length/ Explant (MSL/E) (cm) (Mean±SE*)
1	0.1	9.6 ± 0.4^{bc}	3.5 ± 0.4^{c}
2	0.5	10.8 ± 0.4^{c}	5.8 ± 0.1^{d}
3	1.0	9.6 ± 1.0^{bc}	2.7 ± 0.1^{bc}
4	1.5	8.3 ± 0.7^{b}	2.3 ± 0.2^{ab}
5	2.0	5.8 ± 0.7^{a}	1.4 ± 0.3^{a}

*Values correspond to means±standard error (SE) of three independent experiments with at least 10 replicates. Means in each column followed by same letters are not significantly different according to DMRT at $P<0.05$

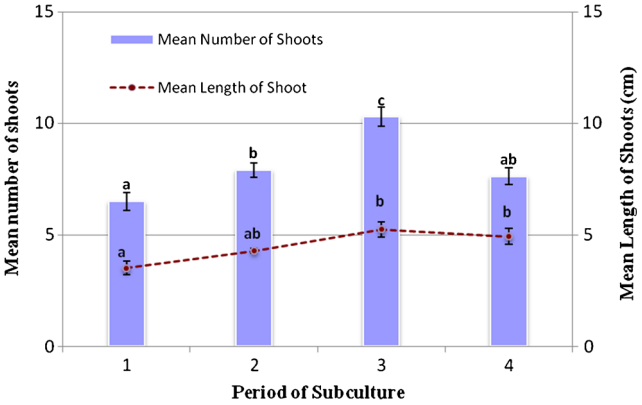

Fig. 2 Effect of subculturing passage on number of shoots per explant and length of shoots per explant in nodal explants of *Boerhaavia diffusa* L. *Bars* represent the mean±SE of three independent experiments, each with 10 replicates. Mean values denoted by the same letter do not differ significantly at $P<0.05$ according to Duncan's multiple range test (DMRT)

could be observed after 8–10 days of culture. However, defoliating the leaves from the young shoots, prolonged washing with tween 20 for 15 mins and use of antifungal Bavistin (0.4 g/L) could eliminate the problem of contamination. Fungal contamination in rainy season may be due to high humidity (Cardoza et al. 1999). Similarly, Bavistin was used effectively to reduce the contamination in *Anacardium occidantale* L (Cardoza et al. 1999). Seasonal contamination in nodal explants of *A. agallocha* was also observed by Nazeem et al. (2007). Incidence of fungal contamination could be substantially controlled by prior fungicidal treatment of the source plant followed by surface sterilization with mercuric chloride wherein the survival rate was 80 percent (Sankar et al. 2007).

Effect of phytohormones on shoot induction

The MS medium was the most suitable medium for callus induction as well as for plant regeneration in *B. diffusa* (Sudarshana et al. 2008). Explants cultured on MS medium supplemented with different phytohormones were significantly affected with respect to the mean no. of shoots per explant (MNS/E) and mean length of shoots per explant (MLS/E) (Table 1). Among the different phytohormones tested, 2 μM of BAP was found to be most effective in shoot induction. The MS medium supplemented with 2 μM of BAP induced maximum seven MNS/E with MLS/E of 4.3 cm and 95 % frequency of shoot induction. As compared to the BAP (1 μM-12 μM) treatment, relatively lower responses in MNS/E, MLS/E and frequency of shoot induction were observed in media supplemented with Kn (1 μM-12 μM) alone or BAP (2 μM) and Kn (2 μM) in combination with NAA (1 μM −5 μM) and IBA (1 μM −5 μM) in nodal explants of *B. diffusa* (Table 1 and Fig. 1a). However, addition of higher concentrations of BAP (14 μM-20 μM) or Kn (14 μM-20 μM) in MS medium resulted in excessive callus growth at the basal end of the explants of *B. diffusa*. (data not shown here). Effectiveness of BAP over kinetin and also basal callus formation at higher concentrations of BAP was observed in *Arnebia hispidissima* (Phulwaria et al. 2013). In the present study, frequency of shoot induction, MNS/E and MLS/E were decreased with increasing concentrations of BAP. Similar to our observation, the favorable response of nodal segments was also reported in a number of plants like *Salvadora persica* (Phulwaria et al. 2011), *Salvadora oleoides* (Shekhawat et al. 2012), *Terminalia bellirica* (Phulwaria et al. 2012a) and *Terminalia catappa* (Phulwaria et al. 2012b). Our observations were superior to earlier report of Sudarshana et al. (2008) and

Table 3 Effect of different phytohormones on root induction in *Boerhaavia diffusa* L

Phytohormones (μM)		% frequency of root induction (Mean±SE*)	Mean number. of rootlets (Mean±SE*)	Mean length of rootlet (cm) (Mean±SE*)
IBA	IAA			
1.0	–	65.7 ± 1.3^{ef}	6.9 ± 0.4^{de}	3.4 ± 0.5^{bcd}
2.0	–	82.5 ± 2.1^{h}	10.0 ± 0.8^{f}	3.7 ± 0.3^{cd}
4.0	–	92.9 ± 1.4^{i}	13.8 ± 1.4^{j}	6.2 ± 0.3^{e}
6.0	–	76.8 ± 1.1^{g}	7.9 ± 1.1^{def}	3.4 ± 0.6^{bcd}
8.0	–	53.0 ± 1.3^{d}	5.3 ± 0.5^{bcd}	2.8 ± 0.3^{abc}
10.0	–	44.7 ± 1.0^{c}	3.2 ± 0.7^{ab}	2.2 ± 0.5^{ab}
–	1.0	62.4 ± 1.0^{e}	5.5 ± 0.7^{bcd}	2.9 ± 0.3^{abc}
–	2.0	67.2 ± 0.6^{f}	6.3 ± 0.4^{cde}	4.6 ± 0.5^{d}
–	4.0	76.5 ± 1.1^{g}	8.5 ± 0.9^{ef}	4.0 ± 0.6^{cd}
–	6.0	52.8 ± 1.2^{d}	4.0 ± 0.9^{abc}	3.6 ± 0.3^{bcd}
–	8.0	39.9 ± 0.9^{b}	3.2 ± 0.5^{ab}	2.6 ± 0.2^{abc}
–	10.0	32.5 ± 0.8^{a}	1.6 ± 0.2^{a}	1.6 ± 0.3^{a}

*Values correspond to means±standard error (SE) of three independent experiments with at least 10 replicates. Means in each column followed by same letters are not significantly different according to DMRT at $P<0.05$

Table 4 List of primers, their sequence and size of the amplified fragments generated by RAPD

Sr. no.	Primer Code	Primer sequence (5'–3')	Total Number of Scorable Bands	Range of the Amplification (bp)
1	P1	GTAACCAGCC	12	200–1500
2	P2	GAACGGACTC	10	200–1000
3	P3	CACCATCCGT	10	400–2,000
4	P4	CTCACCGTCC	11	500–1,500
5	P5	GTTGCCAGCC	12	600–1,500
6	P6	ACGTCCAGAC	12	300–2,000
7	P7	CCCGTCAGCA	13	300–1,500
8	P8	GTGTCGCGAG	13	400–1,500
9	P9	CCCGCTACAC	12	300–1,500
10	P10	GACCGCTTGT	13	400–1000
11	P11	CAAACGTCGG	12	400–1,500

Roy (2008) in terms of frequency of shoot induction from nodal explants of *Boerhaavia diffusa*.

Shoot elongation and multiplication

The MNS/E and MLS/E were observed to be maximum on MS media fortified with 0.5 μM of BAP alone after three weeks of third subculture (Table 2 and Fig. 1b).

Effect of subculture on shoot multiplication and elongation

The subcultures of nodal explant were studied on MS medium supplemented with 0.1–2.0 μM of BAP alone. The shoot multiplication and elongation was considerably affected with subculturing passage. Initially during the first subculture the multiplication rate was low but during the third subculture mean number of shoots were increased to 10 and mean length of shoots per explant was found to be 6 cm (Fig. 2). Similar results of subculturing with low response in initial subculture and maximum in third subculture in micropropagation of *Sapindus trifoliatus* was observed by Asthana et al. 2011. Again we observed that after third subculture there is however, decrease in response of multiplication. Similar to our results Md and Mohammad 2012 also observed low response in nodal explants of *Vitex trifolia* as culture advances.

In vitro rooting and acclimatization of cloned plantlet

When in vitro regenerated shoots (6–7 cm in length) were excised individually and transferred on the half strength MS medium supplemented with phytohormones *viz*., IAA (1 μM-10 μM) and IBA (1 μM-10 μM) the elongated shoots produced in vitro roots. The maximum frequency of root induction, mean no. of roots per explant (MNR/E) and mean length of rootlet per explant (MLR/E) were summarized in Table 3. The results of the present study is in accordance with previous study (Saini et al. 2011) in which the maximum rooting frequency is observed at low concentrations of IBA in *B. diffusa* L. In the present study, we observed that MS medium supplemented with 4 μM IBA alone gives 92.74 % of rooting frequency while other concentrations studied gives low frequency of root induction (Table 3; Fig. 1c). Optimum root induction using IBA alone has been reported for several medicinal plants like

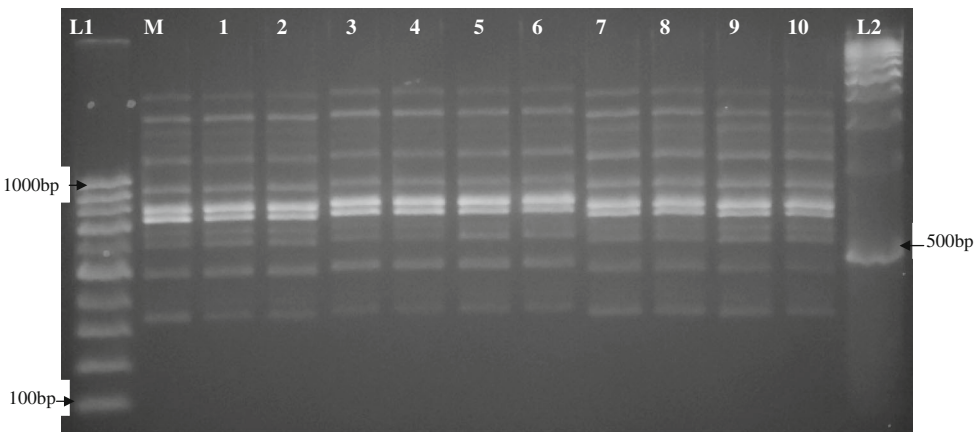

Fig. 3 Polymerase chain reaction (PCR) amplification products obtained with the random amplified polymorphic DNA (RAPD) primer P-9. L1-DNA marker (100 bp ladder), M–Mother plant. Lanes 1–10 represent profile of micropropagated plants of *Boerhaavia diffusa* L. L2-500 bp ladder

Rauvolfia tetraphylla L. (Faisal et al. 2012), *Terminalia catappa* (Phulwaria et al. 2012a). The in vitro rooted plantlets (20–25 days after the subculture) were successfully acclimatized and established in polycups containing mixture of garden soil: peat moss: sand (1:2:1) with 85 % of survivability (Fig. 1e). These plantlets were further established in pots with garden soil in green house for complete acclimatization and finally to field in earthen pots (natural soil) (Fig. 1f).

Assessment of genetic stability

A total of 14 universal RAPD primers were used for generating the RAPD profile of mother plant and in vitro regenerated plants of *B. diffusa*, out of them only 11 RAPD primers gave clear and reproducible bands and these were used to generate RAPD profile (Table 4). The number of scorable bands for each RAPD primer varied from 10 to 12 (Table 4). The 11 RAPD primers produced 105 bands, with an average of 11.81 bands per primer. Each primer generated a unique set of amplification products ranging in size from 200 to 2,000 bp. In the present study, RAPD profile obtained through amplification of genomic DNA of the in vitro field grown plants and that of the mother plant was similar in all the 11 primers tested and produced monomorphic bands confirming the genetic homogeneity of the in vitro raised plants. RAPD analysis of in vitro regenerated and mother plants with primer P-6 (Fig. 3) did not show any detectable polymorphism. The remaining primers (P1-P5 and P7-P11) also produced similar monomorphic band pattern revealing the genetic integrity of in vitro micropropagated plants and thus, revealing that the micropropagated plants are genetically similar during clonal propagation.

RAPD markers are useful for the study of genetic structure of *Boerhaavia* populations (Shukla et al. 2003). RAPD has been proven to be a suitable molecular technique to detect the variation that is induced or occurs during in vitro regeneration of plant species (Shu et al. 2003). Many investigators have reported genetic stability of several micropropagated plants *viz*. *Sapindus trifoliatus* L (Asthana et al. 2011), *Andrographis paniculata* (Dandin and Murthy 2012), *Rauvolfia tetraphylla* L. (Faisal et al. 2012), *Terminalia catappa* (Phulwaria et al. 2012b).

Conclusion

The present study provides the first report on genetic stability of micropropagated plants of *Boerhaavia diffusa* L through nodal explants. The study also describes an efficient plant regeneration method for *B. diffusa* L through nodal explants with good survivability. Monomorphic banding pattern obtained among mother plant and tissue culture-raised plants of *Boerhaavia diffusa* L with RAPD markers confirmed the genetic stability of the plants produced and also ensures the commercial scale utilization of the developed protocol to use this plant as medicinal ingredient and to obtain pharmaceutically important products from the plant.

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