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In vitro propagation of *Chlorophytum borivilianum* (Safed musli*)* through mature explant

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ABSTRACT

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 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.

The basic nutrient medium (MS) Murashige and Skoog (1962) supplemented with different phytohormone concentrations viz. cytokinins “BAP (0.5-5mg/l), kinetin (0.1-0.5mg/l), alone and with combination of auxins (NAA 0.1-1mg/l) with 3% sucrose and 4.5 gm/l agar” were used.

The pH of the medium was adjusted between 5.5-5.8 by using 0.1 N HCl or 0.1 N NaOH before sterilization. The cultures were maintained at a temperature range of 25±2 ^{O}C and the photoperiod of 16 hrs by using white fluorescent light (2000-3000 Lux).

Key words: *Chlorophytum borivilianum,* growth regulators, tissue culture.

INTRODUCTION

Chlorophytum borivilianum commonly known as Safed musli (Described as *Chlorophytum arundinaceous in old literature*) is a threatened species, Nayar and Shastry (1988). The plant belongs to the family Liliaceae is an endangered species valued for the dried fasciculate storage roots. These are reputed to have aphrodisiac properties and form an important ingredient of the herbal tonics prescribed in the Ayurveda system of medicine in India Tuberous roots of *Chlorophytum borivilianum* possess immunomodu-latory 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, Mimaki et al. (1996) and Qiu S-X et al. (2000). The extract of dried root tubers of *C. borivilianum* acts as psycho-stimulant 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 space, Debergh and Zimmerman (1991) Micro-propagation of *C. borivilianum* on solid medium has been reported earlier by Purohit et al. (1994), and Dave et al. (2003). For scale-up cultures using

bioreactors, the use of liquid culture medium has been recommended by Kim et al. (2004), Akita et al. (1994) and Paek et al. (2005).

Most of the medicinal plants have been collected from wild resources and only some species used in large quantities are cultivated systematically. The overexploitation compounded with the rapid afforestation posses a potent danger of extinction of valuable medicinal plants, which may result in the termination of this branch of medicine and related industries. Tissue culture techniques have been reported for conservation and multiplication of several medicinal plants but information on micropropagation of *Chlorophytum borivilianum* is meager so the present study was undertaken.

METHODOLOGY

The explants of *Chlorophytum borivilianum* were collected from the commercial agricultural fields for micropropagation. The entire plant consisting of both the rhizome and shoot were brought to the laboratory in sealed polythene bags. The rhizomes were scrubbed under running tap water with small brush to remove the soil particles present. The roots were removed and the plants consisting of the shoot tip were washed under running tap water for 30 minutes. The explant used for micropropagation was the meristematic part or the shoot tip of the plant. The explant was then taken in a beaker filled with dilute soap solution and washed for 15 minutes then it was subsequently washed with water 3-4 times to remove the soap solution. The explants were treated with 70% alcohol and washed 3 times with sterile distilled water in a laminar air hood. Washed explants were sterilized with 0.1% $HgCl_2$ for 6-10 minutes after $HgCl_2$ treatment, traces of $HgCl_2$ were removed by repeated washing with sterile distilled water.

The basic nutrient medium Murashige and Skoog (1962) supplemented with different concentrations of growth hormones were used for the micropropagation of *Chlorophytum borivilianum*. The pH of the medium was adjusted between 5.5-5.8 by using 0.1 N HCl or 0.1 N NaOH before sterilization. The cultures were maintained at a temperature range of 25±2 ^{O}C and the photoperiod of 16 hrs by using white fluorescent light (2000-3000 Lux). The cultures were observed after 1 week. The media used for micropropagation were supplemented with different concentrations of cytokinins, BAP (0.5-5mg/l, BAP), kinetin (0.1-0.5mg/l), alone and with combination of auxins (NAA 0.1-1mg/l) with 3% sucrose and 4.5 gm/l agar.

RESULTS AND DISCUSSION

A number of scientists are engaged in micropropagation and in vitro preservation of plants that are medicinally important. Commercialization of tissue culture of medicinal plants however has received a poor response compared to ornamental plants. Bonnet and Torrey (1965) succeeded in micropropagation of *Convolvulus arvensis* when they developed plantlets through shoot buds. Chaplot Binita et al. (2005) also studied the effective micropropagation on *Bacopa monniera.*

In the present study shoot tips (explants) of *Chlorophytum borivilianum* were used for micropropagation. The explant remains 80% sterile when treated with 0.1% $HgCl_2$ solution for 6-7 minutes. The highest number of bud break (Initiation) was recorded in BAP (1-3 mg/l) from the apical meristem of the plant in 7 days (Table: 1). All the mediums used with combination of BAP (0.5-3 mg/l) and NAA (0.1-1 mg/l) also give the best results for initiation. The initiated shoots were further used for multiplication in different mediums. Multiple shoots were developed from shoot buds in the number (6-7) in the medium supplemented with BAP (3-5 mg/l) (Fig: 1). The highest number of multiple shoots were obtained in the medium containing BAP (1.5-3 mg/l) in combination with NAA (0.5 mg/l). The number of multiple shoots was recorded (10-15) after 15-20 days. The regenerated shoots usually attained the height of 3-5cm within 15 days on the same media. Krishnan and Seeni (1994) in their study recorded the highest multiplication in *Woodfordia fruiticosa,* when medium was supplemented with a combination of BAP and NAA followed by subculture in media containing BAP alone. Rizvi et al. (2007) also studied the shoot growth and multiplication responses in vitro culture of *Chlorophytum borivilianum.*

Table - 1: Effect of growth regulators on the initiation and multiplication of *Chlorophytum borivilianum.*

M.S.Medium +BAP (mg/l)	% of Initiation	% of Multiplication	No. of shoots	Approx length (cm)	Callusing
0.5	30	10	2-3	2-3	-
1.0	45	10	2-3	2-3	-
1.5	70	80	8-10	2-3	-
2.0	75	85	10-15	4-5	-
2.5	75	85	10-15	4-5	-
3.0	60	50	8-10	4-5	-
3.5	55	30	2-3	2-3	-
4.0	55	30	2-3	2-3	-
4.5	45	10	1-2	1-2	-
BAP + NAA					
0.5+0.1	10	15	1-3	2-3	-
1.0+0.1	10	15	1-4	2-3	-
1.5+0.1	25	20	1-4	2-3	-
2.0+0.1	30	20	2-4	4-5	-
2.5+0.1	35	20	2-4	4-5	-
3.0+0.1	55	40	5-6	4-5	-
0.5+0.5	50	40	5-6	5-7	-
1.0+0.5	70	80	10-12	5-7	-
1.5+0.5	80	80	10-15	5-7	-
2.0+0.5	85	70	8-10	5-7	-
2.5+0.5	65	70	8-10	4-5	-
0.5+0.1	30	30	4-5	2-3	-
1.0+1.0	50	30	5-6	2-3	-
1.5+1.0	50	70	8-10	4-5	-
2.0+1.0	70	50	10-15	4-5	-
2.5+1.0	70	70	8-10	4-5	-
KINETIN					
0.1	-	-	-	-	++
0.2	-	-	-	-	+++
0.3	10	10	1-2	1-2	+++
0.5	10	10	1-2	1-2	++
KINETIN + NAA					
0.1+0.1	-	-	-	-	++
0.1+0.5	-	-	-	-	+++
0.5+0.1	5	-	-	-	+++
0.5+0.5	10	-	-	-	++

Table - 2:Effect of growth regulator (Auxin) on root Initiation of *Chlorophytum borivilianum*

M.S.Medium +Growth Regulator(mg/l)	Day Initiation	%age of Multiplication	No of roots
NAA			
0.1	18	40	2-3
0.3	12	70	4-5
0.5	7	75	5-8
0.8	15	55	4-5
1.0	22	55	2-3
IBA			
0.1	20	70	4-5
0.2	20	70	4-5
0.3	25	80	5-6
0.4	20	70	5-6
0.5	20	50	5-6
MS + Activated Charcoal (mg)			
50	10	70	5-6
100	10	70	5-6
150	15	80	7-8
200	15	80	7-8

In vitro multiplication of *Chlorophytum borivilianum* was also achieved on Murashige and Skoog medium supplemented with 2 mg benzyladenine/litre by Pudake and Dhumale (2003). Among the different combinations involving auxins (IBA or NAA) tested for rooting, IBA (3 mg/l) was relatively better. Earlier Purohit *et al.* (1994) had shown that 22.2 µM BAP concentration in culture medium was best for shoot multiplication and reported increase in shoot multiplication rate. Similarly, Dave et al. have reported less 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. The MS medium supplemented with higher concentration of BAP (3-5mg/l) and NAA (1mg/l) induced callus formation from the entire surface in the culture (Table -1) when the segments were placed on fresh media they developed further adventitious shoots up to 3-5 passages.

During the formation of successful cultures the same explants proliferated large number of multiples. The maximum number of multiplication of *Chlorophytum borivilianum* was standardized on the medium containing BAP (1.5-3mg/l) and NAA (0.1-0.5). The elongated shoots were separated and treated with medium containing auxin (NAA, 0.5-1mg/l, IBA 0.1-0.5mg/l) and activated charcoal for initiating roots (Table.2). The roots were induced within one week in the medium containing 0.5mg/l NAA and the medium with activated charcoal. The %age of root formation in MS medium (full strength) increased in comparison to the MS (half strength) but the roots were thin and stunted. Liquid media were also tried to induce roots and it was found that liquid media were unsuitable for root formation.

Fig. - 1: Multiplication of shoot from SAM

Fig. - 2: Elongated shoots

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ABBREVIATIONS

BAP: 6-benzylaminopurine
NAA: 1-naphthalene acetic acid
MS: Murashige and Skoog (1962) medium.