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DEVELOPMENT OF SHOOT AND ROOT FROM EXPLANTS OF *CHLOROPHYTUM BORIVILIANUM* ON THE BASIS OF GROWTH REGULATOR

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ABSTRACT

Medicinal plants are valuable sources of medicine and many other pharmaceutical products. ***Chlorophytum borivilianum (Safed moosli)*** is an important medicinal plant and used worldwide in drug industry. An overview of tissue culture study on *chlorophytum borivilianum* is presented. The plant is taken from the Patwadangar. The choice of explants is apical bud which is sterilized with tween-20 (surfectent) then with 0.1% bavistin (antifungal) and finaly with surface sterilant (0.1% $HgCl_2$). Now explant inoculated in MS media which have different concentration of growth regulators (NAA, BAP). We observe shooting in 100µl NAA and 1000µl BAP. We observe rooting in the concentration of 100µl of NAA and 20µl of BAP.

Key words: *Chlorophytum borivilianum,* explant

INTRODUCTION

Chlorophytum borivilianum (Safed moosli) is a traditional Ayurvedic medicine since ancient times. Moosli prepared as a paste with goats milk or honey and applied locally over the face, brightens the complexion of the face. It is extensively used by the Ayurvedic practitioners for a wide variety of ailments and particularly an ingredient of aphrodisiac preparations. The roots are widely use as a natural "sex tonic" and is an integral part of more than 100 herbal drug formulations although Indian forest are rich in safed moosli demand is increasing rapidly in India and international drug markets . More than 175 species of chlorophytum have been in world. Thirteen species of *chlorophytum* have reported in India. Following species of chlorophytum are available in India. In nature moosli propogates vegetatively through its fleshy roots, rarely by seed. Seed have poor germination and low viability. *Chlorophytum* is found in soil rich in organic matter. It requires bright sun light for good growth. The crop is popular in rainy season in India and a commercial root harvest can be obtained in 3-4 months. Saponin content may be affected by fungicides. There is now a heavy demand of organically grown safed moosli with high saponin content in national and international drug market. In India based products are coming regularly to the market. The area under this crop is increasing rapidly in India.

It comprises of some important chemical constituent who are essential like carbohydrates (35-45%), alkaloids (15-25%), saponins (2-20%), and proteins (5-10%). It is a rich source of over 25 alkaloids, vitamins, proteins, carbohydrates, steroids, saponins, potassium, calcium, magnesium, phenol, resins, mucilage, and polysaccharides and also contains high quantity of simple sugars, mainly sucrose, glucose, fructose, galactose, mannose and xylose.

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MATERIALS AND METHODS

Plant tissue culture media

Culture media used for the cultivation of plant cells *in vitro* are composed of three basic components:

1. Essential elements, or mineral ions, supplied as a complex mixture of salts;
2. An organic supplement supplying vitamins and/or amino acids; and
3. A source of fixed carbon; usually supplied as the sugar sucrose.

For practical purposes, the essential elements are further divided into the following categories:

1. Macroelements (or macronutrients);
2. Microelements (or micronutrients); and
   An iron source

ESTABLISHMENT OF ASEPTIC CULTURES

Sterilization of plant material

We collected the explants (apical bud) of *chlorophytum borivilianum* from Patwadangar. Sterilization started with the Washing of explants under running tap water for 4-5 times than with distilled water for 2-3 times . Addition of few drops of surfactant (Tween-20) to the solution may enhance the effectiveness of the disinfectant by breaking the surface tension between the water and plant tissue .The explants must be rinsed 2-3 times in distilled water to remove the remaning traces of the disinfectants. Than the explants will be sterilized with 0.1% bavistin which is an anti fungal reagent. Shake it for 1 hour , than wash it with distilled water for 2-3 times and sterilized the explant with 0.1% $HgCl_2$ (sterilants) and finally wash it with autoclaved distilled water for 2-3 times.

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Preparation of media

We used Murashige and Skooge media for in vitro propagation of *chlorophytum borivilianum* which consist of the 3% succrose,(carbon source) 0.1% inositol (stimulate cell growth), 10ml MSI(macroelements),10ml MSII,1ml MSIII(microelements),2ml MSIV(iron source),1ml MSV(organic supplement), 0.8% agar which is used as gelling agent and different concentration of auxin and cytokinin(NAA, BAP). Make up the volume 1000ml and Adjust the pH 5.8 with 1N HCl and 1N NaOH than autoclave the media at 121^{o}c on 15 lbs.

Shoot Induction

Adventitious shoots may be induced by NAA or BAP on the meristmatic regions of leaf and stem .We were induced 1-2mm apical bud, cultured on Murashige and Skoog's mineral salts together with α-napthalene acetic acid (NAA) 100µl, 6 benzyl amino purine (BAP) 1000µl and several organic elements. We developed 7-8mm shoot from 1-2 mm apical bud. Stimulating auxillary branching by in vitro culture of apical bud is the common techniques of micropropagation.(Fig.1)

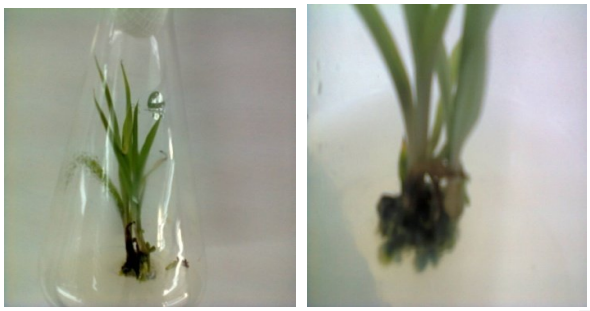
Fig1. Different Stages of shoot development

(A) 5-6 days plant (B) 10-15 days plant (C) 20-25 days plant

Root induction

Shoots transferred to media with low concentrations of cytokinin spontaneously formed the roots. Rooting of regenerated shoots of ***Chlorohytum borivilianum*** was best observed in MS media supplemented with 200µl of NAA and 20 µl BAP. (Fig.2)

Fig1. Multiplication of root



Acclimatization

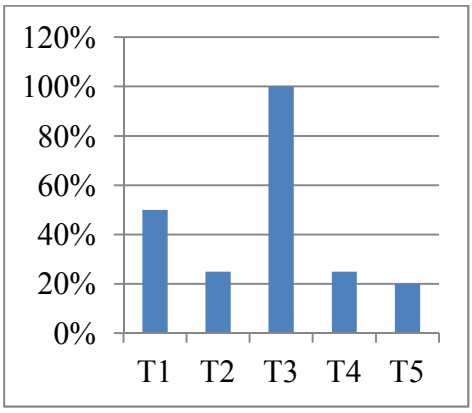
The in vitro developed shoots after rooting are transferred in the pots, containing soil. The pots are then kept under net house condition for better establishment and survival. The survival of plant is recorded after 1 month.

Fig1. Hardening of plant

RESULT AND DISCUSSION

Shooting

$$\% \text{ of bud breaking} = \frac{\text{sum of number of bud breaking explant}}{\text{total number of explants inoculated}} \times 100$$



TREATMENT:

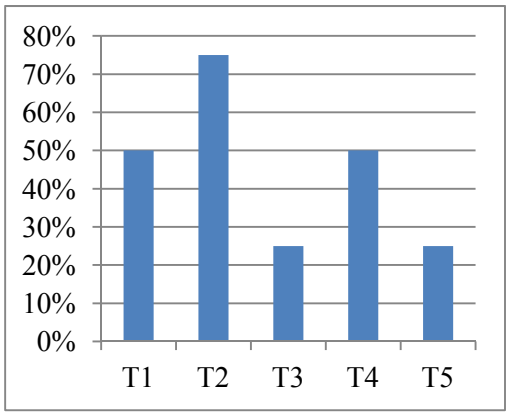
T1= 1000 µl BAP , 20 µl NAA
T2=1000 µl BAP, 100 µl NAA
T3=2000 µl BAP , 20 µl NAA
T4=2000 µl BAP , 100 µl NAA
T5=2000 µl BAP , 200 µl NAA

Shoot development: The data of shoot development of *Chlorohytum borivilianum* was recorded after 4 weeks. *Chlorohytum borivilianum* plant were efficiently regenerated from apical bud. Apical bud (explant) is taken from field grown plants of *Chlorohytum borivilianum* and cultured on MS medium supplimented with various concentrations of cytokinin (BAP) and auxin (NAA). Shoot were developed at 12 -15 days after inoculation.

When we use different concentration of auxin and cytokinin, it shows variable result. When we take BAP concentration 2000µl and NAA concentration 20µl, it shows maximum number of shoot multiplication. When we take BAP concentration 1000µl and NAA concentration kept constant i.e, 20µl, it shows average number of shoot multiplication. When we take BAP concentration 2000µl and NAA concentration 200µl, it shows poor growth of shoots. It means shoot development depend upon high cytokinin concentration (BAP) and low auxin (NAA) concentration.

Rooting

$$\% \text{ of rooting} = \frac{\text{sum of number of root developed}}{\text{total number of shoots inoculated}} \times 100$$



TREATMENT:

T1=20 µl BAP , 100 µl NAA
T2=20 µl BAP , 200 µl NAA
T3=100 µl BAP , 100 µl NAA
T4=100 µl BAP , 200 µl NAA
T5=200 µl BAP ,20 µl NAA

Root development: Effect of auxin (NAA) and cytokinin (BAP) on root development of *Chlorohytum borivilianum* was recorded after 4 weeks. Elongated shoots (3.5cm.) w excised and placed on MS medium

supplimented with various concentrations of auxin and cytokinin for induction of root. Good growth of root is developed when we use 20µl BAP and 200 µl NAA. When we take 20µl BAP and 100 µl NAA, it shows average growth .When we take 100 µl BAP and 200µl NAA , it also shows average growth. Poor growth of root occur when we take 200µl BAP and 20µl NAA.

From above observation, it shows that root development depands upon high concentration of auxin (NAA) and low concentration of cytokinin . When we increase auxin concentration and kept cytokinin concentration low then best growth of root development take place. It means best suitable concentration of root growth is 20µl BAP and 200µl NAA. It shows NAA concentration is responsible for root growth.

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