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INDUCTION OF CALLUS IN chlorophytum borivilianum FOR REGENERATION OF PLANTS AND BIOTECHNOLOGICAL APPLICATIONS

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Chlorophytum borivialianum (Safed Musli) is an endangered Indian medicinal herb, belonging family *Liliaceae.* This species has gained significance due to their valuable medicinal properties of male restorative and rejuvenation. Now-a-days this plant is indiscrimately exploited by people for food medicine and commerce reindering it endangered. To protect the germplasm of this valuable medicinal herb cultural studies was initiated. In the present investigation protocol for induction of callus was standeredize. Cultural studies in Safed Musli was initiated by using floral axis, leaf and stem disc as explant. Explants were surface sterilized by using $Hgcl_2$ (0.01%) and 70% ethylalcohol after Razadan. Explants were cultured on MS media supplemented with various growth hormones. Friable callus was formed after 15 days of inoculation, which after 4-5 days changed in to somatic embroids.

In future, this *invitro* protocol could benefit in crop improvement programs and serve a new source of bioactive compounds from safed musli callus tissue for an various therapeutic applications.

KEYWORDS - Somatic embryoids, Safed Musli, Callus, *Chlorophytum borivilianum*.

INTRODUCTION

Chlorophytum borivilianum, belonging to the family *Liliaceae*, is an endangered medicinal herb. It is valued for its dried fasciculated storage roots which posses immunomodulatory and aphrodisiac properties that form an important ingredient for various herbal tonics.

Although Indian forest are rich in Safed Musli, its demand is increasing rapidly in Indian and International drug markets. Forcign demand has been estimated as 300-700 tones annually (Bordia et al., 1995), a quantity that Indian forest cannot sustain. Moreover abnoxious weeds like *Parthenium* and *Lantana* are taking its place (Oudhia, 1996). This has created a pressure on Indian forests and it is predicted that if steps for timely conservation are not taken, the Indian forests will lose the valuable plants [oudhia, 2001
(b)].

Therefore, to avoid the pressure on the natural forest, attempts have been made to cultivate Safed Musli (Kothari and Singh, 2003; Maiti and Geetha, 2005). However to undertake mass scale cultivation large quantity of quality planting material is required. The tuberous roots of Safed Musli are the only propagules which can either be sold in the market for economic gain or saved for commercial cultivation year after year.

This has created a severe shortage of quality planting material for cultivation. Poor seed set and germination is in
C. borivilianum (Jat and Bordia, 1990; Bordia 1993; Ramawat et al. 1996). So to fill the gap of demand and supply and to provide genetically uniforms planting material from a known source, micro propagation is one of the most desirable option.

MATERIAL AND METHODS

In our investigation, for *C. borivilianum* culture, the ex-plant was obtained from poly-house, situated at garden of Botany Department of Science College, Patna University.

The nodal and internodal portions of floral stem was used as explants. Explant was washed with running tap water for 15 minutes. Then the explants were left for 5-7 minutes in 20 drop savlon and water in beaker. It was again washed for 30 minutes in running water. Then explants were shaken is ethyl alcohol (70%) for 3 Minutes. Again shaken in 0.1% $Hgcl_2$ (w/v) for 3 minutes.

After surface sterilization the explants were rinsed 3 times in sterile distilled water. The culture medium and glass were autoclaved at 120^0c for 20 minutes. Instruments, such as forces, scalpels, and needle were sterilized by dipping in ethanol followed by flaming and cooling. The sterilized explants after washing with sterile distilled water (3-4) times were then implanted on the modified murashige and skoog's (1962) agar-gelled or liquid medium fortified with various concentration combinations of growth hormone and 3% sucrose. The pH of the medium was adjusted to 5.7 before auto calving at 1.04kg/cm^2 pressure and 120^0c temperature for 20 minutes.

The cultures were incubated at 25^0 C to $\pm$ 2^0 C in diffused light under 60-70% RH in culture room

RESULTS

Invitro cultures were established from nodal and internodal portions of floral stem which were used as explants. Explants were cultured on MS medium (Murashige and Skoog"s medium) fortified with various concentrations/combinations of growth hormones and 3% sucrose. In this study different types of auxins (NAA-Naphthalene acetic acid, 2,4 D- 2,4 dichloro phenoxyacetic acid, and IAA-Indolacetic acid) with various concentration were used for inducing callus in safed musli. The results showed that there was a considerable influence of 2,4D on callus induction where both NAA and IAA supplemented media showed no response. Satisfactory results were obtained when explants were cultured on MS medium supplemented with BAP (6-Benzyl aminopurine) and 2,4D (2,4-Dichlorophenoxy acetic acid). When nodal portion of floral stem were cultured on MS medium supplemented with 2.0mg/l BAP (6-Benzyl aminopurine) and 1.5mg/l 2,4-D, 25% of explants showed swelled after 7-10 days of inoculation and after 25-30 days of inoculation friable (loose mass) callus was observed in

swelled part of the explants. Table-1 showed the different concentration of BAP and 2,4D taken together and percentage of response of explants towards callusing. Best callus formation was observed on MS medium supplement with 2.0mg/l BAP and 4.0 mg/l

2,4-D. At higher concentration of 2,4-D (5.0 mg/l) calluising percentage was found to be lower. At the concentration of 2.0mg/l BAP and 4.0mg/l, 2,4-D only 70% explants, callusing was observed.

Response of explants of C. borivilianum on MS basal medium supplemented with different concentration of BAP and 2,4 D taken together.

Sl. No.	Types of basal medium	Types of explants	Days required	No. of tubes inoculated	No. of tubes with positive response in culture	% of explant response	Results
1.	MS + 3% Sucrose + 10% CM + 0.8% Agar 2.0 mg/l BAP + 1.5 mg/l 2,4-D.	Nodal portion of floral stem.	25-30	100	25	25	After 7-10 days of culture swelling appeared in explants then friable callus was appeared in swelled part of explants.
2.	MS + 3% Sucrose + 10% CM + 0.8% Agar + 2.0 mg/l BAP + 2.0 mg/l 2,4-D.	Nodal portion of floral stem.	25-30	100	47	47	After 7-10 days of culture swelling appeared in explants then friable callus was appeared in swelled part of explants.
3.	MS + 3% Sucrose + 10% CM + 0.8 Agar + 2.0 mg/l BAP + 2.5 mg/l 2,4-D.	Nodal portion of floral stem.	25-30	100	62	62	After 7-10 days of culture swelling appeared in explants then friable callus was appeared in swelled part of explants.
4.	MS + 3% Sucrose + 10% CM + 0.8% Agar + 2.0 mg/l BAP + 3.0 mg/l 2,4-D.	Nodal portion of floral stem	25-30	100	65	65	After 7-10 days of culture swelling appeared in explants then friable callus was appeared in swelled part of explants.
5.	MS + 3% Sucrose + 10% CM 0.8% Agar + 2.0 mg/l BAP + 3.5 mg/l 2,4 D.	Nodal portion of floral stem.	25-30	100	72	72	After 7-10 days of culture swelling appeared in explants then friable callus was appeared in swelled part of explants.
6.	MS + 3% Sucrose + 10% CM 0.8% Agar + 2.0 mg/l BAP + 4.0 mg/l 2,4-D.	Nodal portion of floral stem.	25-30	100	75	75	After 7-10 days of culture swelling appeared in explants then friable callus was appeared in swelled part of explants.
7.	MS + 3% Sucrose + 10% CM + 2.0 Mg/l BAP + 5.0 mg/l 2,4-D.	Nodal portion of floral stem.	25-30	100	70	70	After 7-10 days of culture swelling appeared in explants then friable callus was appeared in swelled part of explants.

Table-1

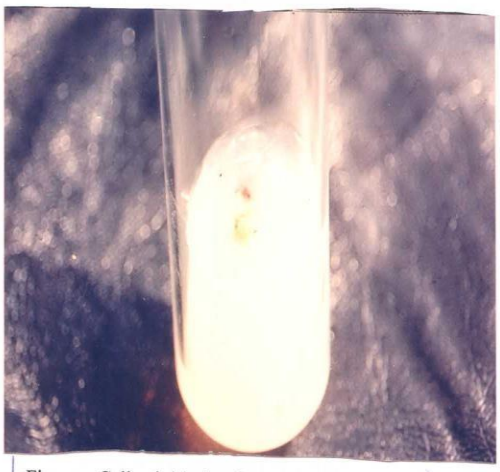

Fig. 1a : Callus initiation from nodal portion of floral stem explant in MS medium supplemented with 2 mg/1BAP (28 days old callus).

Fig.1b : Callus initiation from nodal portion of floral stem explant in MS medium supplemented with 2.0 mg/1 BAP and 4.0 mg/l 2,4-D (28 days old callus).

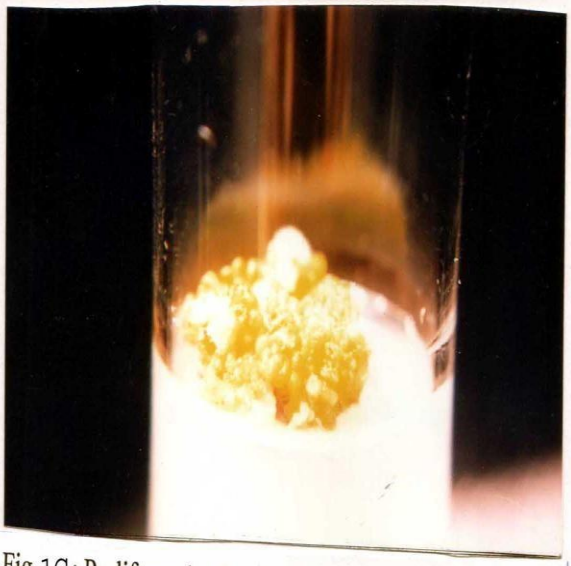

Fig.1C: Proliferated callus from nodal portion of floral stem explant in MS medium supplemented with 2 mg/l BAP and 4 mg/l 2,4-D (35 days old callus).

Fig.1D: Proliferated callus from nodal portion of floral stem explant in MS medium supplemented with 2.0 mg/l BAP and 4 mg/l 2,4-D (49 days old callus).

DISCUSSION

Now-a-days *Chlorophytum borivilianum* is indiscriminately exploited by people for food, medicine and commerces, rendering it endangered. To protect these germplasm from extinction it was thought worthwhile to initiate cultural

studies

In living system, regulation of growth and development has remain an abominable mystery even today. What requires elucidation is, understanding the physicochemical factors triggering the process of morphogenesis. So, this very rigorous exercise was performed on valuable endangered medicinal herb *C. borivilianum*. Keeping in the mind the doctrine of "totipotency" the selection of most favorable nutrient medium was carried out by culturing the leaf and nodal and internodal part of floral stem of *C. borivilianum*.

In the present investigation leaf explants showed little callusing but after sub culturing this callus died. Nodal and intermodal portion of floral stem showed higher percentage of callusing. Nodal portion performed better as explants because callusing percentage was higher in nodal portion than intermodal portion of floral stem.

Direct organogenesis from *C. borivilianum* explants was achieved by Rizvi et al., 2006, Dave et al., 2004; 2003; 2002,; Purohit et al., 2003; 1994. J oshi et al., 2003; and Kukda et al. (1994) established callus culture of *C. borivilianum*. In the present work when explants of *C. borivilianum* were cultured on MS medium supplemented with different concentration of BAP and 2, 4-D taken together callus formation was observed but still no morphogenesis.

Researchers are always in search of certain novel investigations that can have wide range of applications, in the present study callus culture of *C. borivilianum* was established using nodal and internodal portions of floral stem as explants on MS medium supplemented with BAP and 2,4-D either individually or in

combinations. Callus culture serves as a very good system for genetic manipulation of plants which are highly desirable in medicinal plants especially for metabolic engineering. Although the callus organogenetic pathway is not a preferred method for clonal plant propagation.

Agar is most frequently used as gelling agent in tissue culture. It / has been reported to differ in its action from batch to batch (Debergh, 1983) and subsequently show variation in its response due to interaction with media components (Romberger and Tabor, 1971), impurities (Naim et al., 1995) and gelling strength (Debergh, 1983). Agar is generally used at concentration 0.8-1.0 percent (w/v) for culture (Bhojwani and Razdan, 1983). Rizvi et al., (2006) used 0.8 percent w/v agar for the culture of *C. borivilianum*. In the present study at the concentration of 0.8 percent w/v agar, optimum gelling was achieved.

CONCLUSION

Thus, the present protocol is highly useful for transformation or genetic engineering studies. In addition this study facilitiates the possible extraction of bioactive compounds from Safed Musli callus tissue for various therapeutic applications without planting in the fields.

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