IJPSR (2016), Vol. 7, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 December, 2015; received in revised form, 16 January, 2016; accepted, 07 February, 2016; published 01 May, 2016

HIGH FREQUENCY DIRECT SOMATIC EMBRYOGENESIS FROM THE JUVENILE SHOOT BUDS OF *CHLOROPHYTUM BORIVILIANUM* SANT. ET FERNAND AND PRODUCTION OF ARTIFICIAL SEEDS

A. Jha and Y. K. Bansal *

Plant Tissue Culture Laboratory, Department of Biological Science R. D. University, Jabalpur (M. P.) 482001, India

Key words:

Micropropagation, *Chlorophytum borivilianum*, direct somatic embryos, encapsulation, synthetic seeds, tubers

Correspondence to Author:
Y. K. Bansal

Plant Tissue Culture Laboratory, Dept. of Biological Science R. D. University, Jabalpur (M. P.) 482001, India.

Email: yogendrakbansal@rediffmail.com

ABSTRACT: The study presents a protocol for mass propagation of *Chlorophytum borivilianum* through direct somatic embryogenesis. Direct somatic embryos occurred on the surface of juvenile shoot bud sprouts cultured on Murashige and Skoog medium supplemented with 5 mg L-1 BAP, yielding a high frequency of direct somatic embryos. On the other hand, a much lower concentration of BAP (0.5 mg L-1) led to the proliferation of somatic embryos. The conversion of somatic embryos with high MRN and MRL was favoured on BM. Encapsulation of somatic embryos (<5 mm) in calcium alginate beads resulted in the formation of well-formed synthetic seeds. Out of the synthetic seeds prepared from the somatic embryos (direct) using three types of gel matrices viz. DW, BM and SM, the ones formed using SM resulted in the highest frequency of germination (86.1%). Seed coat breakage and germination of synthetic seeds with the emergence of the tap roots were visible within 1-2 weeks of culture on basal medium. The plantlets obtained were successfully hardened on sand-soil-farmyard manure mixture with 85% survival rate. Hardened plantlets developed tubers comparable to the ones obtained in nature grown plants.

INTRODUCTION: *Chlorophytum borivilianum* Santapau & Fernandes (Family: Liliaceae, vern. Safed Musli) is a monsoon herb native to the forests of Rajasthan, Maharashtra, Madhya Pradesh and Gujarat. The plant is highly valued for its dried fasciculated roots that are reported to possess aphrodisiac properties [1]. Besides, the plant is also considered to be a potent energizer and total rejuvenator [2, 3, 4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(5).2040-49

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (5).2040-49

C. borivilianum has a huge market in India and is exported in substantial amounts to many countries in the Gulf, Europe and USA for its use in the production of various herbal products in pharmaceutical, phyto-pharmaceutical and nutraceutical industries. Due to a huge gap in demand and supply, the bulk of industry's demand is met through collection from wild forests, hence threatening its extinction [5]. The restricted distribution and over-exploitation of the plant coupled with low seed set (<20%) and viability and poor seed germination rate has made it rare in the wild [6]. Novel propagation techniques like tissue culture can play an important role in the rapid multiplication of elite clones and germplasm conservation of *C. borivilianum* [7, 8, 9].

Somatic embryogenesis can be defined as the process in which the embryo-like structures are generated from cells other than gametes (i.e. somatic cells) by circumventing the normal fertilization process. Regeneration via somatic embryogenesis is a potential tool for plant tissue cultures and a best known a pathway to induce regeneration from *in vitro* cultures, allowing large-scale propagation of plants, which also helps in germplasm conservation [10].

The process of somatic embryogenesis may be direct or indirect. Indirect somatic embryogenesis involves the de-differentiation of organized tissue into callus prior to embryo production, whereas, direct somatic embryogenesis involves production of embryo from organized tissue without an intervening callus phase [11]. Both somatic and zygotic embryos proceed through a series of identical developmental stages, namely globular, heart, torpedo and cotyledon or plantlet stages for dicotyledons [12] and globular, elongated, scutellar and coleoptilar stages for monocotyledons [13].

An important application of somatic embryos is that it can be utilized for the preparation of artificial seeds or synthetic seeds, which are considered as analogous to the natural seeds. Recently there has been an increased interest in the production and utilization of synthetic seed technology [14, 15, 16, 17]. Synthetic seeds have several advantages, including potential long-term storage, higher scale-up capacity, uniformity in production, potential for automation of the whole production process, seeding of clonal varieties, easy handling and transportation, and may provide a means for maintenance of elite germplasm [18].

The present paper reports high frequency of somatic embryo production in *C. borivilianum* from young shoot bud sprouts and their subsequent synthetic seed production followed by successful *ex-vitro* establishment of the regenerated plantlets.

MATERIALS AND METHODS:

Culture media and growth condition:

The plant material was procured in the form of dry safed musli tubers from Jawaharlal Nehru Agriculture University, Jabalpur (M.P.). The young shoot bud sprouts on the surface of dried tubers during monsoons acted as explants. The explants were first washed in running tap water for 2-3 times and thoroughly washed with 0.01% Labolene (10 min) followed by tap water washing for 2-3 times. The explants were then transferred to laminar air flow hood where they were surface sterilized in 70% ethanol for 5 minutes after which they were washed with distilled water for 2-3 times and then with 0.1% Mercuric chloride (5 min).

Explants were finally rinsed with sterilized distilled water four times and aseptically transferred in the form of explants onto Murashige and Skoog (MS) medium supplemented with 3% (w/v) sucrose, 0.8% (w/v) agar and fortified with different concentrations of PGRs. The pH of the media was adjusted to 5.6 –5.8 before adding agar (8 gm L^{-1}). Media was sterilized at 15 lbs pressure for 15 min by autoclaving. Cultures were incubated at 25 ± 2^0C at photoperiodic cycle of 16 hr light (approx. 1500 lux) and 8 hr dark. All cultures were sub-cultured on the fresh medium at every four week interval. *In vitro* response from each explant was recorded every week.

Somatic embryo induction and development: The young shoot bud explants were inoculated on MS medium supplemented with varying concentrations (0.1, 0.5, 1 and 5 mg L^{-1}) of BAP and KN alone.

After 3-4 weeks on induction media, it was observed that the medium fortified with high concentration of BAP and KN was able to induce embryogenesis effectivly in the young shoot bud sprouts. For the multiplication and growth of somatic embryos, the embryos were next sub-cultured on MS medium supplemented with varying concentrations (0.1, 0.5, 1 and 5 mg L^{-1}) of BAP and KN alone and in combination, whereas, the hormone free medium i.e. basal medium (BM) served as control.

Synthetic seed (synseed) study: The direct somatic embryos (5mm-15mm) obtained in SM were used as the starting material for synthetic seed preparations. The globular embryonic clumps were selected for encapsualtion. The adhering agar as well as necrotic tissues was carefully removed with the help of forceps.

For encapsulation two solutions were prepared.

A. Sodium alginate solution (4 % w/v).

(a) by dissolving sodium alginate in distilled water.

(b) by dissolving sodium alginate in liquid BM (MSM without any PGR).

(c) by dissolving sodium alginate in MSM fortified with BAP (0.5 mg L^{-1})

B. Calcium chloride solution (100 mM):

The solutions were autoclaved for 15 mins. at 121°C temperature and 15 psi pressure. The sterilized solutions were placed in the laminar air flow. The BM was used for the storage of the synthetic seeds and to test their germinability.

The somatic embryos were first immersed in 4% (w/v) sodium alginate gel. They were then dropped in the beaker containing calcium chloride solution. This was done carefully and precisely so as to encapsulate the whole tissue. The flask containing embryos was then placed on the orbital shaker and shaken at 60-80 rpm for 20 mins to complete the complexation process. The calcium chloride solution was later on decanted. The encapsulated embryos were again washed with sterilized double distilled water twice by placing the flask on orbital shaker for 15 min to remove the traces of calcium chloride. The synthetic seeds were finally dried on sterilized filter paper to remove excess water before inoculation on a suitable medium or stored as such at 4^{o}C.

Experimental design, data collection and statistical analysis:

The experiments were set according to completely randomized design. Each experiment consisted of twelve explants per culture tube for each treatment and the procedure was repeated at least thrice. The interaction of hormones on various regeneration parameters such as, frequency of somatic embryogenesis, mean number of somatic embryos was also observed at the end of each subculture. The data was statistically analysed for analysis of variance (ANOVA), and the means were compared at p= 0.05% using Duncan's Multiple Range Test (DMRT) using SPSS Statistical software 20 (IBM Corp.).

Hardening and acclimatization: The plantlets derived both from somatic embryos (Figs. 13 & 14)as well as synthetic seeds were transferred to pots containing autoclaved sand-soil and farmyard mixture [1:1:1], covered with transparent polythenes bearing 6-8 holes (**Fig. 15**) so as to maintain high humidity. Plantlets were irrigated with sterilized distilled water thrice a week. After 10-12 days the polythene covers were removed for an hour each. The exposure time was gradually increased in the following weeks and the polythenes were completely removed after the 6th week (**Figs. 16** & **17**).

Histology:

For histological studies, embryos were fixed in formalin: acetic acid: ethanol (5:5:90 v/v) for 48 h. Tissues were dehydrated stepwise by passing through a graded ethanol series (50, 70, 90 & 100%), followed by embedding in paraffin wax (58-60 °C). Embedded tissues were cut into 10 mm thick sections, using a rotary microtome. The sections were de-waxed and stained with haematoxylin-eosin, mounted with DPX-4 and were observed under microscope.

RESULTS AND DISCUSSION:

Somatic embryo induction and development: The juvenile shoot bud sprouts of *C. borivilianum* cultured on various concentrations of cytokinin (KN, BAP) supplemented medium exhibited embryogenesis after 4 weeks of inoculation from the cut surfaces. KN supplemented medium induced somatic embryogenesis at both 1 mg L^{-1} and 5 mg L^{-1}. On the other hand, BAP at 5 mg L^{-1} showed the highest frequency (80.55) of somatic embryos with an average of 18.44 embryos per explant.

The lower concentrations of BAP, however, failed to induce somatic embryogenesis (**Table 1**). Internal state of explant has been interpreted to be important for somatic embryogenesis by Tisserat and Murashige [19]. There have been a number of reports of the use of young or immature explants in initiating somatic embryogenic response. Higher regenerative capacity of the juvenile explants may

be due to the presence of higher levels of growth promoting substances and absence of inhibitors [20, 21].

TABLE 1: EFFECT OF BAP AND KN ON INDUCTION OF DIRECT SOMATIC EMBRYOGENESIS FROM THE STEM DISC EXPLANTS OF *CHLOROPHYTUM BORIVILIANUM.*

PGRs	Conc. mg L^{-1}	FSE	MSE
Basal Medium (BM)	-	-	-
KN	1.0	30.55 ± 2.26^{a}	4.0 ± 0.09^{a}
KN	5.0	58.33 ± 6.80^{b}	4.7 ± 0.28^{a}
BAP	5.0	80.55 ± 2.26^{c}	18.44 ± 1.37^{b}

For the multiplication and development of direct somatic embryos, BAP proved to be a better cytokinin with the highest number of somatic embryos being obtained at 0.5 mg L^{-1}. This medium supported multiplication of somatic embryos for more than 15 subculture passages (**Fig. 1**) till present. Cytokinins in combination did not produce any significant results. Cytokinins are often regarded as inhibitory to somatic embryogenesis [22, 23].

However, there have been several reports which indicate that this opinion cannot be generalized. In several plant species somatic embryogenesis is induced in the presence of auxin alone or in combination with cytokinins [24, 25, 26, 27] and in a few systems cytokinins alone were found to be effective in somatic embryogenesis [28, 29]. In this study it was found that the young shoot buds induced direct somatic embryogenesis at high BAP concentration (5 mg L-^{1}) while its lower conc. (BAP 0.5 mg L-^{1}) led to more prominent somatic embryo proliferation (**Figs. 3-6**). This finding is in harmony with the results of a similar study in *Phosphocarpus tetragonolobus* [30] *Arachis hypogea* [31] and *Zingiber officinale* [32]. Cytokinins are known for their role in shoot formation. It also stimulates the shoot pole development but the plantlets are found to exhibit stagnation in growth, also the roots remain short and slowly degenerate [33]. Similar pattern has been observed with the plant in question. In the present study, consequently during the span of fifteen subculture passages of direct somatic embryos on MS supplemented with 0.5 mg L-^{1} of BAP the rooting ceased to occur after the fifth subculture passage (**Fig. 1**).

TABLE 2: EFFECT OF BAP AND KN ALONE AND IN COMBINATION ON MULTIPLICATION AND PROLIFERATION OF DIRECT SOMATIC EMBRYOS OF *CHLOROPHYTUM BORIVILIANUM*

PGRs	Conc. mg L^{-1}	FSE	MSE
Basal Medium (BM)		86.08 ± 2.26^{fg}	7.80 ± 0.65^{def}
KN	0.1	49.96 ± 3.96^{b}	1.47 ± 0.14^{a}
	0.5	55.53 ± 2.27^{bc}	2.16 ± 0.2^{ab}
	1.0	61.06 ± 2.27^{bcd}	4.77 ± 0.19^{abcd}
	5.0	66.60 ± 0^{cde}	5.22 ± 1.50^{bcd}
BAP	0.1	63.83 ± 2.27^{bcd}	7.47 ± 0.61^{de}
	0.5	**91.64 ± 3.93^{g}**	**19.45 ± 2.15^{i}**
	1.0	69.40 ± 2.30^{cde}	11.0 ± 1.87^{fg}
	5.0	94.40 ± 2.20^{g}	13.36 ± 0.91^{g}
BAP+KN	0.1+0.1	27.76 ± 2.26^{a}	1.80 ± 0.19^{a}
	0.1+0.5	30.53 ± 2.26^{a}	2.30 ± 0.13^{ab}
	0.1+1.0	91.63 ± 4.10^{g}	11.41 ± 1.05^{h}
	0.1+5.0	61.10 ± 6.0^{bcd}	10.72 ± 0.93^{fg}
	0.5+0.1	72.21 ± 6.01^{def}	3.75 ± 0.37^{abc}
	0.5+0.5	69.44 ± 8.18^{cde}	9.11 ± 0.67^{ef}
	0.5+1.0	63.87 ± 8.19^{bcd}	6.61 ± 0.39^{cde}
	0.5+5.0	80.55 ± 2.27^{efg}	6.61 ± 0.61^{cde}
	1.0+0.1	61.06 ± 2.26^{bcd}	3.25 ± 0.14^{ab}
	1.0+0.5	72.20 ± 2.28^{def}	7.94 ± 0.54^{def}

The values represent the means (±SE) of three independent experiments. At least 12 cultures were raised for each experiment. Mean values within a column followed by the same letter are not significantly different by Duncan's multiple range test (P=0.05%)

The basal medium (BM) was also found to enhance the conversion of the direct somatic embryos into mature plantlets with well- developed shoot and root system and supported tuber formation (**Table 2**). Suprasanna *et al.*[34] also reported good shooting and rooting in *Oryza sativa* embryos after subculturing to BM (**Fig.7-9**). Similarily, in *Epipremnum aureum*, shoots and roots developed on MS medium with no growth regulators [35].

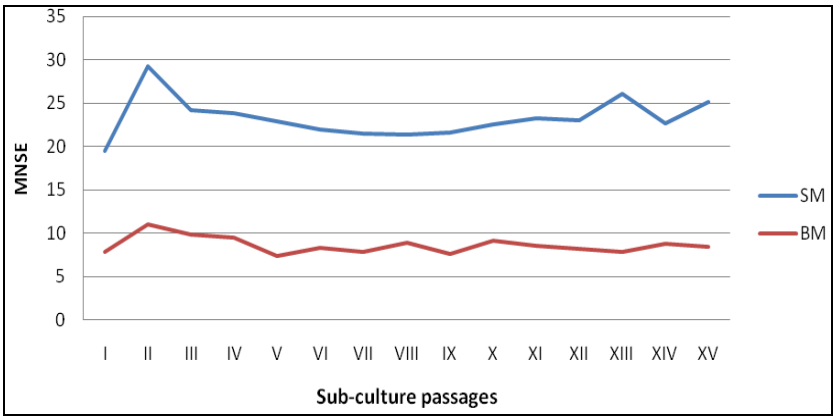

FIG. 1: EFFECT OF SM AND BM ON MEAN NUMBER OF SOMATIC EMBRYOS (MNSE) IN THE SOMATIC EMBRYOS OF *CHLOROPHYTUM BORIVILIANUM* TILL FIFTEEN SUB-CULTURE PASSAGES

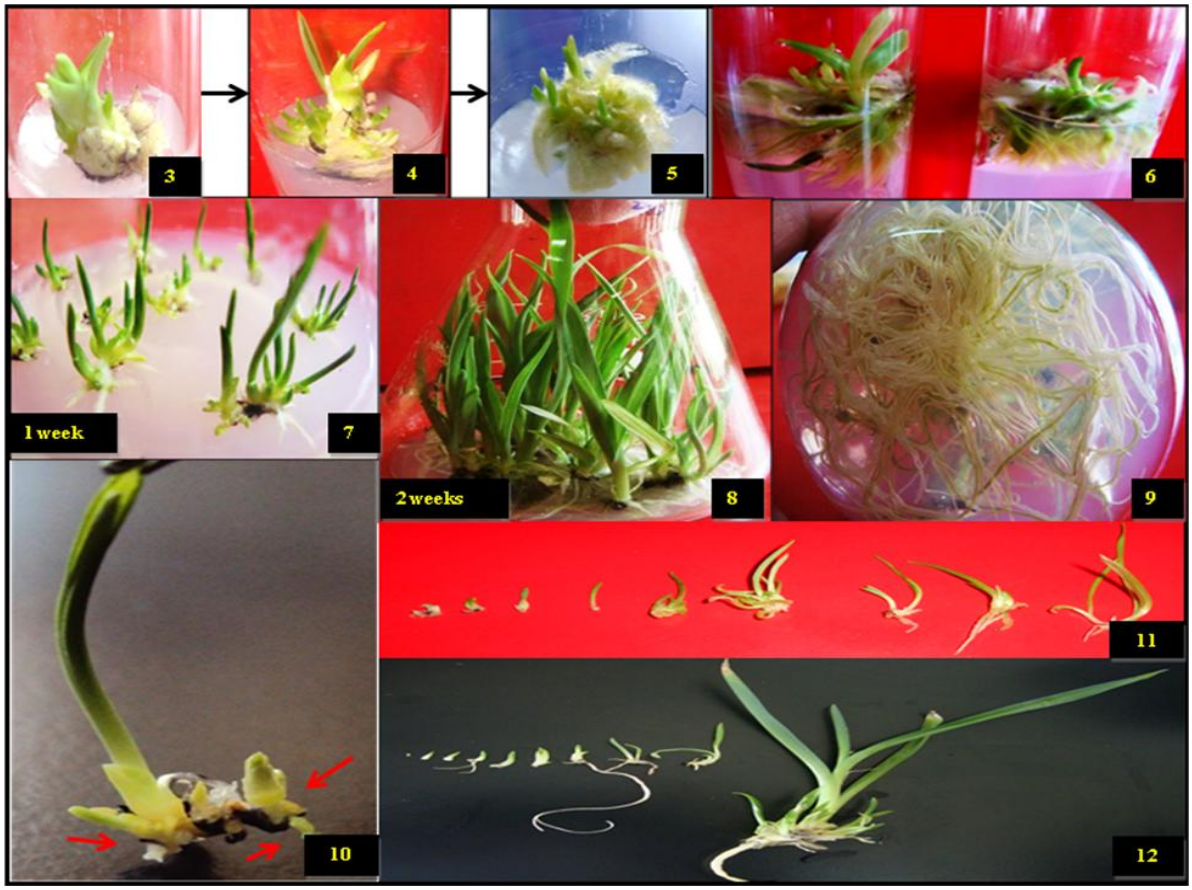

FIG. 3: SWELLING IN THE YOUNG SHOOT BUDS OF *CHLOROPHYTUM BORIVILIANUM* ON BAP (5 mg L^{-1}) SUPPLEMENTED MSM
FIG. 4: FORMATION OF NUMEROUS OFF-SHOOTS ON THE MOTHER EXPLANT ON BAP (5 mg L^{-1}) (2-3 weeks).
FIGS 5& 6:FORMATION OF HAIRY ROOTS UPON FIRST SUB-CULTURE ON BAP (5 mg L^{-1}) (A WEEK AFTER 1ST SUB-CULTURE).
FIGS7& 8: GROWTH AND MULTIPLICATION OF SOMATIC EMBRYOS ON BASAL MEDIUN (BM).
FIG . 9: PROLIFERATING ROOTS OF SOMATIC EMBRYOS UPON FIRST SUB-CULTURE ON BM (2 WEEKS).
FIG. 10: FORMATION OF SOMATIC EMBRYOS (ARROWS) DIRECTLY ON THE EXPLANT SURFACE.
FIGS. 11 & 12 : SEPARATED SOMATIC EMBRYOS ARRANGED IN ASCENDING ORDER.

Synthetic seed study:

Encapsulation of somatic embryos (<5 mm) of *C. borivilianum* resulted in the formation of well-formed synthetic seeds (**Fig.27-29**). To study and optimize their germination, the somatic embryos were gelled in matrices of different composition viz. distilled water (DW), basal medium (BM) and selected medium (SM) and inoculated on to the basal medium (BM). During germination, the synthetic seeds turned light green within a week of inoculation. Seed coat breakage and germination of synthetic seeds were initiated with the emergence of the tap roots which were visible within 1-2 weeks of culture on basal medium (**Fig. 30-32**). Out of the three types of gel matrices used, the SM produced highest frequency of germination (86.1%) when inoculated fresh onto the BM.

In the present study synthetic seed formation was aimed at mass production with a potential of long term storage. Sodium alginate (4%) was used for encapsulation, since, it is considered to be a superior matrix for encapsulation compared to agar. It can be attributed to the difference in permeability of alginate and agar, the former proving better as a protector of the explant than the latter. Sodium alginate has an advantage of being soluble at room temperature and readily forming a gel with $CaCl_2$ [36]. Purohit and Joshi [37] had reported *in vitro* propagation using encapsulated shoot buds. Similar results have also been obtained in mulberry where 4 % sodium alginate formed a clear, uniform capsule during encapsulation and 4% sodium alginate in tea (*Camellia sinensis*) produced artificial seeds having smooth texture appropriate for sprouting [38]. After their storage for 15 days at 4°C, synthetic seeds formed using distilled water as matrix failed to germinate. While those prepared using the basal medium and selected medium showed a germination frequency of 55 % and 70 % respectively (**Fig. 2).**

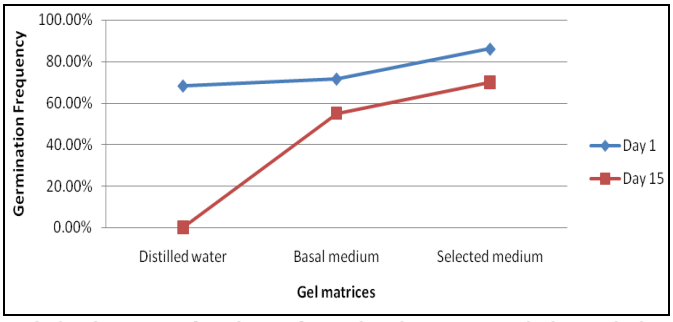

FIG 2: GERMINATION RATE OF SYNTHETIC SEEDS ON BASAL MEDIUM.

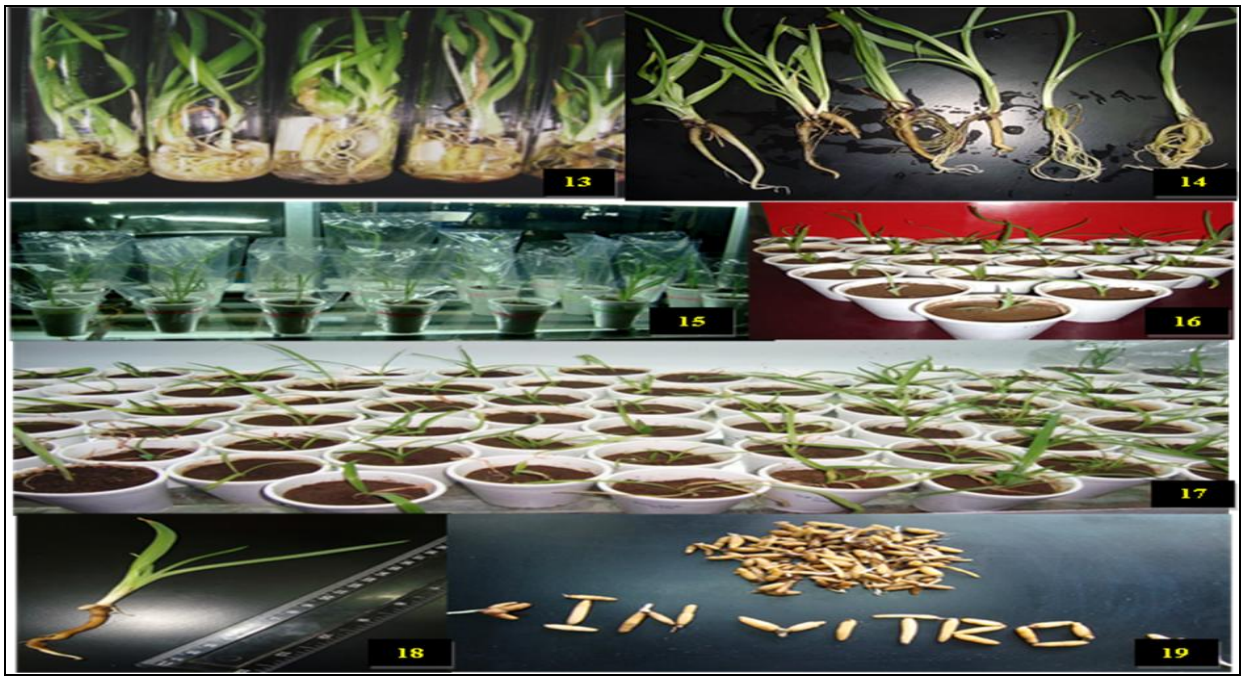

FIG. 13 & 14: PLANTLETS READY FOR HARDENING.
FIG. 15: PLANTLETS TRANSFERRED TO SAND: SOIL: FYM (1:1:1) MIXTURE AND COVERED WITH PERFORATED POLYTHENES. (15 DAYS).
FIG. 16: INTERMITTENT REMOVAL OF POLYTHENE COVERS (15-22 DAYS).
FIG. 17: COMPLETE REMOVAL OF POLYTHENE COVERS AFTER SUCCESSFUL HARDENING (15-22 DAYS).
FIG.18: A THREE MONTH OLD HARDENED PLANT BEARING A WELL-DEVELOPED TUBER.
FIG.19 - HARVESTED TUBERS FROM THE HARDENED PLANTS OF *C. BORIVILIANUM*.

Hardening and acclimatization:

Hardening and acclimatization of the *in vitro* grown plantlets is of prime importance for their survival and field establishment. Micropropagated plants often do not survive acclimatization or they resume growth only a few days after soil transfer due to sub-optimal conditions during the preceeding stages of multiplication, rooting and acclimatization [39, 40, 41]. The plantlets undergo morphological and physiological adaptations enabling them to survive in the natural environment [9]. Tissue culture-based propagation techniques in *C. borivilianum* using various explants in solid and liquid media [42, 43] reported so far have met with limited success because of high mortality at transplantation stage due low establishment in soil, cytological instability and early loss of regeneration potential of the *in vitro* cultures.

Contrary to these reports the regenerated plantlets of *C. borivilianum* in the present case were successfully established in soil with 85% survival rate. The plants regenerated *in vitro* exhibited morphology similar to the naturally propagated ones. Besides, the plantlets developed tubers comparable to the ones obtained in nature (**Fig. 18 &19)**.

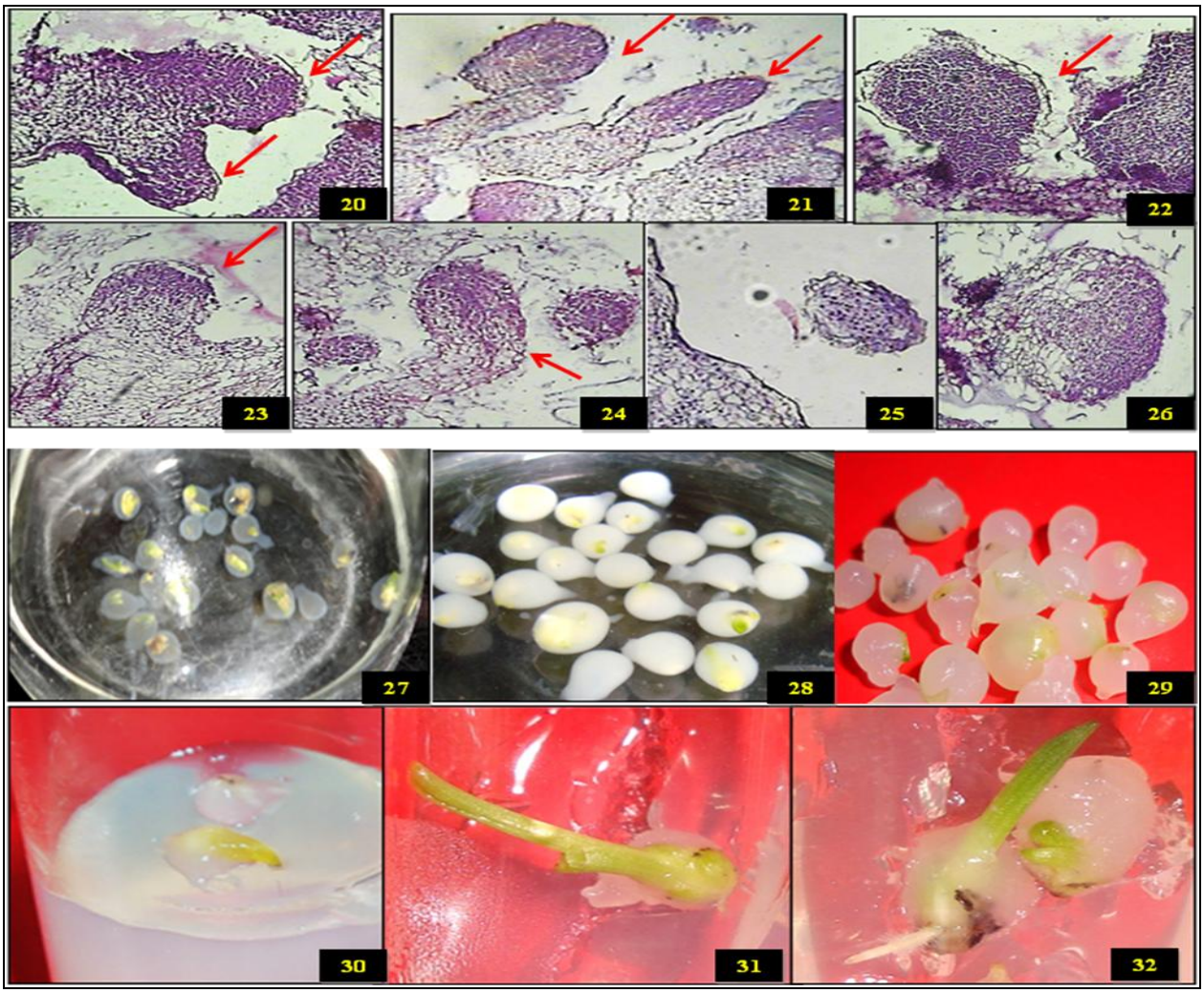

FIG 20 – 24 : GLOBULAR SOMATIC EMBRYOS OF *CHLOROPHYTUM BORIVILIANUM* SHOWING DENSELY STAINED MERISTEMATIC CELLS JOINED TO THE MATERNAL TISSUE THROUGH SUSPENSOR CELL (ARROWS) (400X).
FIG. 25: DETACHED GLOBULAR SOMATIC EMBRYOS OF *CHLOROPHYTUM BORIVILIANUM* (400X).
FIG.26: PROTRUBERANCES ON THE SUB-EPIDERMAL LAYER LEADING TO THE FORMATION OF GLOBULAR SOMATIC EMBRYOS (100X).
FIG. 27:ENCAPSULATED SOMATIC EMBRYOS BEFORE COMPLETE COMPLEXATION WITH $CaCl_2$ (100MM).
FIGS. 28 & 29: ENCAPSULATED SOMATIC EMBRYOS AFTER COMPLETE COMPLEXATION WITH $CaCl_2$ (100MM).
FIG. 30 : GERMINATION OF SYNTHETIC SEED ON BM WITH SM AS GEL MATRIX (5 DAYS).
FIG. 31: ELONGATION OF SYNTHETIC SEED.
FIG. 32: ROOT FORMATION IN THE SYNTHETIC SEED ON BM (8-9 DAYS).

Histological study: Histology of the embryogenic tissue of *Chlorophytum borivilianum* in the present study confirmed that the induction of the development process was embryogenic and not organogenic in nature. The presence of suspensor like stalk could be seen in the early stages similar to those found in zygotic embryos. Embryogenic regions arised from the mitotically more active sub-epidermal layer through the proliferation of fast dividing cells (**Fig. 20-24**).

Additional evidence of the acquisition of embryogenic competence by somatic cells was the formation of a typical meristematic zone in the somatic embryos of the plant under study. Late-globular stage somatic embryos without vascular connection with the parental tissue were also observed in the histological sections culture (**Fig. 25 & 26**). The presence of these globular embryo clusters suggests the occurrence of secondary embryogenesis in *C. borivilianum.* Rapid mitotic division, small size, dense cytoplasm, a large nucleus with prominent nucleolus, small vacuoles and abundant starch deposits are common attributes of embryogenic cells [44]. These embryogenic characteristics were also reported for *Coffea arabica* [45], *Cocos nucifera* [46], *Araucaria angustifolia* [47], *Bactris gasipaes* [48].

CONCLUSIONS: The present paper therefore reports a high frequency of direct somatic embryogenesis from the young shoot buds of *Chlorophytum borivilianum***.** Regeneration through direct somatic embryogenesis occurred from the young shoot bud explants with BAP (5.0 mg L^{-1}). Once induced much lower concentration of BAP (0.5 mg L^{-1}) was required for further growth. The conversion of somatic embryos with high MRN and MRL was favoured on BM. It took aound 3-4 months of culture to obtain a complete plantlet from the direct somatic embryos.

The production and encapsulation of somatic embryos allows mass propagation as well as having a potential for automation of the whole production process for elite genotypes of *C. borivilianum* by *in vitro* techniques. Out of the synthetic seeds prepared from the somatic embryos (direct) using three types of gel matrices viz. DW, BM and SM, the ones formed using SM resulted in the highest frequency of germination (86.1%).

C. borivilianum is used as a chief ingredient in over a hundred Ayurvedic formulations, as a rejuvenator, vitalizer and health-tonic, a curative for pre-natal and post-natal problems, a restorative for immunity-improvement and as a remedy for diabetes and arthritis and as a potent aphrodisiac. Extensive research on this plant will thus provide new avenues to the pharmaceutical industries involved in manufacture of herbal products.

ACKNOWLEDGEMENT: The senior author acknowledges the financial assistance provide by the M. P. Council of Science and Technology, Bhopal, M.P., India.

REFERENCES:

1. Kirtikar KR and Basu BD: Liliaceae: *Chlorophytum*. Indian medicinal plants 1975; 2508-2509.
2. Govindrajan R, Sreevidya N, Vijaykumar M, Thakur M and Dixit VK: In vitro antioxidant activity of ethanolic extract of *Chlorophytum borivilianum*. Natural Product Science 2005; 11: 165-169.
3. Visavadiya NP, Narasimhacharya AVRL: Ameliorative effect of *Chlorophytum borivilianum* root on lipid metabolism in hyperlipaemic rats. Clinical and Experimental Pharmacology and Physiology 2007; 34(3):244–249
4. Thakur M, Chauhan NS, Bhargava S and Dixit VK: A comparative study on aphrodisiac activity of some Ayurvedic herbs in male albino rats. Archives of Sexual Behavior 2009; 38:1009–15.
5. Rizvi MZ, Kukreja AK and Knanuja SPS: In vitro culture of *Chlorophytum borivilianum* Sant. et. Fernand. in liquid culture medium as a cost effective measure. Current Science 2007; 92(1):87-90.
6. Biswas S, Jain S and Pal M: Research needs and priorities for conservation of Indian medicinal flora. The Indian Forester 2003; 129: 85-92.
7. Dave A, Joshi N and Purohit SD: In vitro propagation of *Chlorophytum borivilianum* using encapsulated shoot buds. European Journal of Horticultural Science 2004; 69 (1): 37-42.
8. Kothari SK: Safed musli (*Chlorophytum borivilianum*) revisited. Journal of Medicinal and Aromatic Plant Science 2004; 26: 60-63.
9. Jha A and Bansal YK: Induction of photoautotrophy in *Chlorophytum borivilianum* Sant. et Fernand, regenerated in vitro. Journal of Phytology 2012; 4(1):26-31.
10. Jimenez VM: Regulation of in vitro somatic embryogenesis with emphasis on the role of endogenous hormones. Revista Brasileira de Fisiologia Vegetal 2012; 13(2): 196-223.
11. Slater A, Scott NW and Fowler MR: Plant Biotechnology Oxford University Press, England 2003.
12. Mandal AKA and Gupta SD: Direct somatic embryogenesis of safflower-a scanning electron microscopic study. Current Science 2002; 83:1138-1140.

13. Godbole S, Sood A, Thakur R, Sharma M and Ahuja PS: Somatic embryogenesis and it's conversion into plantlets in a multipurpose bamboo, *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro. Current Science 2002; 83:885-889.
14. Ganapathi TR, Srinivas L, Suprasanna P and Bapat VA: Regeneration of plants from alginate-encapsulated somatic embryos of banana cv. Rasthali (*Musa* spp. AAB group). In Vitro Cellular and Developmental Biology of Plant 2001; 37:178–181.
15. Nyende A B, Schittenhelm S, Mix-Wagner G and Greef JM: Production, storability and regeneration of shoot tips of potato (*Solanum tuberosum* L.) encapsulated in calcium alginate hollow beads. In Vitro Cellular and Developmental Biology of Plant 2003; 39: 540–544.
16. Nyende AB, Schittenhelm S, Mix-Wagner G and Greef JM: Yield and canopy development of field grown potato plants derived from synthetic seeds. European Journal of Agronomy 2005; 22:175–184.
17. Huda A, Bari MA and Rahman M: Asexual propagation of eggplant (*Solanum melongena* L.) through encapsulated axillary buds. Plant Tissue Culture and Biotechnology 2009; 19:263-288.
18. Singh B, Sharma S, Rani G, Virk GS, Zaidi AA, Nagpal A: In vitro response of encapsulated and non-encapsulated embryos of Kinnow mandarin (*Citrus nobilis* Lour x *C. deliciosa* Tenora). Plant Biotechnol Report 2007; 1:101-107.
19. Tisserat B and Murashige T: Repression of asexual embryogenesis *in vitro* by some plant growth regulators. In Vitro 1977; 13: 799-805.
20. Gugsa L and Kumlehn J: Somatic Embryogenesis and Massive Shoot Regeneration from Immature Embryo Explants of Tef. Biotechnology Research International 2011; Article ID 309731, 7 pages doi:10.4061/2011/309731.
21. Yang J, Wu S, and Li C: High efficiency secondary somatic embryogenesis in *Hovenia dulcis* Thunb. through solid and liquid cultures. The Scientific World Journal 2013; Article ID 718754, doi:10.1155/2013/718754
22. Rugini E: Somatic embryogenesis in Olive (*Olea europaea* L.) In: Jain S, Gupta P and Newton R (eds) Vol.2 Kluwer Academic Publishers, The Netherlands 1995: pp.171-189.
23. Wongtiem P, Courtois D, Florin B, Juchaux M, Peltier D, Broun P and Ducos JP: Effects of cytokinins on secondary somaticembryogenesis of selected clone Rayong 9 of Manihot esculenta Crantz for ethanol production. African Journal of Biotechnology 2011; 10(9), 1600-1608.
24. Gaj MD: Factors influencing somatic embryogenesis induction and plant regeneration with particular reference to *Arabidopsis thaliana* (L.) Heynh. Plant Growth Regulation 2004; 43: 27–47.
25. Paul S, Dam A, Bhattacharya A and Bandhopadhyay TK: An efficient regeneration system via direct and indirect somatic embryogenesis for the medicinal tree *Murraya koenigii*. Plant Cell Tissue and Organ Culture 2011; 105: 271–283.
26. Pinto DL P, de Almeida AMR, Rego MM, da Silva ML, de Oliveira EJ and Otoni WC: Somatic embryogenesis from mature zygotic embryos of commercial passion fruit (*Passiflora edulis* Sims) genotypes. Plant Cell Tissue and Organ Culture 2011; 107: 521–530
27. Bao Y, Liu G, Shi X, Xing W, Ning G, Liu J and Bao M: Primary and repetitive secondary somatic embryogenesis in *Rosa hybrid* 'Samantha'. Plant Cell Tissue and Organ Culture 2012; 109: 411–418.
28. Nanda RM and Rout GR: In vitro somatic embryogenesis and plant regeneration in *Acacia arabica*. Plant Cell Tissue and Organ Culture 2003; 73: 131–135.
29. Carra A, De Pasquale F, Ricci A and Carimi F: Diphenylurea derivatives induce somatic embyrogenesis in *Citrus*. Plant Cell Tissue and Organ Culture 2006; 87:41–48
30. Dutta Gupta S, Ahmed R and De DN: Direct somatic embryogenesis and plantlet regeneration from seedling leaves of winged beans, *Psophocarpus tetragonolobus* (L.) D. C. Plant Cell Reports 1997; 16: 628-631.
31. Venkatchalam P, Kavikishor PB, Geetha N, Thangavelu M and Jayabalan N: A rapid protocol for embryogenesis from immature leaflets of ground nut (*Arachis hypogea* L.) In Vitro Cellular and Developmental Biology of Plant 1999; 35: 409-412.
32. Guo Y and Zhang Z: Establishment and plant regeneration of somatic embryogenic cell suspension cultures of the *Zingiber officinale* Rosc. Scientia Horticulturae 2005; 107:90–96.
33. Godbole S, Sood A, Thakur R, Sharma M and Ahuja PS: Somatic embryogenesis and it's conversion into plantlets in a multipurpose bamboo, *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro. Current Science 2002; 83:885-889.
34. Suprasanna P, Ganapathi, TR, Rao PS: Establishment of embryogenic callus, somatic embryos and plant regeneration in Indica rice. Journal of Genetics and Breeding 1995; 49: 9-14
35. Zhang Q, Chen J and Henny RJ: Direct somatic embryogenesis and plant regeneration from leaf, petiole, and stem explants of Golden Pothos. Plant Cell Reports 2005; 23:587–595.
36. Piccioni E and Standardi A: Encapsulation of micropropagation of buds of six wood species. Plant Cell Tissue and Organ Culture 1995; 42: 221-226.
37. Purohit SD and Joshi N: *In vitro* propagation of C*hlorophytum borivilianum* using encapsulated shoot buds. European Journal of Horticultural Science 2004; 69(1): 37-42.
38. Mondal TK, Bhattacharya A, Sood A and Ahuja PS (2002) Factors affecting germination and conversion frequency of somatic embryos of Tea (*Camellia sinensis* (L.) O. Kuntze. Journal of Plant Physiology 2002; 159: 1317–1321.
39. Preece JE and Sutter EG: Acclimatization of micropropagated plants to the greenhouse and field. In: Debergh PC and Zimmerman RH (eds) Micropropagation, Kluwer Academic publishers, Netherlands 1991;.71-93.
40. Pospisilova J, Solarova J and Catsky J: Photosynthetic response to stress during in vitro cultivation. Photosynthetica: 1992; 26: 3-18.
41. Kozai T and Smith MAL: Environmental control in plant tissue culture - general introduction and overview. In: Aitken-Christie J, Kozai T and Smith MAL (Eds.), Automation and Environmental Control in Plant Tissue Culture, Kluwer Academic Publishers, Dordrecht - Boston – London, 1995; pp. 301-318.
42. Purohit SD, Dave A and Bilochi G: Scaling up production and field performance of micropropagated medicinal herb 'safed musli (*Chlorophytum borivilianum*). In Vitro Cellular and Developmental Biology of Plant 2003; 39: 419-424.
43. Rizvi MZ, Kukreja AK and Knanuja S P S: *In vitro* culture of *Chlorophytum borivilianum* Sant. et Fernand. in liquid culture medium as a cost effective measure. Current Science: 2007 92(1): 87-90.

44. Maheshwaran G and Williams EG: Origin and immature embryos of *Trifolium repens* in vitro. Annals of Botany 1985; 56: 619-630.
45. Quiroz FFR, Fuentes CFJ, Rojas HR and Loyola VM: Histological studies on the developmental stages and differentiation of two different somatic embryogenesis systems of *Coffea arabica*. Plant Cell Reports 2002; 20(12): 1141-1149.
46. Fernando JA, Vieira MLC, Machado SR and Appezzato-da-Gloria B: New insights into the *in vitro* organogenesis process: the case of *Passiflora.* Plant Cell Tissue and Organ Culture 2007; 91:37–44.
47. Steiner N, Vieira FN, Maldonado S and Guerra MP: Carbon source affects morphogenesis and histo-differentiation of *A. augustifolia* embryogenic cultures. Brazilian Archives of Biology and Technology 2005; 48:895–903.
48. Steinmacher DA, Cangahuala-Inocente GC, Clement CR and Guerra MP: Somatic embryogenesis from peach palm zygotic embryos. In Vitro Cellular and Development Biology of Plant 2007; 43:124–132.

How to cite this article:
Jha A and Bansal YK: High Frequency Direct Somatic Embryogenesis of the Juvenile Shoot Buds of *Chlorophytum Borivilianum* Sant. Et Fernand and Production of Artificial Seeds. Int J Pharm Sci Res 2016; 7(5): 2040-49.doi: 10.13040/IJPSR.0975-8232.7(5).2040-49