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RESEARCH ON IN-VITRO **CLONAL MULTIPLICATION PROTOCOL IN VARIOUS CULTIVARS O F *CHLOROPHYTUM.***

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Abstract:

*Chlorophytum co*mmonly known as 'Safed Musli' has become an endangered species due t o its over exploitation, used as tonic and aphrodisiac. Root of the plant contains steroidal sa ponin as secondary metabolite. It has a worldwide demand in ayurvedic and allopathic syst ems of medicine to cure general debility, fatigue, weakness and male sterility. Although thi s species has been brought under commercial cultivation but the systematic research on coll ection and evaluation of germplasm still in demands.A tissue culture method has been deve loped for its large scale multiplication and conservation. Micropropagation using stem disc as explant has been achieved on Murashige and Skoog (MS) medium containing 5.0 mg.L-1 BAP. Survival rate of these rooted plantlet in soil is about 80 %. A comparative studies h ave been carried out to compare the protocol in different cultivars of *Chlorophytum.*

Keywords- *Safed Musli, saponin, Micro propagation, Murashige and Skoog medium*

Introduction:

'Safed musli' is an endangered medicinal plant of Liliaceae family belonging to genus *Chlorophytum.* The tuberous roots of the plant are used in ayurvedic tonic and has aphrodisiac property due to the presence of saponin. Safed musli has wide distribution in India, mainly in the southern Rajasthan, Western MP and North Gujarat (Borodia *et.al.*).More than 175 species of *Chlorophytum* have been reported in world, about 13 species of genus are found in India out of which roots of species *C. borivillianum* and *C. tuberosum* only are used as safed musli in many ayurvedic preparations. It has a worldwide demand in ayurvedic and allopathic systems of medicine to cure general debility, fatigue, weakness and male sterility (Bhagat. *et.al*). In view of its day-to-day increasing demand not only by the user of our country but of abroad too, it may be a dollar-earning crop.

Population of the plant is dwindling at an alarming rate. In nature the plant is propagated by seeds and has become an endangered species due to low seed set, viability and germination, associated with over exploitation from the wild strands. The planting material/ tubers as well as seeds have dormancy for a certain period.

Owing to its enormous uses, its worldwide demand is estimated to be 35000 tons annually as compared to current annual production of 5000 tons. The immediate task is to conserve and multiply the plant in bulk amount required for its domestication to meet the present demand. In recent years a large-scale propagation of plant has been developed by use of the tissue culture technique and the number of plant species being enabled to multiply by this technique annually. Plant tissue culture has been successfully used to micro-propagate medicinal plants. Plantlet regeneration has been reported through apical meristem *in C. Comosum.* Plantlet regeneration and bulbil formation has been reported through leaf and stem explant in *Curculigo orchiodes* (Suri *et al*.1999).

In the present study, an efficient and rapid *In-vitro* micro propagation method of Safed musli (*Chlorophytum borivillianum)* has been established through optimization of medium and cytokinin and auxin ratio for shoot proliferation and root formation.

Material & Methods:

Leaf segment, root segment and stem disc of two different collection of *Chlorophytum borivillianum* (Fig-A, Fig.-B) were collected from mature plants as explants from the field and brought to the laboratory. The source tissues were kept under running tap water (5 mints) and washed with a 5 % laboline detergent for 5-10 mints. Different concentration and combination of sterilent tried for the surface sterilization of explants. After washing with detergent surface sterilization was done with 1 % bleaching powder solution with

0.1% amplicillin for 20 mints. After removing few scales and waste part, explants were washed three times with sterile water. After washing explants immersed in 0.2% mercuric chloride solution with few drops of tween 20 for 15 min. in laminar flow. Last treatment given by 70% ethanol for 60 seconds. Explants were inoculated after washing with sterile water and drying with sterile blotting paper.

The materials were cut into appropriate sizes (leaf- 1cm., root segment 1 cm, stem disc) and cultured on MS medium (Murashige and Skoog, 1962). The medium was used at different strengths. The medium was supplemented with 3 % sucrose and gelled with 0.8% agar. Basal medium was supplemented with different concentration of growth regulators (2,4-D, Kin, 2iP,NAA,BAP) either alone or in combination for shoot initiation as well as shoot multiplication. The pH of the medium was adjusted to 5.8 before autoclaving at a pressure of 15 lbs for 15 mints. The cultures were incubated at 25 +- 2^0C with 16/18 hours photoperiod under white fluorescent tubes. Experiments were set up in a completely randomized design. Minimum 6 explants were used for each treatment and all treatments were repeated twice. Data were presented in mean number of shoot. Plantlets were transferred to small pots containing soil and sand (1: 1) and subsequently to the field.

Result:

In this experiment most of the explants were contaminated with fungi and thus explants were sterilized with different concentration and combination of sterilent. Strong treatment has been given to explants to protect them for contamination. Shoot initiation was observed only in stem disc explant. No response has been seen in leaf as well as root segment. Growth regulators NAA, 2,4-D,BAP, 2iP and Kin. either alone or in combination tested in the present study (Table 1). Callus development has been seen on MS medium enriched

with 2,4-D(1-2.5mgL^{-1}) (Fig.-C). Cream coloured callus was not found suitable for shoot initiation as well as multiplication. Good response has been seen by Stem disc (crown) on the medium containing different concentration of BAP. BAP in higher amount is the best for shoot initiation in comparison to 2,4-D, Kin, 2iP. MS medium supplemented with 5.0 mgL^{-1} BAP was best for shoot initiation. Mean number of shoot initiated in MS medium supplemented with 5.0mgL^{-1} BAP is 7.8 in sample 1 and 6.6 in sample 2, which is highest among others (Fig.D,). More than 80% response has been seen in 5.0 mgL^{-1} BAP treatments. These cultured shoots were used for sub culturing. MS medium supplemented with 5.0 mgL^{-1} BAP used as a parent media. Different concentration and combination of growth regulators tried for multiple shoot formation. Auxin and Cytokinin ratio has given average response of 75% with 6-8 mean numbers of shoots. When sub culturing has been done on to the medium of same composition (5.0 mgL^{-1} BAP) resulted in an increase in size and extra ordinary response has been seen. 100% response with more than 15 mean number of shoots in sample 1 and 17 in sample 2 generated from the MS medium supplemented with 5.0 mgL^{-1} BAP (Fig.-E). 75 % response reported when BAP concentration increased after 5.0 mgL^{-1} BAP. (Table 2).

Healthy roots were found when shoots transferred to half strength MS medium supplemented with 500mgL^{-1} charcoal (Fig.-F.1,2). Plantlets developed through tissue culture were successfully transferred in soil and sand (1: 1) with high rate of survivability (80%) (Fig.-G).

Tissue culture has been used to accelerate plantation development, to shorten breeding cycle and to rapid multiplication. The best use of micro propagation technique is to

overcome dormancy problem. The plantlets obtained by this technique are expected to be pathogen free.

Discussion:

Due to large scale and indiscriminate collection of the wild material and insufficient attempts either to allow its replenishment or its cultivation *Chlorophytum* is rapidly disappearing from nature Growth regulators NAA, 2,4-D, BAP, 2iP and KIN either alone or in combination tested in the present study. Response has been recorded by Stem disc (crown) on the medium containing different concentrations of BAP. BAP in higher amount was found best for shoot initiation in comparison to 2,4-D, KIN, 2iP. MS medium supplemented with 5.0 mgL^{-1} BAP was best for shoot initiation. Mean number of shoot initiated in MS medium supplemented with 5.0 mgL^{-1} BAP is 7.8 in sample1 and 6.6 in sample 2. More than 80% response has been found in 5.0 mgL^{-1} BAP treatments. These cultured shoots were used for sub culturing. MS medium supplemented with 5.0mgL^{-1} BAP used as a parent media. Different concentrations and combinations of growth regulators tried for multiple shoot formation. Average response of 75% with 6-8 mean numbers of shoots was found in auxin and cytokinin ratio. When sub culturing was done on to the medium of same composition (5.0mgL^{-1} BAP) resulted in an increase in size and extra ordinary response was found. 100% response with more than 15 mean numbers of shoots in sample 1 and 17 in sample 2 generated from the MS medium supplemented with 5.0 mgL^{-1} BAP. 75 % response reported when BAP concentration increased more than 5.0 mgL^{-1} BAP. Healthy roots were found when shoots transferred to half strength MS medium supplemented with 500 mgL^{-1} charcoal. Plantlets developed through tissue culture were successfully transferred in soil and sand (1: 1) with high rate of survivability (80%) Arora

et al., (1999) developed an improved method for large-scale rapid multiplication of *C. borivillianum* through somatic embryogenesis. Somatic embryos were obtained on MS medium containing 2.25 micro molar 2,4-D and 1.5 micro molar kinetin. The technique developed is highly efficient to get miniature plantlets for field transfer in 2 months, starting from callus.

Tissue culture has been used to accelerate plantation development, to shorten breeding cycle and to rapid multiplication. The best use of micro propagation technique is to overcome dormancy problem. Plant tissue culture has been successfully used to micro-propagate medicinal plants. Plantlet regeneration has been reported through apical meristem in *C. comosum.* Plantlet regeneration and bulbil formation has been reported through leaf and stem explant in *Curculigo orchiodes* (Suri *et al*., 1999).

(Table 1)- Effect of different concentrations and combinations of phytohormones on shoot initiation

Hormone Concentration (mg/1)	Sample 1		Sample 2	
	% response	Mean no. of Shoots initiated	% response	Mean no. of shoots initiated
2,4 D				
0.5	25	2.0	25	1
1.0	20	2.0	20	1.5
1.5	50	3.5	50	4
2.0	25	2.0	25	2.5
2.5	25	1.0	25	1
2,4 D + KIN				
1.0+1.0	20	1.0	20	1
1.0+1.5	50	2.5	50	1
1.0+2.0	25	2.0	25	2.0
1.0+2.5	40	2.5	40	2.0
2iP				
0.5	20	4.0	20	0
1.0	33.3	5.5	33.3	1
1.5	40	2.0	40	0
2.0	40	1.0	40	2
KIN				
0.5	25	1.0	25	3
1.0	33.3	2.0	33.3	0
1.5	40	1.0	40	0
2.0	40	2.5	40	0
2.5	50	2.0	50	2.0
NAA+KIN				
1.0+1.0	40	1.5	40	1.5
1.0+1.5	20	1.0	20	3.5
1.0+2.0	25	2.0	25	2.5
1.0+2.5	40	2.5	40	2.5
BAP				
1.0	50	3.5	66.6	3.0
1.5	50	3.33	50	2.5
2.0	80	2.25	50	3.0
2.5	75	5.0	60	3.33
3.0	83.3	6.6	60	3.66
4.0	80	5.0	75	4.3
5.0	**83.3**	**7.8**	**83.3**	**6.6**
6.0	75	5.0	75	5.5
8.0	75	4.3	75	3.0
10.0	50	2.5	75	3.33

(Table 2) Effect of subculture media on multiple shoot formation.

Hormone Concentration (mg/1)	Sample 1		Sample 2	
	Mean no. of shoots initiated	% Response	Mean no. of shoots initiated	% Response
NAA+BAP				
1.0+0.5	6.66	75	6.0	50
1.5+1.0	3.33	75	6.0	33.3
1.0+2.0	4.00	50	3.5	66.6
1.0+2.5	3.33	75	3.5	66.6
NAA+KIN				

1.0+0.5	3.5	66.6	3.0	75
1.0+1.0	3.00	75	1.5	50
1.5+1.5	2.33	75	2.0	33.3
1.0+2.0	2.00	75	3.0	50
1.0+2.5	2.00	50	3.0	50
BAP				
0.5	6.66	75	2.0	25
1.0	8.00	75	3.5	66.6
1.5	7.5	66	3.0	66.6
2.0	6.00	75	2.0	66.6
2.5	6.0	66	2.5	33.3
3.0	6.5	50	7.0	75
4.0	6.6	75	7.6	75
5.0	**14.75**	**100**	**17.0**	**100**
6.0	8.6	75	9.0	75
7.0	8.33	75	9.3	75
8.0	9.00	75	6.6	75
10.0	5.00	75	9.3	75

Protocol for Multiplication of *Chlorophytum borivillianum*.

Explant (Stem disc)

Surface sterilization

Culture initiation (MS medium + 5.0 mg./l. BAP, 7.8 mean no. of shoots)

Shoot Multiplication (MS medium + 5.0 mg./l. BAP, 15 mean no. of shoots)

Rooted plantlets

Field establishment

In-vitro regeneration potential of different *Chlorophytum* genotypes

To study the *in-vitro* regeneration potential of different *Chlorophytum* genotypes, all the collected genotypes were cultured on the standardized medium. Among the three only two responded on the same standardized medium (Table 4.16). 50.0-percentage response with 2.55 mean numbers of shoots was recorded for M4 and 60.00 percent response with 3.33 mean numbers of shoots were recorded for M5. Genotype M3 not responded in the standardized medium (5.0 mg/l BAP). It has been concluded that *in-vitro* regeneration technology can be successfully employed for *Chlorophytum* plantlets, if careful selection of proper combination and concentrations of phytohormones for explants is made for specific genotype.

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Fig.-B

Fig.-E

Fig.-G