Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2010, 1 (2): 41-46

Pelagia Research Library
ISSN: 0976-8610
CODEN (USA): AASRFC

Micropropagation of *Chlorophytum borivilliens* through direct organogenesis

Manisha Sharan[1], Indira L. Dhumne[1], Madhuri Sharon [2]*

[1]*GUFIC Applied Research & Education Foundation, 11th Road, MIDC, Marol, Andheri (E), Mumbai – 400 093, India*
[2]*N.S.N.Research Center, SICES College of Arts Science & Commerce, Jambhul Phata, Ambernath (W) 421 505, Maharashtra, India*

ABSTRACT

Commercially viable protocol for in vitro propagation of Chlorophytum borivilliens was achieved through direct organogenesis from shoot apex explants. Cultures were initiated on MS medium supplemented with 2 mg l^{-1} benzyl adenine (BA). From one shoot apex, 250 shoots proliferated when it was transferred to MS medium supplemented with 3 mg l^{-1} benzyl adenine in six months time. Ninety eight percent of the regenerated shoots rooted within a month on MS medium supplemented with 0.05 mg l^{-1} naphthalene acetic acid. Rooted plants showed 100 % survival on transfer to in vivo conditions. From one plant in 7 months, 2200 plants were micropropagated and ready for field transfer.

Key words: Plant Regeneration; Apical meristem; *In Vitro* Culture;

INTRODUCTION

Roots of *Chlorophytum borivilliens* (family – Liliaceae) commonly known as safed moosali are extensively used in many Ayurvedic medicines and the leaves are used as vegetable [1]. The secondary metabolites or active ingredients found in this plant are sapogenins and steroids [2].

For medicinal purposes earlier requirement of *C. borivilliense* was mostly met by collecting it from wild. However, increasing demand and depletion of natural sources has initiated cultivation of this plant. Since the plant is propagated and cultivated using meristematic tissues situated at the top of tuberous roots, and roots are required for medicinal purposes, there has been a scarcity of planting material for cultivators. This has prompted the need for development of a commercially viable protocol for micropropagation of this plant. There has been attempt [3], to

micropropagate *C. borivilliens*. An effort to repeat their experiments did not yield the same results. Hence we tried to develop a commercially viable micropropagation protocol and the results obtained by us are presented in this paper.

MATERIALS AND METHODS

Explant Collection and Surface Sterilization

Crown meristem of *C. borivilliens* plants, having nine tuberous roots, was harvested in October from a farm in Gujarat, India. Healthy plants were selected for *in vitro* culture and washed thoroughly with tap water to remove soil adhered to it. Approximately 2 cm pieces of root top with crown meristem were cut and kept in 2% Tween-20 solution on a shaker for 30 minutes and then washed with demineralized water. Explants were dipped in a solution containing 0.1% Bavistin (a fungicide from BASF India) + 0.25% Chloramphenicol (a bactericide from Ranbaxy India) and kept on a shaker for 30 min. The crown part of tuberous roots was cut 1 cm below the apex and surface sterilized in a Laminar flow bench. Each explant was washed and treated separately, because being a sub-soil explant; it tended to have heavy surface microbial presence. Explants were dipped into 1% sodium hypochlorite solution for 10 min and then rinsed with autoclaved distilled water. They were then immersed into 0.2% mercuric chloride solution for 3 min and then rinsed thrice with sterile distilled water. The explants were further trimmed to 0.5 cm cube, without damaging the apical meristem.

Micropropagation was achieved in 3 stages. The basal medium for all the 3 stages was MS medium [4] having 3% sucrose and solidified with 0.8% agar (Himedia, India).

Culture initiation

Cultures were initiated separately in 25 X 150 mm borosilicate test tubes, by incorporating 2,4-dichloro acetic acid (0.5, 1.0 or 2.0 mg l^{-1} 2,4-D), triacontenol (1.0, 2.0 or 5.0 mg l^{-1}), 6-furfuryl amino purine (0.5, 1.0 or 2.0 mg l^{-1} kinetin), 2-iso pentenyl adenine (0.1, 0.5 or 1.0 mg l^{-1} 2 iP/l) or 6-benzyl adenine (0.5, 1.0, 2.0, 3.0 or 4.0 mg l^{-1} BA) into MS medium having 3% sucrose. The temperature of the growth room was maintained at 24 ± 2^0C. Cultures were incubated in dark. Initiation of culture was monitored visually as well as by observing the sections of cultures under the microscope.

Proliferation and Shoot Elongation:

After 4 weeks of incubation, the initiated cultures were transferred to MS medium augmented with 2.0, 3.0 or 4 mg l^{-1} BA and 3% sucrose; for further proliferation of shoot buds and their elongation. Various concentrations of coconut water was also tried to enhance the rate of elongation. Cultures were shifted to growth room having same temperature but 3000 lux light intensity. Cultures were exposed to 16 hours of light period followed by 8 hours of dark period daily.

Rooting of Regenerated Shoots:

For rooting, regenerated shoot clusters were cut into single plants and sub-cultured on to medium containing various auxins such as indole acetic acid (0.5, 1.0, 1.5 or 2.0 mg l^{-1} IAA), naphthalene acetic acid (0.01, 0.05,0.1 or 0.5 mg l^{-1} NAA) or indole butyric acid (0.5, 1.0, 2.0 or 5.0 mg l^{-1} IBA). Light intensity growth room was reduced to 2000 lux.

A regular subculture at an interval of 4 weeks was done through out the culture period.

Hardening of Rooted Plants

Regenerated plantlets were hardened in a 50 cavity portray having 1" X 1" X 1.5" cavity size, in a green house with a fan-pad cooling system and fogger to control temperature and relative humidity. The green house temperature did not exceed 29^{o}C. The maximum day light intensity during hardening was 12,000 to 14,000 Lux. Immediately after transfer to the green house plantlets were kept at 100% relative humidity for first 3 days and then a RH of 60–70% was maintained. The substrate used for hardening of regenerated plantlets was steam sterilized 50:50 soil : cocopeat.

RESULTS AND DISCUSSIONS

C. borivilliens was found to be very selective in their requirement of type and concentration of plant growth regulator for initiation of cultures. Culture initiation was noticed in 3 weeks, only from the explants inoculated on to medium containing 2 mg l^{-1} BA only (Table 1). Microscopic observation of section of cultures revealed it multiple shoot formation from the apical meristem (Figure 1.d).

A week after transferring the cultures to light, 8 -10 shoot buds appeared directly from the explants. Continued cultures on the same medium did not result in any further proliferation of shoot buds. No other treatment successfully induced formation of more shoot buds.

When the initiated cultures (Fig. 1a) were transferred to higher concentration (3 and 4 mg l^{-1}) of BA; proliferation began within a month from cultures growing on 3 mg l^{-1} (Fig. 1b). Cultures did not proliferate at 2 or 4 mg l^{-1} BA (Table 2). Induction of direct organogenesis from apical meristem has been reported earlier in monocots [5] but such concentration and PGR specificity are not very common, only 2 mg l^{-1} BA could initiate the culture. Though 3 mg l^{-1} BA could not initiate the culture, but was able to induce bud proliferation and elongation. As can be seen in the figure 1b many shoot meristem or shoot buds arose from the apical meristem as well as the leaf primordia. Such concentration specificity was not reported by [3] as they got culture initiation, proliferation and shoot elongation on the same medium containing 22.2 μM BA. Neither Kinetin nor 2-iP was found to initiate or proliferate the cultures. 2,4-D and triacontenol also failed to induce any growth or callus formation.

A four-fold increase in number of shoot buds (Fig. 1b and 1c) per subculture was noticed upto 4 subcultures (i.e. by 150th day) in proliferation medium and then it declined to 3. Hence at this stage that shoots were transferred to rooting medium. Later the shoot multiplication rate declined to 2 per sub-culture (Table 2). By 150th day good growth of leaf was observed. Shoots near the periphery of test tubes showed better leaf growth suggesting that higher light intensity was needed for better leaf growth. Therefore, at this stage cultures were kept at 3500 Lux light intensity. Requirement of higher light intensity for better leaf growth in monocots (date palm) has been reported earlier [6]

As mentioned above, plants with 9 roots were selected for isolating the meristem. Therefore, from one plant 9 meristematic shoot apex was taken. From one meristem after approximately

250 shoots differentiated by 160th day. 98% of shoots rooted in medium containing 0.05 mg l^{-1} NAA. Though 90% rooting occurred on 1 and 2 mg l^{-1} IBA also, but the roots were very thin and fewer in number. Only 0.05 mg l^{-1} NAA produced healthy tuberous roots and the maximum (28) number of roots (Table 3, Fig. 1d). These results are not at all matching with those of Purohit et al (1994) who got best rooting on medium containing 3/4th MS supplemented with 9.8µM IBA and very thin and short roots with profuse callusing on medium containing NAA. Unlike Purohit et al (1994), no rooting was observed when shoots were inoculated on medium containing any of the tested concentration of IAA. Moreover the maximum number of roots that could be produced from each shoot in the present work was much higher (28) than what was obtained (16) by [3]. At the rate of 98% rooting; from one explant 245 plants were produced in 6 months time.

Direct regeneration of healthy tuberous roots from regenerated shoots was another unique feature of this plant. Such tuberous root formation has been observed in another liliaceous plant *Asparagus* [7], but only after elaborate pulse and pre-culture treatment with auxins, whereas in *C. borovilliens* tuberous roots regenerated in high numbers on auxin (0.05 mg l^{-1} NAA) containing MS medium. Induction of direct organogenesis from apical meristems obviously presents a clonal propagation protocol.

One hundred percent survival was achieved in the green house under the controlled conditions using a soil + cocopeat substrate. Two hundred forty five plants were regenerated from a single shoot apex in seven months time. From one plant we had 9 shoot apices, that makes it production of 2200 plantlets from each plant.

Table 1. Effect of various concentrations of BA supplemented MS medium on the initiation of culture from apical meristem explants of *Chlorophytum borivilliense* as recorded on 3^{0th} day. Results are mean of 10 observations.

BA (mg l^{-1})	Number of shoot buds regenerated per explant
0.5	0
1.0	0
2.0	3.4 (SD ± 0.1550)
3.0	0
4.0	0

Table 2. Proliferation of shoot buds initiated on medium containing 2 mg l^{-1} BA; on transfer to higher concentrations of BA as recorded on 60th, 90th and 120th day i.e. after 2nd, 3rd, and 4th subculture; and leaf growth as recorded on 120th day. Results are mean of 20 observations.

BA (mg l^{-1})	Number of shoot buds on			Leaf growth recorded on 150th day	
	60th day	90th day	120th day	Leaf No.	Leaf length
1.0	0	-	-	-	-
2.0	4	-	-	3	2.5 cm
3.0	16	64	250	3	5 cm
4.0	4	-	-	-	-

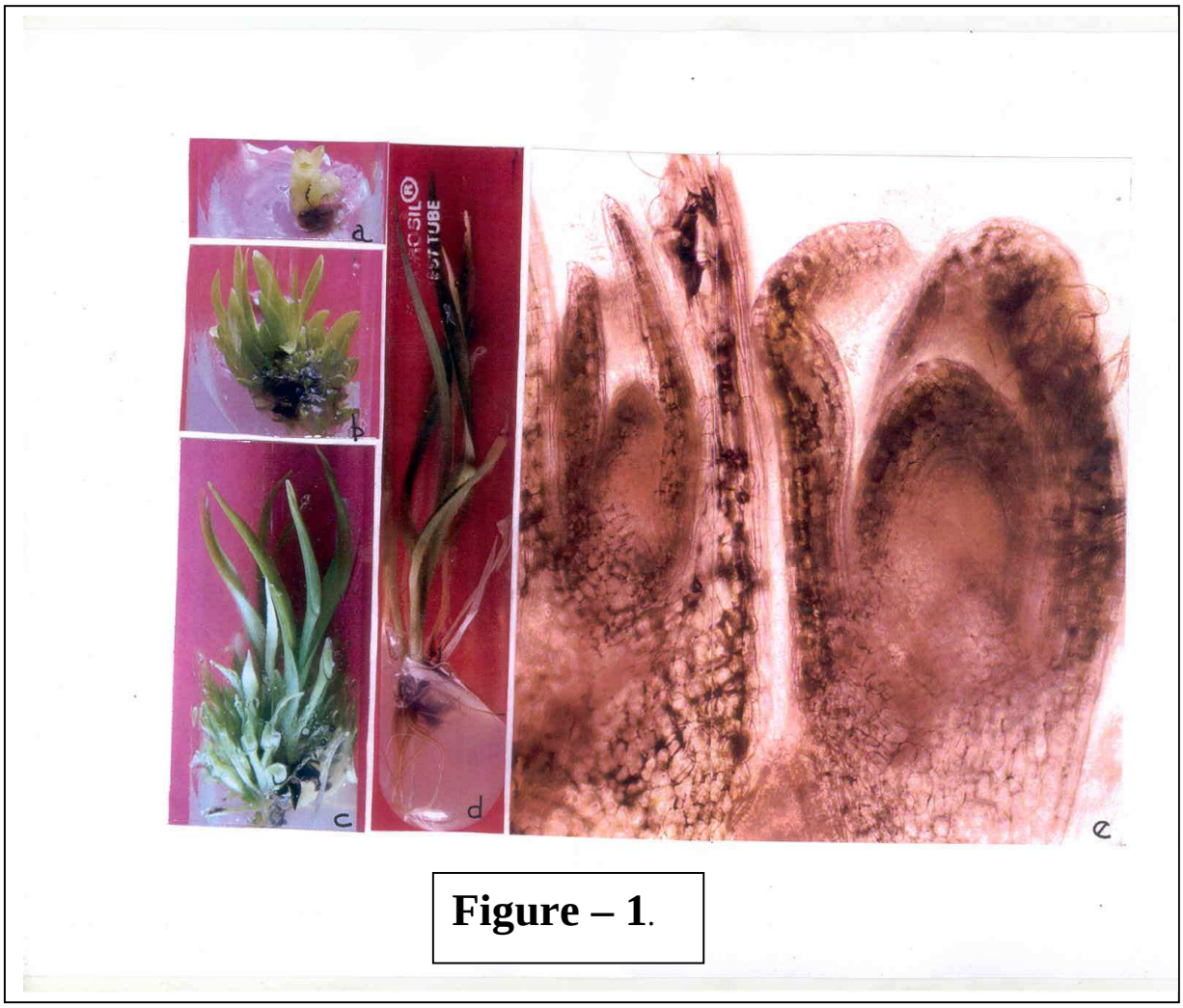

Figure – 1.

Fig-1 Various stages of plant regeneration of *Chlorophytum borivilliens* from apical meristem

(a) 30 days old culture showing shoot bud initiation on gelled MS medium containing 2 mg l^{-1} BA,
(b) Proliferated shoot buds from initiated cultures, cultured on gelled MS medium supplemented with 3 mg l^{-1} BA.
(c) Elongating shoots cultured on gelled MS medium augmented with 3 mg l^{-1} BA
(d) In vitro rooting of shoots cultured on gelled MS medium supplemented with 0.05 mg l^{-1}NAA.
(e) Micro-photograph of apical meristem differentiating into shoots taken on 30th day after inoculation

Table 3. Effect of auxins (NAA and IBA) on *in vitro* rooting and root growth of regenerated shoots, 30 days after transfer to rooting medium. Results are mean of 100 values

MS medium supplemented with	%Rooting response by regenerated shoots	Average Root Growth		
		Root Number ± S.D.	Root Length (cm) ± SD	Root Width (cm)
0.01 mg l^{-1} NAA	80	23.5 ± 0.0221	2.0 ± 0.0310	>0.05
0.05 mg l^{-1} NAA	98	28.0 ± 0.0645	2.25 ± 0.037	0.1
0.1 mg l^{-1} NAA	84	24.5 ± 0.0418	1.5 ± 0.0325	>0.05
0.5 mg l^{-1} NAA	85	23.5 ± 0.0600	1.5 ± 0.0325	>0.05
1.0 mg l^{-1} IBA	90	15.0 ± 0.0764	1.1 ± 0.0387	>0.05
2.0 mg l^{-1} IBA	90	13.0 ± 0.0712	1.4 ± 0.0381	>0.05
3.0 mg l^{-1} IBA	90	10.0 ± 0.0721	1.5 ± 0.0412	>0.05

Acknowledgements
Authors wish to acknowledge financial and scientific support rendered by the authorities of GAREF and to Shri Jayesh P. Choksi M.D. GUFIC BIOSCIENCES, Mumbai for his constant support and encouragement.

REFERENCES

[1] Shingade, M.Y.; Chavan, K.N.; Gupta, D.N.; *J. Food. Sci. Technol*. **1995,** 32 (5): 429-31.
[2] Kitply Industry, Agro-forestry Division Report. Presence of hezoxymine & asparagin in *Chlorophytum*.
[3] Purohit, S; Dave, A.; Kukda, G; *Plant Cell, Tissue and Organ Culture* **1994** 39: 93-96.
[4] Murashige, T; Skoog, F, *Physiol. Plant*. **1962** 15: 473 – 497.
[5] Takayama, S.; Miswa, M.; *Scientia Hortica*, **1983,** 18: 353-362.
[6] Sharon, M.; Shankar, C.; *Indian J. Plant Physiol*. **1999** 4: 323-326.
[7] Watanabe, S.; Imakawa, S.; Yakuwa, T.; *J. Fac. Agr. Hokkaido Univ*. **1991** 64 (4): 292-303.