Factors affecting *in vitro* propagation and field establishment of *Chlorophytum borivilianum*

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Abstract

The effect of plant growth regulators (PGRs), gelling agents, sucrose and heat shock on shoot multiplication, shoot growth, rooting and subsequent survival of *Chlorophytum borivilianum* Sant. *et* Fernand was evaluated. Benzyladenine (BA) was found to be better cytokinin over kinetin (KIN) for shoot multiplication. Sucrose concentrations from 116 - 290 mM in the basal medium (BM) promoted shoot multiplication. Heat shock (50 °C, 1 h) also promoted shoot multiplication at these sucrose concentrations on both BM medium and BM supplemented with 5.0 μM BA. Beneficial effect of sucrose was also observed on rooting of shoots on BM as well as BM supplemented with 5.0 μM indole-3-butyric acid (IBA). *Phytagel* as a gelling agent was found to be more effective for shoot proliferation and growth compared to agar. Amongst various soil mixtures tested, higher survival of plants was observed in soil containing *Vermicompost*. It was interesting to note that a maximum plant survival (> 95 %) was observed when plants were directly transferred to net-house (irradiance reduced to 50 % with green net, without humidity and temperature control) than poly-house (with humidity and temperature control). Random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) analysis of regenerated plants showed genetic similarity to mother plant.

Additional key words: acclimatization, heat shock, ISSR, micropropagation, RAPD, sucrose.

Introduction

Chlorophytum borivilianum is a traditional medicinal plant belonging to family *Liliaceae*. Poor regeneration and seed germination in the natural habitat was responsible for being endangered species (Narasimham and Ravuru 2003). Subsequently, attempts were made for the conservation of this species through conventional methods (Kothari and Singh 2003, Maiti and Geetha 2005) and *in vitro* approaches, which focussed on development of efficient micropropagation protocol (Dave *et al.* 2004, Dave and Purohit 2002, Purohit *et al.* 1994a,b, 2003, Rizvi *et al.* 2007). However, there are clone specific variations and still these protocols are required to be refined for commercial micropropagation (Dave *et al.* 2003). Therefore, the present study was aimed to investigate the role of some of the important factors such as plant growth regulators (PGRs), gelling agents, sucrose concentration, heat shock treatment, *etc*., which may improve *in vitro* cultivation of *C. borivilianum* and to develop simple and efficient acclimatization procedure. The uniformity and quality of propagated plantlets was proved by using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers.

Material and methods

Elite cultivar of *Chlorophytum borivilianum* Sant. *et* Fernand (safed musli) was collected from the farmer's field and maintained in earthen pots. Stem discs with shoot buds were used as explants. All chemicals (analytical

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Abbreviations: BA - benzyladenine; BAM - basal MS medium supplemented with 5.0 μM BA; BIBA - basal MS medium supplemented with 5.0 μM IBA; BM - basal MS medium; HS - heat shock; IBA - indole-3-butyric acid; ISSR - inter simple sequence repeat; KIN - kinetin; PGRs - plant growth regulators; PCR - polymerase chain reaction; RAPD - random amplified polymorphic DNA.

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grade) used were from *HiMedia Laboratories* (Mumbai, India). Explants were treated with 10 % (v/v) aqueous sodium hypochlorite for 12 min, washed thoroughly with sterile distilled water and disinfected with 0.04 % (m/v) mercuric chloride solution with 0.1 % (v/v) *Tween 80* for 7 min and then repeatedly washed with sterile distilled water. After surface disinfections, explants were transferred to 250 cm^3 culture bottles (*Borosil*, Mumbai, India) containing 50 cm^3 of basal medium (BM; Murashige and Skoog 1962) containing 58 mM sucrose (pH 5.8), 2.5 μM benzyladenine (BA) and 0.65 % (m/v) agar and autoclaved at 121 ºC for 20 min. The cultures were incubated at temperature of 25 ± 1 ºC, 16-h photoperiod and irradiance inside the vessel of 42 μmol m^{-2} s^{-1} (cool fluorescent tubes, *Philips*, Kolkata, India). Shoots proliferated from these stem discs were multiplied on basal medium supplemented with 5.0 μM BA (BAM).

The effect of different concentrations (0.5 - 12.5 μM) of BA and kinetin (KIN) was studied on shoot multiplication. Five shoots were inoculated in each culture vessel. Number of shoots per explant and average shoot length was recorded after three weeks of subculture. The effect of different sucrose concentrations (58, 116, 174, 232 and 290 mM) was studied in BM, BAM or BM supplemented with 5.0 μM IBA (BIBA). After one week of subculture, half of the cultures from each treatment were subjected to heat shock by exposing to 50 ºC for 1 h. The effect of two commonly used gelling agents, agar (0.65%, m/v) and *Phytagel* (0.22 %, m/v) was tested on shoot growth, multiplication and rooting of microshoots on BM or BAM containing 58 mM sucrose.

Acclimatization of plantlets was carried out in poly-house with controlled temperature and humidity using soil mixtures of sand, soil, *Vermicompost*, *Perlite*, *Soilrite* and *Vermiculite* in different combinations. During the initial periods, 90 % humidity was maintained, and slowly reduced to 40 % over a period of one week. In another experiment, a comparative study was carried out under poly-house (with humidity and temperature control) and open net-house (irradiance reduced to 50 % with green net and without any control of temperature and humidity) conditions. In all experiments at least 20 plantlets were transferred for each treatment and experiments were repeated twice.

Clonal fidelity of micropropagated plants was tested using RAPD and ISSR markers. Genomic DNA was isolated from leaves of randomly selected plants following 3 weeks of acclimatization using CTAB method as described by Doyle and Doyle (1990). Quality of the DNA was checked on a 0.7 % (m/v) agarose gel by loading an aliquot of samples. PCR amplification was performed using 5 RAPD decamer primers and 8 ISSR (16 - 20 nucleotide) primers (Table 1). The reaction mixture consisted of 1.0 unit of Taq DNA polymerase, 100 μM dNTP mixture, 0.002 cm^3 reaction buffer (10×), 10 nmol primer and water was added to make up the volume to 0.02 cm^3. Amplifications were performed in thermal cycler model *GeneAmp® 9700* (*Applied Biosystems*, San Francisco, USA). Amplification conditions were: denaturation at 94 ºC 4 min, 41 cycles of 94 °C 1 min, 36 °C 45 s (52 °C in case of ISSR) and 72 °C 1.5 min with final extinction at 72 °C 5 min. The amplified products were separated on a 1.2 % (m/v) agarose gel and viewed under UV transilluminator (*Gel Doc Mega*, *Biosystematica*, UK) following ethidium bromide staining.

Unless otherwise stated, all experiments were conducted taking four replicates with five explants in each culture vessel and repeated four times. Data were analysed by analysis of variance and the means were compared with Tukey-Kramer test using *GraphPad Prism 4* software.

Table 1. Sequence of various primers used in ISSR and RAPD profiling of regenerated plants and the number of bands amplified and their size range.

Primers	Primer sequence	Number of bands	Size range [bp]
ISSR-1	$(CA)_8CG$	7	500- 700
ISSR-2	$(GA)_8CG$	3	600-1000
ISSR-3	$(GA)_8TC$	10	300-1500
ISSR-4	$(AC)_8GCGC$	4	600-1000
ISSR-5	$(AC)_8$	4	400-1500
ISSR-6	$(CA)_8TG$	4	1000-1400
ISSR-7	$(CA)_8GC$	6	550-1700
ISSR-8	$(GA)_8TA$	3	250-1500
RAPD-1	AGCGCCATTG	8	400-3000
RAPD-2	CTTCCCCAAG	2	400-1000
RAPD-3	AGGGCGTAAG	7	400-1200
RAPD-4	CTGGGGGACT	4	1000-1500
RAPD-5	GGTCTACACC	7	500-1500

Results and discussion

Cultures were established using stem disc explant containing shoot buds as reported by Dave *et al.* (2003). Shoots sprouted from the buds were grown and subsequently maintained on MS medium supplemented with 5.0 μM BA for at least 2 - 3 cycles before experimentation. Earlier, various explants were used to establish culture, which include seeds (Purohit *et al.* 1994b), shoot buds (Purohit *et al.* 2003) and shoot meristems from stem discs (Lattoo *et al.* 2006). For clonal propagation of elite plants, the use of vegetative tissue as explant is important, because seed raised populations are likely to show variations. Due to the preformed apical meristems, stem discs are better choice for culture establishment and plants multiplied from such tissues are likely to be true-to-type.

Both cytokinins BA and KIN promoted shoot

multiplication, however, BA was found to be more effective than KIN (Table 2) in agreement with previous reports (Dave and Purohit 2002, De Bruyn and Ferreira 1992, Rao and Purohit 2006, Rout *et al.* 2008). Higher cytokinin concentrations (> 2.5 μM) promoted shoot multiplication, and at lower concentrations (< 1.0 μM) shoot elongation was observed. Similar effects of lower concentrations of cytokinin on shoot elongation have been reported in safed musli (Dave and Purohit 2002). In the present investigation, shoot elongation was also achieved on PGR-free medium. Cytokinins in general are known to suppress apical dominance and thus stimulate shoot multiplication. The observed shoot growth in the absence of cytokinin could be due to release from the inhibition of apical dominance (Cline *et al.* 1997).

Sucrose concentration, PGRs and heat shock influenced shoot multiplication, growth and rooting of microshoots. In the absence of heat shock, higher sucrose concentrations (>116 mM) significantly promoted shoot multiplication on basal medium (BM), however, this effect was not observed on medium supplemented with BA (BAM) (Table 3). The cultures which were subjected to heat shock, showed the beneficial effect of sucrose on shoot multiplication both on BM and BAM. Following heat shock, on medium containing 290 mM sucrose, a maximum of 6.1 shoots per explants was multiplied on BM and 10.1 shoots per explants on BAM. The synergistic effect of BA, sucrose concentration and heat shock on shoot multiplication seen in the present study seems to be a novel observation. The beneficial effect of higher sucrose concentration in medium supplemented with BA on shoot multiplication was observed in gladiolus (De Bruyn and Ferreira 1992). In the present study, increased shoot growth was observed following heat shock on BM at sucrose concentrations 58 - 116 mM, whereas, sucrose concentrations higher than 174 mM and heat shock inhibited shoot growth (Table 3). Interaction of different sucrose concentrations and heat shock on regulation of morphogenesis was also reported in gladiolus (Kumar *et al.* 1999, 2002).

Table 2. The effect of different concentrations of benzyladenine (BA) and kinetin (KIN) on shoot induction and growth. Values are the means ± SE of four experiments, each treatment consisted of 4 replicates with five explants in each culture vessel. Data were scored three weeks after subculture. Values sharing a common letter within the column are not significantly different at $P < 0.05$.

Cytokinin	Conc. [μM]	Number of shoots [explant^{-1}]	Shoot length [cm]
Control	0.0	3.7 ± 0.2 fg	4.8 ± 0.1a
BA	0.5	3.9 ± 0.1fg	4.1 ± 0.1bc
	1.5	4.4 ± 0.1ef	3.4 ± 0.1de
	2.5	5.4 ± 0.2d	2.9 ± 0.1f
	5.0	7.3 ± 0.3b	1.8 ± 0.1h
	12.5	8.7 ± 0.1a	1.2 ± 0.1i
KIN	0.5	3.4 ± 0.2g	4.3 ± 0.1ab
	1.5	4.9 ± 0.2fg	3.6 ± 0.1cd
	2.5	4.7 ± 0.1de	3.0 ± 0.1ef
	5.0	6.2 ± 0.1c	2.3 ± 0.1g
	12.5	6.8 ± 0.2bc	1.9 ± 0.1gh

Sucrose concentration, IBA and heat shock increased root induction in microshoots in terms of percentage of shoot rooting and number of roots per rooted shoot. In the cultures which were not subjected to heat shock, higher rooting frequency was observed on medium containing higher sucrose concentrations (> 174 mM) and on medium supplemented with IBA (BIBA) (Table 4). The beneficial effect of sucrose on rooting has been reported earlier in rose (Rahman *et al*. 1992) and gladiolus (Kumar *et al.* 1999). This beneficial effect of higher sucrose concentration on rooting in the presence of IBA could be due to requirement of higher energy during rooting phase of microshoots. An increase in reducing sugars content has been shown to initiate rooting in cutting following auxin application (Haissig 1982). Beneficial effect of sucrose on rooting of cutting has also been reported by Nanda *et al.* (1968).

Table 3. The effect of sucrose concentration and heat shock on shoot induction and growth on basal medium (BM) and basal medium supplemented with 5.0 μM BA (BAM). Values are the mean ± SE of four experiments; each treatment consisted of four replicates with five shoots in each vessel. Heat shock given to 50 % cultures after 7 d of inoculation. -HS: cultures not subjected to the heat shock, +HS: cultures subjected to the heat shock. Data were recorded three weeks after subculture. Values sharing a common lower case letters within the column and upper case letter within the row are not significant at $P < 0.05$.

Sucrose conc. [mM]	Number of shoots [clump^{-1}]				Shoot length [cm]			
	-HS		+HS		-HS		+HS	
	BM	BAM	BM	BAM	BM	BAM	BM	BAM
58	3.7±0.20bB	6.7±0.26aA	3.8±0.19dB	6.4±0.25dA	4.4±0.14aB	1.5±0.08cD	5.8±0.08aA	1.9±0.09dC
116	3.9±0.22bB	6.4±0.25aA	4.4±0.21cdB	7.2±0.23cdA	3.6±0.12bB	2.2±0.11bC	4.3±0.07bA	2.5±0.07cC
174	4.8±0.21aC	6.6±0.28aB	5.1±0.19bcC	7.5±0.21bcA	3.0±0.11cA	2.5±0.12abB	2.8±0.11cAB	2.9±0.13bAB
232	5.1±0.22aC	6.4±0.26aB	5.5±0.16abBC	8.6±0.35bA	2.4±0.11dC	2.9±0.11aB	2.2±0.10dC	3.9±0.07aA
290	5.6±0.26aB	6.0±0.27aB	6.1±0.25aB	10.1±0.36aA	1.5±0.07eB	2.6±0.08abA	1.6±0.10eB	2.7±0.08bcA

Table 4. The effect of sucrose concentrations and heat shock on rooting of microshoots of *C. borivilianum* on basal MS medium (BM) and MS medium supplemented with 5.0 μM IBA (BIBA). Heat shock given to 50 % cultures after 7 d of inoculation. Other details as in Table 3.

Sucrose conc. [mM]	Number of roots [explant^{-1}] -HS BM	BIBA	+HS BM	BIBA	Shoots rooted [%] -HS BM	BIBA	+HS BM	BIBA
58	2.4±0.16dB	3.4±0.16cA	2.3±0.19dB	3.3±0.19cA	35.0±1.9dB	41.9±1.7cA	40.6±1.5cAB	45.0±1.3dA
116	2.9±0.20cdB	3.6±0.16cAB	3.4±0.16cAB	3.7±0.19cA	37.5±2.0dB	37.5±2.0cB	43.1±1.8cB	61.9±1.7cA
174	3.6±0.23bcB	4.1±0.22cB	4.2±0.21cB	5.6±0.23bA	54.4±2.3cC	69.4±2.4bAB	65.0±1.9bB	76.2±1.6bA
232	4.4±0.26abC	6.4±0.27aA	5.4±0.21bB	7.1±0.23aA	63.1±2.0bC	75.0±1.9abAB	70.0±2.3abB	81.9±1.7bA
290	4.7±0.30aC	5.0±0.19bC	6.5±0.28aB	7.6±0.23aA	71.9±1.9aC	80.0±2.1aB	72.5±2.0aC	95.0±1.6aA

Table 5. The effect of agar (0.65 %, m/v) and *Phytagel* (0.22 %, m/v) on shoot proliferation in *C. borivilianum* on basal medium (BM) and basal medium supplemented with 5.0 μM BA (BAM). Values are the mean of four experiments, each treatment consisted of 4 replicates with five explants in each culture vessel. Data were scored 3 weeks after subculture. The values are mean ± SE. Only on BM medium with agar 60 % of shoots rooted and number of roots per explant was 3.25 ± 0.25.

Gelling agent	Medium	Number of shoots [clump^{-1}]	Shoot length [cm]
Agar	BM	3.37 ± 0.24	7.25 ± 0.47
	BAM	6.90 ± 0.35	1.62 ± 0.23
Phytagel	BM	4.70 ± 0.41	13.75 ± 0.47
	BAM	12.51 ± 1.04	22.82 ± 1.11

The heat shock treatment to cultures significantly increased rooting of shoots in most of the sucrose concentrations used on BM and BIBA medium. In gladiolus cultures, Kumar *et al.* (1999) have shown the synergistic effect of sucrose and heat shock on rooting of microshoots. These heat shock induced developmental events may result from the induction of heat shock proteins as developmental role of heat shock proteins was speculated (Schnall *et al.* 1991, Vierling 1991). In the present investigation, about 95 % of shoots were rooted following heat shock on medium containing 290 mM sucrose and 5.0 μM IBA, however, in the absence of IBA a maximum of 72 % shoots were rooted (Table 4) indicating the synergistic effect of sucrose concentration, IBA and the heat shock on rooting. The number of roots per rooted shoot was also significantly higher on medium supplemented with IBA following heat shock. The promotory effect of sucrose concentration on medium supplemented with IBA following heat shock treatment seems to be a novel finding in this study. Sucrose dependent changes in endogenous contents of indole-3-acetic acid (IAA) and polyamines following heat shock has been reported in gladiolus cultures (Kumar 1996), and in the present study, the observed effect of heat shock on morphogenesis could be due to the alteration in the endogenous pool of some of the PGRs.

The effect of two commonly used gelling agents (agar and *Phytagel*) was studied on shoot multiplication, shoot growth, and rooting of shoots. The shoot multiplication and shoot growth was better on medium gelled with *Phytagel* both on BM and BAM when compared to medium gelled with agar (Table 5). The beneficial effect of *Phytagel* over agar on shoot multiplication and growth has also been observed in *Rosa damascena* (Kumar *et al.* 2003). Agar has been reported to cause water stress in the cultures of potato (Gopal *et al.* 2008). Previous studies have indicated the inhibitory effect of agar on the growth of some cultures (Debergh and Maene 1984, Nairn 1988). On BM gelled with agar, about 60 % shoots rooted, whereas on medium gelled with *Phytagel* root induction was not observed. Similar effect of *Phytagel* and agar on rooting of microshoots have been reported in *Rosa damascena* (Kumar *et al.* 2003).

Amongst different potting mixtures used, *Vermicompost* was beneficial for plant survival. Maximum survival of about 60 % was achieved in a mixture of *Vermicompost* and *Perlite* (1:1; m/m). The beneficial effect of *Vermicompost* on growth and acclimatization has also been observed in *Bacopa* (Sharma 2005). When different environmental conditions were compared for hardening, it was observed that the plants which were kept in net-house showed much higher survival (> 95 %) as compared to those which were kept in poly-house with controlled humidity (90 %) and temperature (25 ± 3 °C). This might be due to the faster adaptation of plants to *ex vitro* conditions in net houses (covered only with green nets) and further lower humidity in net house might discourage microbial attack. This is important not only because of higher survival but also it results in lower input of energy during acclimatization, which help in substantially reducing production cost of plant for commercial micropro-pagation.

The RAPD and ISSR analyses of the regenerated plants showed similarity with mother plant indicating the clonal nature of the propagated material. In all 13 primers used (5 RAPD and 8 ISSR) for amplification, banding patterns were similar in regenerated plants to that of mother plant (Fig. 1). The utility of RAPD and ISSR

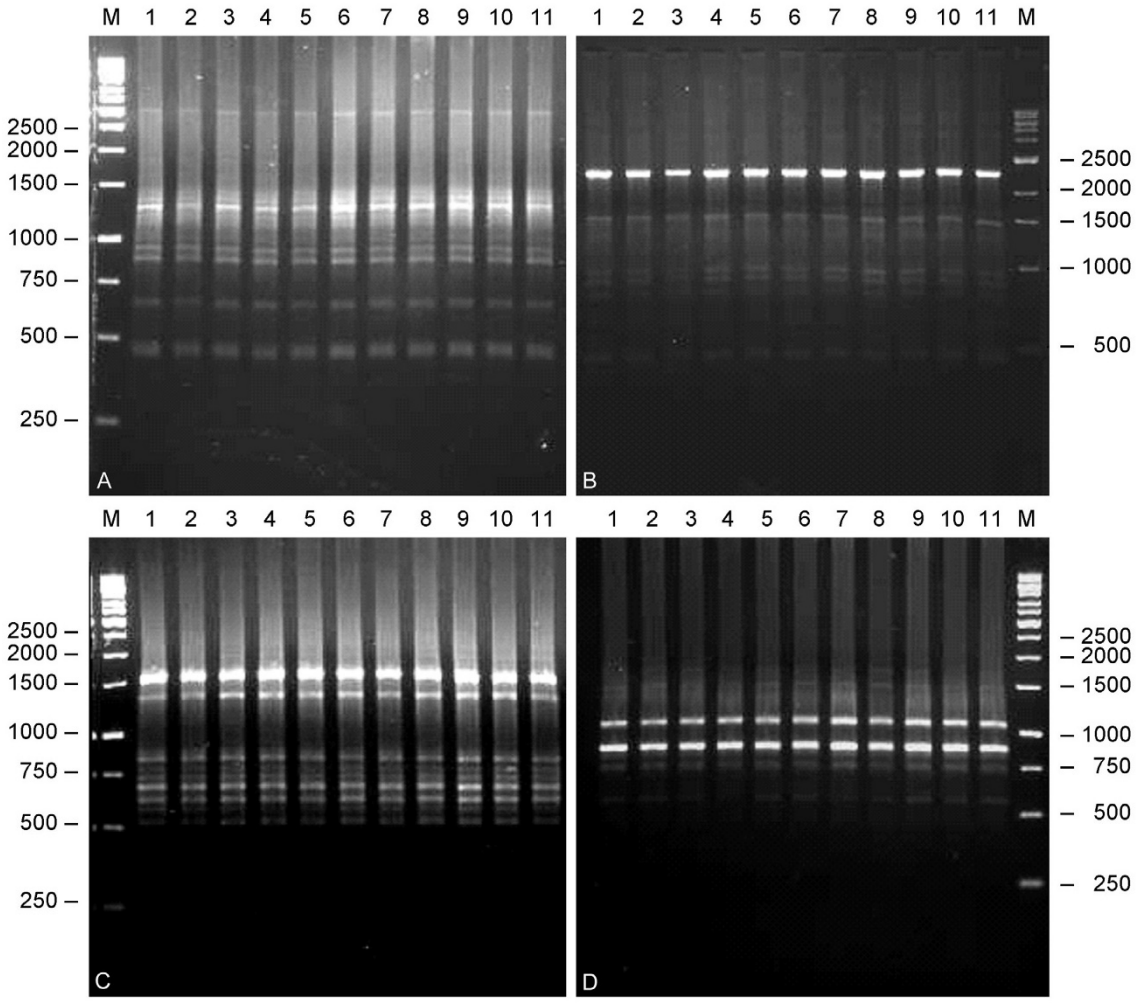

Fig. 1. RAPD profiles of micropropagated plantlets and mother plant of *C. borivilianum* using RAPD primers (*A* - RAPD-1, *B* - RAPD-3) and ISSR primers (*C* - ISSR-1, *D* - ISSR-7). *Lane* 1 - mother plant; *lanes* 2 to 11 - regenerated plants; *lane M* - molecular mass markers [kb]

markers for the molecular analysis of micropropagated plants for testing clonal fidelity has been well documented (Gupta and Varshney 1999, Darokar *et al.* 2001, Joshi and Dhawan 2007, Chandrika *et al.* 2008, Tyagi *et al.* 2010). The clonal fidelity of *in vitro* regenerated plants of safed musli was tested using RAPD markers (Lattoo *et al.* 2006). In the present study the regenerated plants following different cycles (10 - 15) of subculture show high degree of genetic uniformity, which might be due to the use of stem discs as explants. These explants have preformed shoot buds and plants regenerated from these tissues have been reported to show very little genetic variations (Mathur *et al.* 2008).

The present study reveals that use of higher sucrose concentration along with BA promoted higher shoot multiplication and with IBA stimulated rooting of shoots. The heat shock treatment enhanced the shoot multiplication rates to double and also have beneficial effect on rooting of shoots. Rooted plants can be efficiently acclimatised under net house. This has potential to cut down the cost of regenerated plants by about 30 %. The RAPD and ISSR analyses revealed that regenerated plants showed similarity with mother plant, which suggest clonal fidelity and it can be said that the regenerated plants are of true-to-type.

References

Chandrika, M., Thoyajaksha, Ravishankar Rai, V., Ramachandra Kini, K.: Assesment of genetic stability of *in vitro* grown *Dictyospermum ovalifolium*. - Biol. Plant. **52**: 735-739, 2008.

Cline, M., Wessel, T., Iwamura, H.: Cytokinin/auxin control of apical dominance in *Ipomoea nill*. - Plant Cell Physiol. **38**: 659-667, 1997.

Darokar, M.P., Khanuja, S.P.S., Shasany, A.K., Kumar, S.: Low

levels of genetic diversity detected by RAPD analysis in geographically distinct accessions of *Bacopa monnieri.* - Genetic Resour. Crop Evol. **48**: 555-558, 2001.

Dave, A., Bilochi, G., Purohit, S.D.: Scale-up production and field performance of micropropagated medicinal herb 'Safed Musli' (*Chlorophytum borivilianum*). - *In Vitro* cell. dev. Biol. Plant. **39**: 419-424, 2003.

Dave, A., Joshi, N., Purohit, S.D.: *In vitro* propagation of *Chlorophytum borivilianum* using encapsulated shoot buds. - Eur. J. hort. Sci. **69**: 37-42, 2004.

Dave, A., Purohit, S.D.: Factors influencing *in vitro* culture of 'Safed Musli' (*Chlorophytum borivilianum*). - Oikoassay **15**: 19-27, 2002.

De Bruyn, M.H., Ferreira, D.I.: *In vitro* corm production of *Gladiolus dalenii* and *G. tristis.* - Plant Cell Tissue Organ Cult. **31**: 123-128, 1992.

Debergh, P.C., Maene, L.: Pathological and physiological problems related to the *in vitro* culture of plants. - Parasitica **40**: 69-75, 1984.

Doyle, J.J., Doyle, J.L.: Isolation of plant DNA from the fresh tissue. - Focus **12**: 13-15, 1990.

Gopal, J., Iwama, K., Jitsuyama, Y.: Effect of water stress mediated through agar on *in vitro* growth of potato. - *In Vitro* cell. dev. Biol. Plant. **44**: 221-228, 2008.

Gupta, P.K., Varshney, R.K.: Molecular markers for genetic fidelity during micropropagation and conservation. - Curr. Sci. **76**: 1308-1310, 1999.

Haissig, B.E.: Carbohydrate and amino acid concentration during adventitious root primordium development in *Pinus banksiana* Lamb. cuttings. - Forest. Sci. **61**: 13-19, 1982.

Joshi, P., Dhawan, V.: Assessment of genetic fidelity of micropropagated *Swertia chirayita* plantlets by ISSR marker assay. - Biol. Plant. **51**: 22-26, 2007.

Kothari, S.K., Singh, K.: Production techniques for the cultivation of safed musli (*Chlorophytum borivilianum*). - J. Hort. Sci. Biotechnol. **78**: 261-264, 2003.

Kumar, A.: Studies on *in vitro* propagation, biochemistry and field evalution of two economically important plants: *Rosa damascene* Mill. and *Gladiolus* spp. - Ph.D. Thesis, Kumaun University, Nainital 1996.

Kumar, A., Palni, L.M.S., Nandi, S.K.: The effect of light source and gelling agent on micro propagation of *Rosa damascena* Mill. and *Rhynchostylis retusa* (L.) Bl. - J. Hort. Sci. Biotechnol. **78**: 786-792, 2003.

Kumar, A., Palni, L.M.S., Sood, A., Sharma, M., Palni, U.T., Gupta, A.K.: Heat shock induced somatic embryogenesis in callus cultures of gladiolus in the presence of high sucrose. - J. Hort. Sci. Biotechnol. **77**: 3-78, 2002.

Kumar, A., Sood, A., Palni, L.M.S. Gupta, A.K.: *In vitro* propagation of *Gladiolus hybridus* Hort.: synergistic effect of heat shock and sucrose on morphogenesis. - Plant Cell Tissue Organ Cult. **57**: 105-112, 1999.

Lattoo, S.K., Bamotra, S., Dhar, B.S., Khan, S., Dhar, A.K.: Rapid plant regeneration and analysis of genetic fidelity of *in vitro* derived plants of *Chlorophytum arundinaceum* Baker – an endangered medicinal herb. - Plant Cell Rep. **25**: 499-506, 2006.

Maiti, S., Geetha, K.A.: Characterization, genetic improvement and cultivation of *Chlorophytum borivilianum* – an important medicinal plant of India. - Plant Genet. Resour. **3**: 264-272, 2005.

Mathur, A., Mathur, A.K., Verma, P., Yadav, S., Gupta, M.L., Darokar, M.P.: Biological hardening and genetic fidelity testing of micro-cloned progeny of *Chlorophytum borivilianum* Sant. *et* Fernand. - Afr. J. Biotechnol. **7**: 1046-1053, 2008.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassay with tobacco tissue culture. - Physiol. Plant. **15**: 473-497, 1962.

Nairn, B.: Significance of gelling agents in a production tissue culture laboratory. - Comb. Proc. Int. Plant Propag. Soc. **37**: 200-205, 1988.

Nanda, K.K., Purohit, A.N., Mehrotra, K.: Effect of sucrose, auxins and gibberelic acid on rooting of the stem segments of *Populus nigra* under varying light conditions. - Plant Cell Physiol. **9**: 725-743, 1968.

Narasimham, K.R.S.L., Ravuru, B.K.: A note on some endangered medicinal plants as NTFPs of Eastern Ghats, Andhra Pradesh. - EPTRI-ENVIS Newslett. **9**: 11-12, 2003.

Purohit, S.D., Dave, A., Bilochi, G., Joshi, N.: Opportunities for scaling-up production and commercial cultivation of micropropagated safed musli (*Chlorophytum borivilianum*). - In: Mathur, A.K., Dwivedi, S., Patra, D.D., Bagehi, G.D., Sangwan, N.S., Sharma, A., Khanuja, S.P.S. (ed.): Proceedings of First National Interactive Meeting on Medicinal and Aromatic Plants. Pp. 387-398. CIMAP, Lucknow 2003.

Purohit, S.D., Dave, A., Kukda, G.: Micropropagation of safed musli (*Chlorophytum borivilianum*), a rare Indian medicinal herb. - Plant Cell Tissue Organ Cult. **39**: 93-96, 1994a.

Purohit, S.D., Dave, A., Kukda, G.: Somatic embryogeny and plantlet regeneration in safed musli (*Chlorophytum borivilianum*). - Indian J. Plant Genet. Resour. **7**: 65-71, 1994b.

Rahman, S.M., Hoasian, A.K.M., Rafiul, I., Joarder, O.I.: Effect of media composition and culture condition on *in vitro* rooting of rose. - Sci. Hort. **52**: 163-169, 1992.

Rao, M.S., Purohit, S.D.: *In vitro* shoot but differentiation and plantlet regeneration in *Celastrus paniculatus* Willd. - Biol. Plant. **50**: 501-506, 2006.

Rizvi, M.Z., Kukreja, A.K., Khanuja, S.P.S.: *In vitro* culture of *Chlorophytum borivilianum* Sant. *et* Fernand in liquid culture medium as a cost effective measure. - Curr. Sci. **92**: 87-90, 2007.

Rout, G.R., Mahato, A., Senapati, S.K.: *In vitro* clonal propagation of *Nyctanthes arbor-tristis*. - Biol. Plant. **52**: 521-524, 2008.

Schnall, J.A., Hwang, C.H., Cooke, T.J., Zimmerman, J.L.: An evaluation of gene expression during somatic embryogenesis of two temperature sensitive carrot variants unable to complete embryo development. - Physiol. Plant. **82**: 498-504, 1991.

Sharma, N.: Micropropagation of *Bacopa monneiri* L. Penn. – an important medicinal plant. - M.Sc. Thesis, Thapar University, Patiala 2005.

Tyagi, P., Khanduja, S., Kothari, S.L.: *In vitro* culture of *Capparis decidua* and assessment of clonal fidelity of the regenerated plants. - Biol. Plant. **54**: 126-130, 2010.

Vierling, E.: The role of heat shock proteins in plants. - Annu. Rev. Plant Physiol. mol. Biol. **42**: 579-620, 1991.