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PROSPECTS OF USING MS FOR *IN-VITRO* PROPAGATION OF *CHLOROPHYTUM BORIVILIANUM*

Rashmi Arnold*

Department of Botany, Govt. Model Science College, Rewa, M.P.

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***Correspondence for Author**
Rashmi Arnold
Department of Botany, Govt. Model Science College, Rewa, M.P.

ABSTRACT

Traditional medicine system provided effective platform for the discovery of many new drugs. Further, the increased cost of health care and side effects of allopathic drugs has become a driving force in the shift towards greater recognition between diet and health care. *Chlorophytum borivilianum* is such a better option in the current context which can be taken up as a medicinal and nutritional diet in the form of powders and chips etc. *Chlorophytum borivilianum* has many additional beneficial effects including properties of adaptogen, immunomodulation and anti-aging and is also a rich source of zinc and iron. The present study is an attempt to evolve an efficient system for mass multiplication of this important medicinal plant *Chlorophytum borivilianum* Sant.et Fernands within a short period of time with objectives of induction of adventitious organogenetic differentiation from explants, establishment of shoot tip culture involving axillary/ apical bud, elongation and growth of *in vitro* regenerated shoots and induction of rooting in *in-vitro* regenerated shoots.

KEYWORDS: *Chlorophytum borivilianum,* Micropropagation, explants etc.

INTRODUCTION

Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. The current accepted modern medicine or allopathy has gradually developed over the years by scientific & observational efforts of scientists.[1] Medicinal plants are an integral component of research developments in the pharmaceuticals industry. Such research focuses on the isolation and direct use of active medicinal constituents or on the development of semi-synthetic drugs, or still again on the active screening of natural products to yield synthetic pharmacologically-active compounds.[2]

Chlorophytum borivilianum Santapau & Fernandes (Liliaceae) also known as 'Safed Musli' is a traditional rare Indian medicinal herb which has many therapeutic applications in Ayurvedic, Unani, Homeopathic and Allopathic systems of medicine. Its roots (tubers) are widely used for various therapeutic applications.[3] It is used to cure physical illness and weakness, as an aphrodisiac agent and revitalizer, as general sex tonic, remedy for diabetes, arthritis and increasing body immunity, curative for natal and postnatal problems, for rheumatism and joint pains, increase lactation in feeding mothers, as antimicrobial, anti-inflammatory, antitumor agent, also used in diarrhea, dysentery, gonorrhea, leucorrhea etc.[4] It has spermatogenic property and is found useful in curing impotency, now it is considered as an alternative 'Viagra'. Its root contains steroidal and triterpenoidal saponins, sapogenins and fructans which act as therapeutic agents and play vital role in many therapeutic applications.[5]

It is also reported to cure diabetes, arthritis and increasing general body immunity. However, in recent years its effectiveness in increasing male potency has become very popular and is now considered as an alternative to Viagra'. The roots are reported to contain 42% of carbohydrates, 8–9% of proteins, 3–4% fibres4. Ethanolic extract of the tubers of C. borivilianum and its sapogenin were evaluated for their immunomodulatory activity. The assessment of immunomodulatory activity was carried out by determining the effect of azathioprine induced myelosuppresion and administration of extracts on hematological and serological parameters.[6] Administration of extract greatly improved survival against Candida albicans infection. An increase in delayed type hypersensitivity response, % neutrophil adhesion and in-vivo phagocytosis by carbon clearance method was observed after treatment with extracts.[7]

One of the most exciting and important aspects of *in vitro* cell and tissue culture approach is the capability to regenerate and propagate plants from cultured cells and tissues. Micropropagation involves the production of plants from very small plant parts, tissues, or cells grown aseptically in test tubes or other containers where the environment and nutrition can be rigidly controlled [8].Micropropagation is used routinely to generate a large number of high-quality clonal plants, including medicinal, agricultural, ornamental and vegetable species, and in some cases also plantation crops, fruits and vegetable species.[9,10] Micropropagation has significant advantages over traditional clonal propagation techniques. These include the potential of combining rapid large-scale propagation of new genotypes, the

use of small amounts of original germplasm and the generation of pathogen-free propagules.[11, 12]

MATERIAL AND METHODS

Explant collection

The actively growing plant of *Chlorophytum borivilianum* Sant et Fernard for the experiments were collected from Jawaharlal Nehru Krishi Vishwavidyalaya, (JNKV) Jabalpur and these plants were maintained and grown at Garden of Department of Botany, Govt. Model Science college, Rewa, Madhya Pradesh. Stem disc of healthy growing 10-15 cm of plant were excised and used as explants.

Explant preparation

Stem disc of healthy growing 10-15 cm of plant were excised from mother plant having characters like young, healthy, diseased free, about 1 cm long were selected for carrying out study as young cells are supposed to have retained their totipotency grown in pots.

Surface Sterilization

Surface sterilization is necessary in order to disinfect the explants before it was placed over media. The explant were placed in different bottles and covered with net and washed for 30 minutes under running tap water to remove all the dust particles and microbes from the surface. In the next step explants were soaked in an aqueous soap solution containing 1% Labolene (Qualigens) for 5-7 minutes and then washed with distilled water. Explants were treated with 2% Bavistin (antifungal) for 10 minutes and antibiotic for 5 minutes. This was followed by gentle wash in double distilled water for 5 minutes for two cycles.

Culture Medium

The composition of the nutrient medium is an important aspect of successful plant regeneration schedule. *In vitro* studies on *Chlorophytum borivilianum* were carried on Murashige and Skoog's medium (MSM) [13]. The cultures were maintained in culture tubes and conical flasks and were kept in the culture room at a temperature of 25±2ºC, relative humidity (RH) of 60-70% and a light intensity of approx. 2500 lux provided by cool, white, fluorescent tubes under a photoperiod of 16/8 hr (light/dark). Cultures were maintained through regular monthly subcultures. The cultured tissues were aseptically transferred on to fresh media without being subjected to chemical sterilization. To achieve shoot elongation, the multiple shoot clusters were dissected into finer units. The adhering agar and necrotic

tissues were removed without damaging the shoot primordial/buds. These units were finally recultured on to fresh media having appropriate plant growth regulators (PGRs). Subcultures were also performed as and when necessary after evaluation of growth changes.

Statically analysis

The morphogenetic responses of the explants were assessed and recorded regularly up to a maximum of 30 passages. For each treatment, 12 replicates were maintained and each experiment was repeated at least three times. The results were noted in the form of Mean ± Standard Error (SE) [13, 14] which was calculated with the help of Standard Deviation (SD) as follows:

$$SD = \sqrt{\frac{n\sum fx^2 - (fx)^2}{n(n-1)}}$$

$$SE = \frac{SD}{\sqrt{n}}$$

Where n = No. of observations

fx = Each observation.

Various explants viz. leaf, apical bud and axillary bud were used for the induction of multiple shoots. The frequency of shoot elongation, mean number of shoots and mean length of shoots was calculated from the buds showing signs of break in the following manner

$$\text{Frequency of bud break (FBB)} = \frac{\text{No. of explants exhibiting bud break}}{\text{Total No. of explants inoculated}} \times 100$$

$$\text{Frequency of bud elongation (FBE)} = \frac{\text{No. of buds elongated}}{\text{Total No. of explants exhibiting bud break}} \times 100$$

$$\text{Mean number of shoot (MNS)} = \frac{\text{No. of shoots obtained}}{\text{No. of shoots elongated}}$$

$$\text{Mean shoot length (MSL)} = \frac{\text{Total shoot length obtained}}{\text{No. of shoots elongated}}$$

$$\text{Frequency of rooting (FR)} = \frac{\text{No. of shoots rooted}}{\text{No. of shoots elongated}} \times 100$$

The effect of interaction of BAP and Kn and explant on various parameters viz. the frequency of bud break and bud elongation and mean number of shoots and shoot length was observed. A total of 5 conc. of BAP (0.5.1.0, 2.0, 3.0, 4.0, 5.0 mg/l) were used. There were three replicates for each treatment and a total of 12 treatments were performed for each parameter. And the experiment was performed according to randomized factorial design to study the effect of Auxins viz. IBA (alone) on stem disc and their interactions on frequency of Root Number (FNR) and frequency of Root Length (FRL).

RESULTS AND DISCUSSIONS

Effect of BAP

In this experiment effect of different conc. of BAP were studied on different parameters. Stem disc stayed fresh in BAP supplemented medium till the next subculture and Kn supported the elongation of the explants. Stem disc were inoculated on MS medium supplemented with 3% vitamins, 3% sucrose, 0.8% agar and different concentration of BAP (cytokinin) on various parameters were studied. BAP is a plant growth regulator play important role in initiation of explant. The effect of the different concentration of BAP was found to be not significant for all the parameters. Frequencies of bud break and bud elongation and mean number of regenerated shoots and shoot length were observed at 5mg/l BAP. i.e. frequencies of bud break is 62% and bud elongation is 86% and mean shoot number is (5.75) and mean shoot length is (4.46). While at concentration lower than 5.0 mg/l treatment resulted decline in all parameters (Table-1).

Effect of Kn

Kn is a plant growth regulator play important role in initiation and multiplication of explant. The effect of the different concentration of BAP was found to be significant for all parameters after 3-4 weeks of inoculation. Maximum frequencies of bud break and bud elongation and mean number of regenerated shoots and shoot length were observed at 5mg/l BAP. i.e. maximum frequencies of bud break is 72% and bud elongation is 85% and mean shoot number is (9.25) and mean shoot length is (4.21), while at concentration lower than 5.0 mg/l treatment resulted decline in all parameters (Table-2).

Effect of IAA on rooting

IAA is a plant growth regulator play important role in the rooting of plants. No significant differences in rooting were observed by various concentration of IAA i.e. 0.5, 1.0, 1.5, 2.0, 2.5 mg/l. Frequencies of Root No. observed in 2.0 mg/l i.e. (4.08) and frequencies of Root

length observed in 2.0 mg/l i.e. (3.78). While at concentration lower then 2.0mg/l treatment resulted decline in all parameters (Table-3).

Table-1 Effect of BAP on initiation and multiplication of *Chlorophytum borivilianum*

BAP(mg/L)	FBB	FBE	MNS	MSL
0.00	22.56	36.44	1.80±0.5149	2.12±0.079
0.5	24.26	42.48	2.22±0.452	2.26±0.022
1.0	36.42	52.88	3.58±0.5146	2.42±0.0462
2.0	44.58	68.56	4.16±0.3892	2.54±0.0667
3.0	52.22	78.68	4.75±0.4523	3.02±0.0522
4.0	58.92	80.02	5.58±0.5149	3.52±0.0574
5.0	62.56	86.48	5.75±0.4523	4.46±0.0792

Table-2 Effect of Kn on initiation and multiplication of *Chlorophytum borivillianum*

Kn(mg/L)	FBB	FBE	MNS	MSL
0.00	24.16	38.46	2.16±0.572	1.91±0.0256
1.0	39.76	56.32	3.17±0.5773	2.53±0.0651
2.0	48.52	70.02	4.580.5149	2.74±0.066
3.0	68.56	82.24	4.91±0.6685	3.15±0.0674
4.0	70.78	84.56	7.08±0.5149	3.77±0.0754
5.0	72.24	85.94	9.25±0.4522	4.51±0.0718

Table-3 Effect of Auxins (IAA) on Rooting of *Chlorophytum borivilianum*

IAA (mg//l)	FRN	FRL
0.5	3.2±0.61	3.53±0.079
1.0	3.43±0.52	3.69±0.12
1.5	3.77±0.68	3.49±0.078
2.0	3.78±0.57	4.08±0.09
2.5	3.63±0.58	3.25±0.1

CONCLUSION

Chlorophytum borivilianum is generally found in forests and are members of a special group of Ayurvedic herbs known as Vajikaran Rasayana, which are used for improving potency and alleviating sexual dysfunction. This protocol can be successfully used in large scale commercial multiplication of elite *Chlorophytum borivilianum* clones. By using the protocol developed through this effort, it is possibly produce 500-650 plants from a single stem disc through micropropagation genetic schedule within 6 months of culture period. It is estimated that the number of plants could be more than double if the primary stem disc (explant) are further multiplied for an additional month to get the viable secondary plantlets.

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