

***In vitro* culture of *Chlorophytum borivilianum* Sant. et Fernand. in liquid culture medium as a cost-effective measure**

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***Chlorophytum borivilianum*, commonly known as safed musli is an endangered medicinal herb valued for its dried, fasciculated storage roots which possess immunomodulatory and aphrodisiac properties that forms an important ingredient of herbal tonics. Efficient and cost-effective micropropagation of *C. borivilianum* employing liquid medium has been achieved. In the case of employing liquid medium; while on the one hand lesser amount of medium is consumed, on the other hand, shoot growth and multiplication response were better than in solid medium. About 7.5-fold increase in shoot multiplication could be obtained in liquid medium against 4.5-fold on agar solidified medium. Use of liquid culture medium resulted up to 92.31% reduction in single shoot production cost compared to solid medium.**

Keywords: *Chlorophytum borivilianum*, liquid medium, multiple shoots, safed musli.

TUBEROUS roots of *Chlorophytum borivilianum* (commonly known as safed musli) (family Liliaceae) possess immunomodulatory and adaptogenic properties and are used to cure impotency, sterility and enhance male potency. The main active principles of roots, saponins are stimulants and metabolic enhancers and have been shown to possess anti-tumour activity^{1,2}. The extract of dried root tubers of *C. borivilianum* acts as psychostimulant and has a beneficial effect on the brain and human body by increasing alertness, mental ability, intelligence and sexual characters. Due to its therapeutic activity and diversified uses, demand for *C. borivilianum* is increasing in Indian and the international market. Its seeds have poor germination percentage (11–24%), low viability and long dormancy period. Safed musli is propagated vegetatively by fleshy tuberous roots bearing shoot buds. Due to large-scale and indiscriminate collection of its roots for gainful trade and insufficient attempts either to allow its replenishment or its cultivation, *C. borivilianum* has been enlisted in the list of National Medicinal Plant Board as one of the prioritized plant species. There is need for commercial cultivation of this species. Micropropagation technology is advantageous

due to production of high-quality disease-free, true-to-type plants independent of seasonal and other environmental conditions in a comparatively smaller space³, but higher cost of plant production has always limited the use and exploitation of this technique at industrial level⁴. To overcome this limitation, cost-reduction strategies have been employed⁵. Micro-propagation of *C. borivilianum* on solid medium has been reported earlier^{6,7}. For scale-up cultures using bioreactors, the use of liquid culture medium has been recommended^{8–10}.

The physical state of the culture medium and its composition affect the *in vitro* growth of plants to a great extent. The most commonly used gelling agent—agar (adding up to 65% of the cost of the culture medium) results in local accumulation of heat and hinders the access of dissolved oxygen to the cultured cells¹¹. By employing liquid culture medium, reduction in plant production cost can be achieved^{12,13}. The advantages of liquid culture medium for enhancing shoot proliferation and growth have been reported in several plant species^{14–16}. Liquid cultures are generally more desirable than solid support medium because of higher growth rates resulting from high medium to tissue contact, and can be employed in bioreactors for large-scale multiplication of plants. The response of cultured tissue to media manipulation and selection pressure is also more rapid.

In the present study shoot growth and multiplication response were compared on solid and liquid culture medium for achieving a cost-effective method of multiple shoot production of *C. borivilianum*.

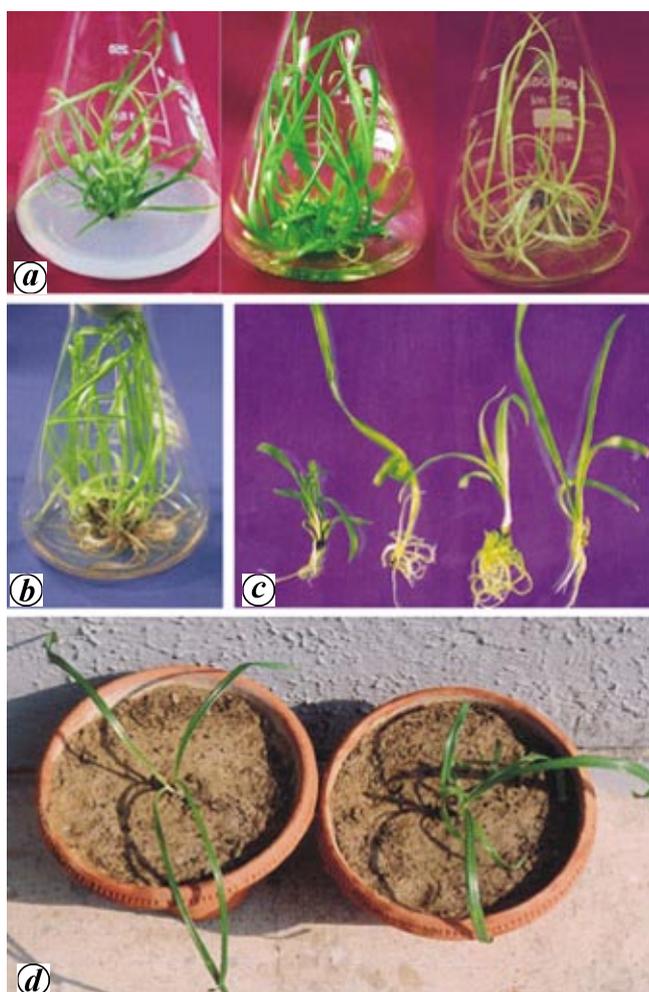
Plants of *C. borivilianum* collected from natural habitat in Rajasthan were maintained in CIMAP botanical garden. *In vitro* cultures were established from young shoot apices obtained from the tuberous roots of these field-grown plants. Shoots grew in clusters from these cultured shoot apices. Each shoot of a cluster was separated and trimmed from the top leaving behind about 1–1.5 cm portion along with shoot apex. Such prepared explants were used throughout the study. Murashige and Skoog¹⁷ (MS) basal medium supplemented with 3% w/v sucrose, 22.2 μM 6-benzylaminopurine (BAP) and with 0.8% w/v agar or without agar (liquid medium) was used. In our earlier experiments, amongst a wide range of BAP (2.2–40.0 μM) used to test the efficacy on *in vitro* shoot multiplication, BAP at 22.2 μM level exhibited optimal response. Next, 55 ml of solid or 30 ml of liquid medium was dispensed in 250 ml conical Erlenmeyer culture flask. The pH of the medium was adjusted to 5.80 ± 0.1 using 0.10 N HCl and/or 0.10 N NaOH prior to autoclaving at 121°C temperature and 15 lb pressure for 20 min. Two explants (inoculum density) were inoculated per culture flask. All aseptic cultures were maintained under 16 h photoperiod at $25 \pm 2^\circ\text{C}$ temperature. The liquid cultures were incubated in agitated (70 rpm) as well as static conditions. Observations were recorded after 40 days of culture period. For root induction in *in vitro* regenerated shoots, three-fourths strength MS liquid medium was

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Table 1. Effect of physical state of medium on growth and shoot multiplication of *in vitro* cultured shoots of *Chlorophytum borivilium*

Growth parameter(s)	Physical state of culture medium		
	Solid	Static liquid	Agitated liquid
No. of shoots/flask	9.00* ± 1.00	8.20 ± 1.30	15.00 ± 1.22
No. of shoots having different lengths (cm)			
0.00–3.00	5.00	1.40	1.60
3.10–6.00	3.00	4.20	4.00
6.10–9.00	1.00	2.60	7.00
9.10–12.00	0.00	0.00	2.40
Shoot length (cm)	2.80 ± 0.42	4.94 ± 0.17	6.50 ± 0.79

*Average value ± SE (standard error).

**Figure 1.** *In vitro* shoot growth and multiplication in *C. borivilium*: (a) solid (control), liquid agitated and liquid static (from left to right); (b–c) rooting in shoots regenerated in agitated liquid medium; and (d) *In vitro* raised *C. borivilium* plants in earthen pots after 8 weeks.

supplemented with 9.8 μM indole-3-butyric acid (IBA). All experiments were repeated twice. Observations are mean of five replicates per treatment.

The observations revealed that liquid culture supported better shoot multiplication and growth of regenerated shoots than solid medium (Table 1). An average of 15 shoots per flask (7.5-fold increase over two shoots/flask inoculum density) could be obtained in liquid medium against nine shoots (4.5-fold increase) on agar solidified medium during the same culture duration. Earlier Purohit *et al.*⁶ had shown that 22.2 μM BAP concentration in culture medium was best for shoot multiplication and reported fourfold increase in shoot multiplication rate. Similarly, Dave *et al.*⁷ have reported only 3.5-fold increase in shoot multiplication on BDH agar-gelled MS medium containing 22.2 μM BAP, while other gelling agents did not perform as well for shoot multiplication. In the present study the average length of shoots recorded on liquid medium was 6.50 cm against 2.80 cm on solid medium. Thus, liquid culture medium not only supported multiplication of shoots but shoot growth was also better compared to solid medium (Figure 1a). Agitated liquid culture supported better shoot growth and multiplication than static liquid culture (Table 1).

In another experiment, the effect of different ratios of inoculum density and volume of culture medium on shoot growth and multiplication of *C. borivilium* revealed that two shoots/culture flask inoculum density resulted in about 7.0- to 7.5-fold increase in total number of shoots per flask in 30 to 50 ml medium (Table 2). Use of 30 ml of culture medium/flask was optimal volume for multiplication of shoots, resulting in 7.5-fold increase in shoot number/flask. Higher inoculum density exhibited only 2–5-fold increase in shoot multiplication. It was observed that when inoculum density increased beyond six shoots per culture flask, percentage response decreased thereby reducing the total number of shoots produced per culture flask. Thus, only 30 ml liquid medium per 250 ml Erlenmeyer flask is required against 55 ml solid medium per 250 ml Erlenmeyer flask, where only nine shoots/flask could be obtained after 40 days of culture duration. In this way, 33 and 18 culture flasks per litre of culture medium can be prepared employing liquid and solid culture medium respectively, which will ultimately result in more number

Table 2. Effect of inoculum density vs volume of culture medium on *in vitro* shoot growth and multiplication of *C. borivilianum*

Inoculum density [†]	Growth parameter	Volume of medium used in culture flask (ml)				
		10	20	30	40	50
2	No. of shoots/flask	2.00* ± 0.00	7.60 ± 0.89	15.00 ± 1.22	15.40 ± 1.67	14.40 ± 1.14
	Shoot length (cm)	0.94 ± 0.08	3.59 ± 0.30	6.50 ± 0.79	3.22 ± 0.61	4.54 ± 0.60
4	No. of shoots/flask	2.40 ± 0.54	8.00 ± 1.00	19.80 ± 2.16	14.00 ± 2.00	20.60 ± 2.30
	Shoot length (cm)	0.82 ± 0.13	5.01 ± 0.52	3.69 ± 0.28	4.36 ± 0.64	3.70 ± 0.73
6	No. of shoots/flask	3.80 ± 0.83	10.60 ± 1.51	14.40 ± 1.67	16.80 ± 1.92	23.00 ± 2.44
	Shoot length (cm)	1.27 ± 0.25	3.40 ± 0.63	4.73 ± 0.77	5.78 ± 0.69	5.02 ± 0.64
8	No. of shoots/flask	4.00 ± 0.70	10.00 ± 1.58	14.40 ± 1.67	17.00 ± 1.58	21.00 ± 2.12
	Shoot length (cm)	1.20 ± 0.15	3.50 ± 0.38	5.00 ± 0.52	5.20 ± 0.80	5.40 ± 0.56
10	No. of shoots/flask	3.00 ± 0.70	11.00 ± 1.58	14.00 ± 1.14	16.00 ± 1.67	18.00 ± 1.67
	Shoot length (cm)	0.85 ± 0.07	3.50 ± 0.48	4.60 ± 0.40	5.00 ± 0.53	5.10 ± 0.54

[†]No of shoots inoculated; *Average value ± SE (standard error).

Table 3. Comparative cost of shoot production in *C. borivilianum* on solid and liquid media

Cost analysis	Physical state of medium		
	Solid	Liquid (agitated)	Per cent cost reduction
Cost of 1 l medium* (Rs)	41.88	7.56	81.95
Volume of medium used/flask (ml)	55.00	30.00	–
Cost of medium/flask (Rs)	2.30	0.23	90.00
Approximate cost of single shoot (Rs)	0.26	0.02	92.31

*Cost of chemical ingredients (Hi-Media-make according to price list 2004–05) used according to Mura-shige and Skoog¹⁷ for preparation of 1 l MS basal culture medium.

of shoots thereby resulting in lower cost of production in liquid culture medium.

Amongst various (0.2–24.6 µM) levels of IBA added to the basal liquid culture medium, *in vitro*-regenerated shoots exhibited optimal root induction and growth in three-fourths strength basal MS liquid medium supplemented with 9.8 µM IBA (Figure 1 b, c). *In vitro*-regenerated plants exhibited 80% survival rate (Figure 1 d). Thus, based on the cost of 30 ml of liquid MS culture medium/flask employed in the experiments using Hi-Media-make chemical ingredients, the production cost of single shoot on liquid medium is Rs 0.02 compared to Rs 0.26 on solid medium, thereby reducing the production cost of a single shoot in liquid medium by 92.31% of the production cost on solid medium (Table 3). In the present study by employing liquid medium, while on one hand lesser amount of medium is consumed, on the other hand, shoot growth and multiplication response were better than that observed on solid medium. Simultaneously in liquid medium by excluding agar, shoot production cost is also reduced. Role of liquid versus agar-gelled media in mass propagation and *ex vitro* survival of banana has been well documented¹³. Recently, emphasis on the physical form of the culture medium has received much attention and use of liquid medium for *in vitro* micropropagation is advocated as one of the strategies of reducing the cost of *in vitro*-regenerated plantlets.

Advantages of liquid media for enhancing shoot propagation have also been reported for *Allium sativum*¹⁶. Further, handling of liquid medium is comparatively easier than solid medium, which saves labour and energy. Thus, due to higher response of shoot growth and multiplication, liquid culture medium can be useful for large-scale multiplication of *C. borivilianum*.

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Patterns of plant species diversity in the forest corridor of Rajaji–Corbett National Parks, Uttaranchal, India

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Patterns of vegetation diversity in the various strata (trees, shrubs and herbs) were analysed in the Rajaji–Corbett corridor forest. Mean species numbers of all the vegetation strata were correlated with each other. The seedlings and saplings showed weak correlation with herbs and shrubs, proving that some species of

herbs and shrubs are detrimental for regeneration of trees. The α -diversity is highest for trees, while shrubs showed the highest β -diversity. Mosaic diversity values were low indicating that the study area had a relatively simple landscape with few dominating environmental gradients.

Keywords: Corridor forest, mosaic diversity, Shannon–Wiener index, similarity, species richness.

THE broad stratification of vegetation of any area into the following strata, viz. trees, shrubs and herbs is the first and foremost feature that one notices while attempting to characterize the vegetation. Forest and woodland ecosystems extend over *ca.* 37% of the earth's terrestrial surface¹, where the distinction of canopy strata has long been used for the description of vegetation^{2,3}. While diversity indices have been computed for various forest types by various workers, less effort has been devoted to the description of patterns of diversity in these conspicuous strata of vegetation. This issue is important because it may reveal ecological processes responsible for plant community structure. The present communication focuses on the vertical distribution of various measures of diversity in the corridor forest of northwestern India based on the study of the various patterns of plant species diversity such as the α , β , γ and compositional pattern diversity. These parameters were analysed and their distribution was studied across the different strata.

A variety of factors contribute to the diversity of plants in a region. Plant species diversity is affected by several topographic gradients and climatic variations. It is generally observed that areas with high species diversity are found in the middle latitudes, particularly in the tropics because of the congenial climatic, edaphic and other factors prevailing therein.

The most widely used indices for measurement of diversity are the 'information theory indices'. Among the various such indices, the Shannon–Wiener index is most commonly used. This index has been used for the present study, since sampling was done randomly and also because it is the most widely used measure of diversity and thus the findings of the present work could be easily compared with other studies done in the surrounding areas. Species richness is essentially a measure of the number of species in a defined sampling unit. This is the basic component of diversity of any community and is relatively simple to measure. Species richness measures also provide an easily comprehensible expression of diversity. The β -diversity can be defined as the 'extent of species replacement or biotic change along environmental gradients'⁴. Studies by Whittaker have established the importance of identifying β - and α -diversity as components of overall plant diversity. Accurate measurement of β -diversity is important because: (i) it indicates the degree to which habitats have been partitioned by species; (ii) values of β -diversity can