Int. J. Mendel, Vol. 35(1-2), 2018 & 36(1-2), 2019 151-163 ISSN0970-9649

MICROPROPAGATION OF *Chlorophytum borivilianum* SANT. et. FERNAND THROUGH CALLUS CULTURE

Sushma Singh and A.K. Gupta *

Key words : *Chlorophytum borivilianum*, micropropagation.

Chlorophytum borivilianum (Family Liliaceae), commonly known as Safed Musli is an important medicinal herb. Mainly its tuberous roots are used in herbal medicines, which are widely used as natural "sex tonic".
Due to its economic potential, this plant is now-a- days indiscriminately exploited by people for commercial purposes rendering the species endangered. Now-a-days biotechnological inventions are being used to conserve such valuable germplasm. Callus culture serves as a very good system for genetic manipulation of plants which are highly desirable in medicinal plants especially for metabolic engineering. For the purpose of micropropagation, cultural studies were initiated. In the investigation protocol for micropropgation of *Chlorophytum borivilianum* was standardized.

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 is rich in Safed Musli, its demand is increasing rapidly in Indian and International drug markets. Foreign demand has been estimated as 300-700 tones annually (Bordia et al., 1995), a quantity that Indian forest cannot sustain. Moreover obnoxious 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. There is poor seed set and germination 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 uniform planting material from a known source, micropropagation is one of the most desirable options.

MATERIALS AND METHODS

In our investigation, for *C. borivilianum* culture, the explant 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% Hgcl2 (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°c for 20 minutes. Instruments, such as forceps, 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 and combinations of growth hormone and 3% sucrose. The pH of the medium was adjusted to 5.7 before autocalving at 1.04kg/cm^2 pressure and 120°c temperature for 20 minutes.

The cultures were incubated at 25° C to + 2° 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. 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

***Department of Botany, Patna University, Patna, Bihar 800 005, India**

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 2,4-D taken together and percentage of response of explants towards callusing. Best callus formation was observed on MS medium supplemented 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.

Table-1

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/1 BAP +1.5 mg/12, 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/1 BAP +2.0 mg/1 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/1 BAP +2.5 mg/12, 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/1 BAP +3.0 mg/12, 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/1 BAP +3.5 mg/12, 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/1 BAP +4.0 mg/12, 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/1 BAP +5.0 mg/12, 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

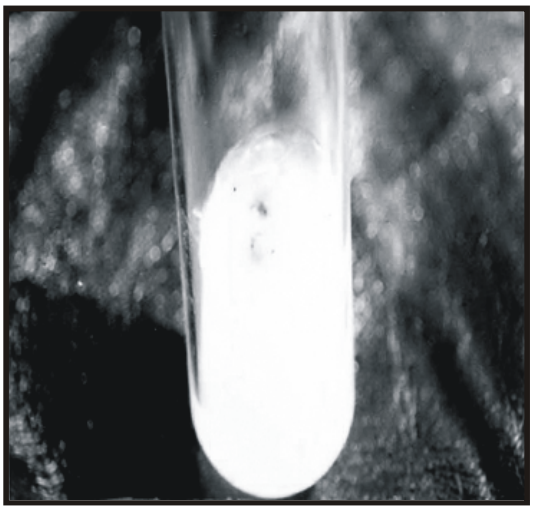

Fig. 1a : Callus initiation from nodal portion of floral stem explant in MS medium supplemented with 2 mg/1 BAP (28 days old callus).

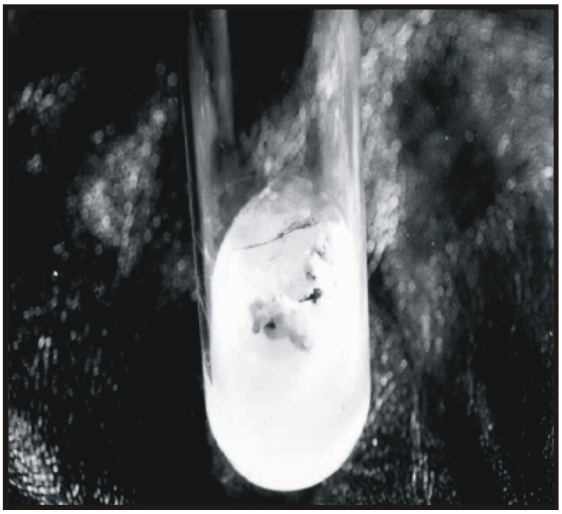

Fig. 1b : Callus initiation from nodal portion of floral stem explant in MS medium supplemented with 2.0 mg/1 BAP and 4.0 mg/12, 4-D (28 days old callus)

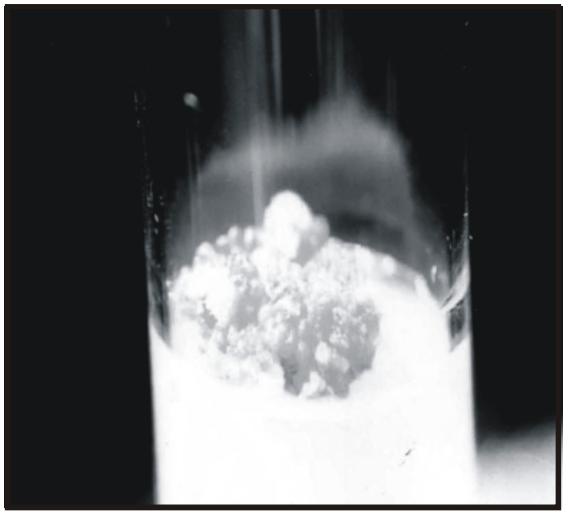

Fig. 1c : Proliferated callus from nodal portion of floral stem explant in MS medium supplemented with 2 mg/1 BAP and 4mg/12, 4-D (35 days old callus).

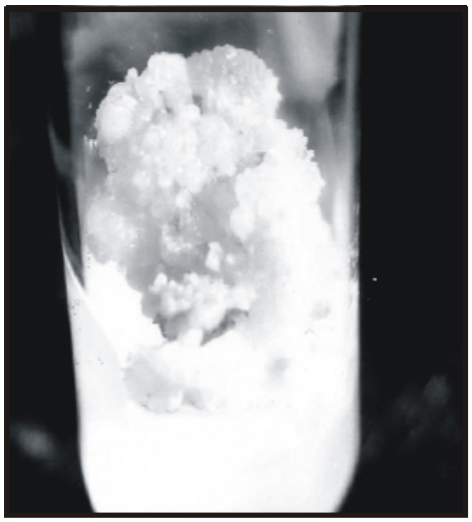

Fig. 1d : Proliferated callus from nodal portion of floral stem explant in MS medium supplemented with 2.0 mg/1 BAP and 4 mg/12, 4-D (49 days old callus).

Shooting of *in vitro* produced callus

In the present investigation shoot initiation from callus and shoot proliferation were achieved by using a range of combination of BAP and Kinetin (Table-2). In the present investigation BAP alone was able to initiate and proliferate the shoot but kinetin alone was not capable to initiate and proliferate the shoot. It was observed that 82% callus showed shooting when the callus was cultured with 4.0mg/l BAP and 1.0mg/l kinetin. Maximum shoot proliferation (25-30 shoots per tube) was observed at this concentration.

Table-2

Response of callus of C. borivilianum on MS basal medium supplemented with different concentration of BAP and Kinetin taken together

Sl. No	Types of basal medium	No. of tubes inoculated	No. of tubes with positive response towards shooting	% of explant response	No. of shoots in each tube	Days required
1	MS +3% sugar +10 CM +0.8% Agar +1.5 mg/1 BAP +1.0mg/1 Kinetin.	100	38	38	11-15	20-25
2	MS + 3% Sugar +10% CM +0.8% Agar +2.0 mg/1 BAP +1.0 mg/1 Kinetin.	100	41	41	14-17	20-25
3	MS + 3% Sugar +10% CM +0.8% Agar +2.5 mg/1 BAP +1.0 mg/1 Kinetin.	100	53	53	14-20	22-27
4	MS + 3% Sugar +10% CM +0.8% Agar +3.0 mg/1 BAP +1.0 mg/1 Kinetin.	100	70	70	14-22	25-27
5	MS + 3% Sugar +10% CM +0.8% Agar +3.5 mg/1 BAP +1.0 mg/1 Kinetin.	100	75	75	20-25	25-30
6	MS + 3% Sugar +10% CM +0.8% Agar +4 mg/1 BAP +1.0 mg/1 Kinetin.	100	82	82	25-30	25-30

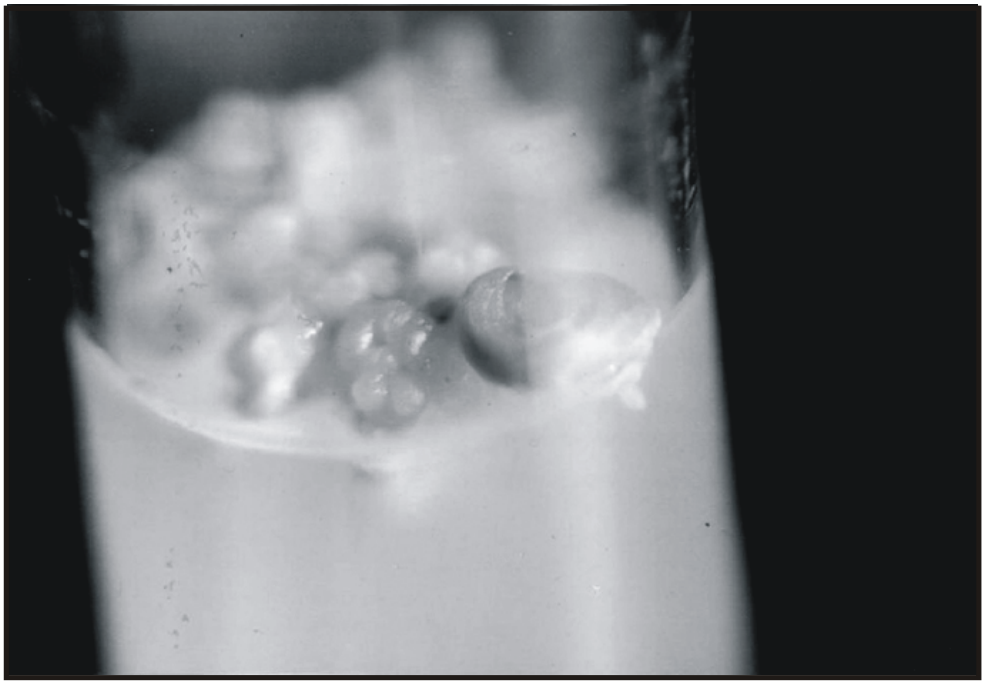

Fig. 2a : Shoot initiation from callus in MS medium supplemented with 2.5 mg/1 BAP and 1.0 mg/1 Kinetin (15 days old culture)

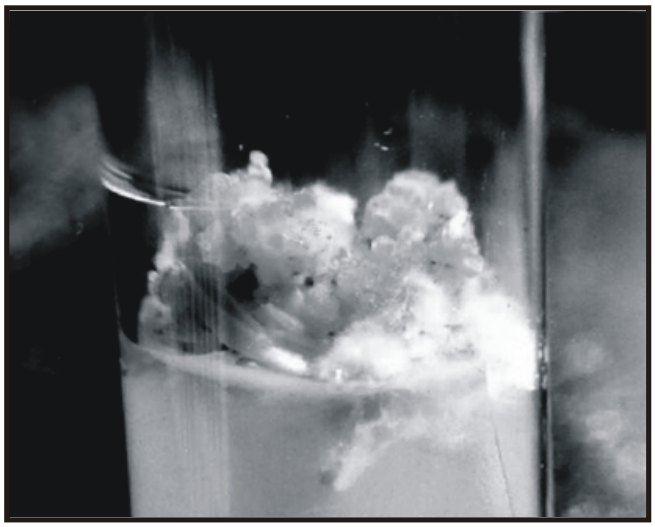

Fig. 2b : Shoot initiation from callus in MS medium supplemented with 3.5 mg/1 and 1.0 mg/1 Kinetin (21 days old culture).

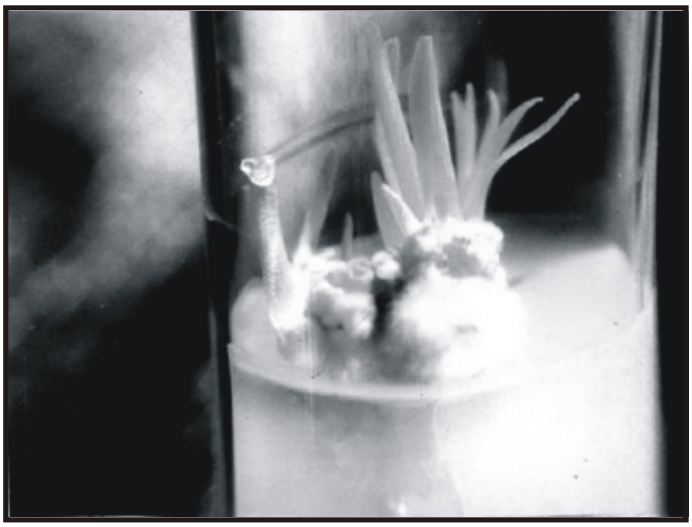

Fig. 2c :Shoot initiation from callus in MS medium supplemented with 3.5 mg/1 BAP and 1.0 mg/1 Kinetin (28 days old culture).

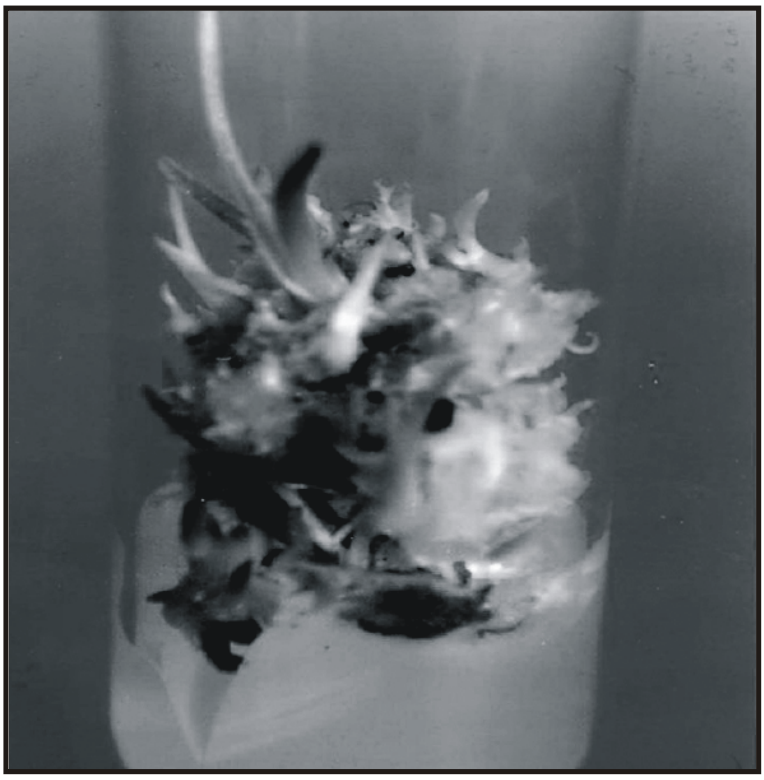

Fig. 2d : Profuse shooting from callus in MS medium supplemented with 3.5 mg/1 BAP and 1.0 mg/1 Kinetin (42 days old culture).

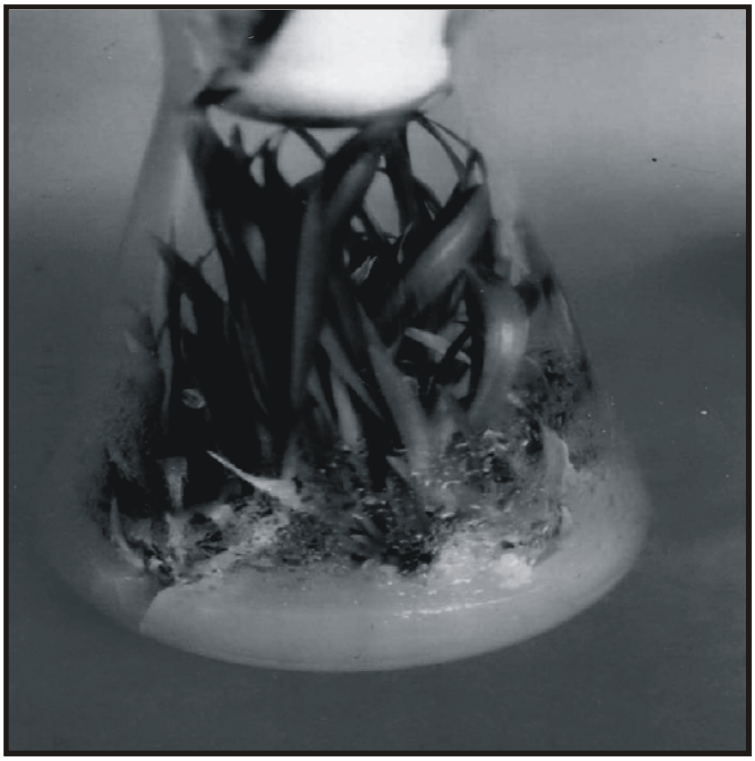

Fig. 2e : Profuse shooting from callus in MS medium supplemented with 4.0 mg/1 BAP and 1.0 mg/1 Kinetin (42 days old culture).

Rooting of *In vitro* develop shoots

To induce rooting in the regenerated shoot, it was excised from the multiple culture and transferred on to liquid culture medium fortified with various concentrations/ combinations of rooting hormone. IBA (Indole -3- butyric acid) and IAA(indole-3-acetic acid) have recorded same capacity for root initiation where as NAA (Naphthalene acetic acid) has slightly better response for rooting. Better results were recorded when combination of two rooting hormone were supplemented in MS medium in different concentration. Table -3 reveals the effects of rooting phytohormones on shoots. Best rooting response was observed on MS medium supplemented with 1.5mg/IAA, 1.5 mg/l NAA and 2.0mg/l BAP. At this concentration rooting frequency was noted to 72%.

Table-3

Response of shoots of C. borivilianum of MS medium supplemented with BAP and two different rooting hormones in different concentration.

Sl. No	Types of basal medium	No. of tubes inoculated	No. of tubes with positive response towards rooting	% of shoots response towards rooting	Days required
1	MS +3% sugar +10% CM +1.5 mg/1 IBA +1.0 mg/1 NAA +2.0 mg/1 BAP.	100	65	65	30-35
2	MS +3% sugar +10% CM +1.5 mg/1 IBA +1.5 mg/1 IAA +2.0 mg/1 BAP.	100	70	70	30-35
3	MS +3% sugar +10% CM +1.5 mg/1 IAA +1.5 mg/1 NAA.	100	72	72	30-35

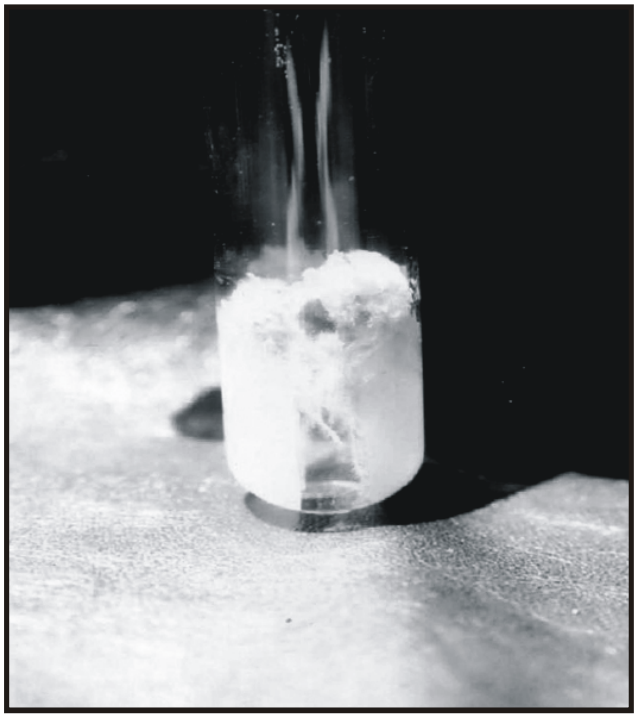

Fig. 3a : Profuse rooting from callus in MS medium supplemented with 1.5 mg/1 IBA (28 days old culture).

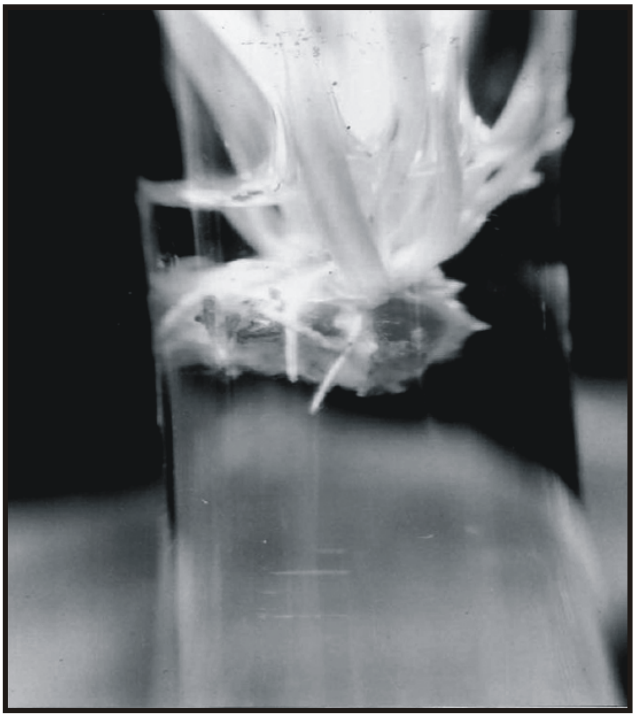

Fig. 3b : Profusely grown fibrous root from shoot culture in MS medium supplemented with 1.5 mg/1 IBA, 1.5 mg/1 NAA and 2.0 mg/1 BAP (42 days old culutre).

Hardening of In vitro produced plantlets

The water holding capacity of soil is an important parameter, therefore in order to find out the suitable soil moisture, plant was transferred to plastic pot having different soil mixture. It was observed that vermicompost manure has beneficial effect on survival rate as well as subsequent growth of the plant. After 45 days of culture of microshoots on rooting medium sufficient rooting plantlets were transplanted to plastic pot containing garden soil and vermicomost manure (1;1). Rooted plantlets that were from culture tubes to plastic pots containing mixture of 1:1 ratio of soil : vermicompost for their hardening period to their final transfer to the soil, showed 50% survival in polyhouse.

Fig. 4a : Plastic pots showing *In vitro* raised young plants in culture environment.

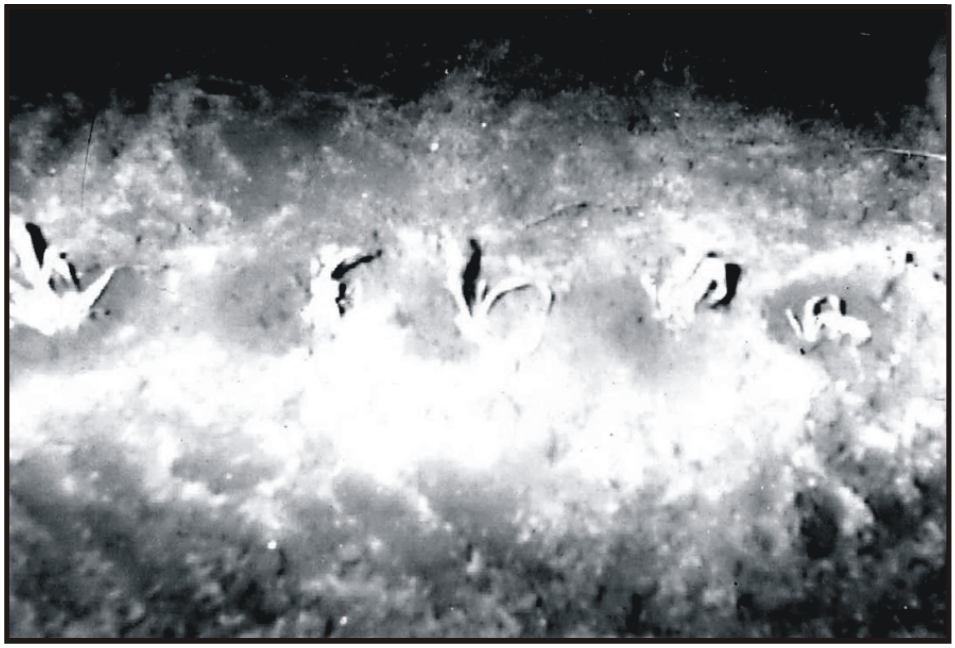

Fig. 4a : *In vitro* raised plants transferred to soil in natural environment.

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 mind the doctrine of totipotency the selection of most favourable 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 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. In the present study, for shooting, agar medium was used, but for rooting mostly agar less (liquid) medium was used.,

For shoot proliferation, growth regulators especially cytokinins (Lane, 1979; Stoltz, 1979; Bhpjwani 1980; Garland and Stoltz, 1981) are one of the important factors affecting the response. A range of cytokinins (*Kinetin, BA, BAP,* 2-ip and Zeatin) have been used in micropropagation work (Bhojwani and Razdan, 1983). In white clover (Bhojwani, 1981), hybrid willow (Bhojwani, 1980) and Chickpea (Barna and Wakhlu, 1994), BA was the most effective cytokinin for the shoot tip, meristem and bud culture. At higher levels, cytokinins tend to induce adventitious bud formation (McComb, 1978; Zimmerman and Broome, 1980). Purohit et al. (1994) used MS media supplemented with 22.2 M BA for shoot multiplication of C. borivilianum. Joshi et al. (2003), Suri et al. (1998) utilized MS media supplemented with 5.0 mg/L). The combination of 4 mg/l BAP and 1 mg/l Kinetin was found to be optimum. In the present investigation, it was observed that 82 per cent callus showed shooting when these callus were cultured on MS medium supplemented with 4.0 mg/l BAP and 1.0 mg/l Kinetin. In the present study shooting was callus mediated not direct from explants. Purohit et a1. (1994), Suri et al. (1998), Joshi et a1.(2003) and Rizvi et a1. (2006) observed directed organogenesis (shooting) from explants of *C. borivilianum*.

Rooting response of microshoots is reported to be controlled by growth regulators in the medium (Bhojwani and Razdan, 1983) basal salt composition (Garland and Stoltz, 1981; Zimmerman and Broome, 1981), genotype (Rines and McCoy, 1981) as well as cultural condition (Murashige, 1977). Auxins are known to induce rooting in the microshoots shoots (Blackesly and Chandwett, 1993) Bonza et al. 1994). NAA and IBA are most commonly used for root induction (Bhojwani and Razdan, 1983).

In the present project for rooting mostly liquid medium was used. In the present investigation IBA, IAA and NAA was used either individually or in combination of two for Invitro rooting of microshoots of C. borivilianum. 72 percent rooting was observed when microshoots were cultured on MS medium supplemented with 1.5 mg/l IAA + 1.5 mg/l NAA and 2.0 mg/l BAP. In the present project it was observed combination of two rooting hormones gave better result than single hormone.

Hardening of tissue culture plants is the most crucial steps in micropropagation. The plants produced are very soft to ambient environmental condition (Bhojwani and Razdan, 1983) during acclimatization. Under these conditions the leaves of plants develop cuticle and its photosynthetic stem starts functioning. The most crucial stage is during first 10 days in polyhouse.

The water holding capacity of soil is an important parameter, therefore during hardening experiments various soil mixture were used. In the present study it was observed that soil mixtures containing Vermicompost showed better results. It may be due to the high organic content present in the mixture, that supported the plant growth. The role of organic matter present in soil in hardening of *C. borivilianum* had been specified by Oudhia et al. (2001).

In present investigation rooted plantlets that were transferred from culture tubes to plastic pots containing 1:1 ratio of soil:

vermicompost for their hardening prior to their final transfer to the soil, showed 50 percent survival in polyhouse.

CONCLUSION

The present research was undertaken to explore the possibility of cloning strategies for large scale production of *In vitro* regenerated and improved *C. borivilianum* plants.

Except MS medium, none of the nutrient medium formulations supported the growth in culture. Although MS basal medium too, did not support any morphogenetic response.

The objectives of the present study was to standardize conditions for establishment of axenic culture from elite germplam, shoot proliferation , rooting of microshoots, hardening and transfer of plants to soil.

REFERENCES

Bhojwani, S.S., and Razdan, M.K. (1983). Plant tissue culture Theory and Practice, Elsevier, Amsterdam, London, New York, Tokyo.

Bordia, P.C., Joshi A., and Simlot M.M. (1995). Safed Musli. P. 429- 451. In: K.L. Chadha and Rajendra Gupta (eds.), Advances in horticulture Vol. II Medicinal and aromatic plants, Malhotra Publ. House, New Delhi.

Bhojwani, S.S. (1980). Micro propagation method for hybrid willow (Salix matsudanax alba NZ-10002), N.Z.J. Bot., 18: 209-21.

Barna, K.S., and Wakhlu, A.K. (1994). Whole Plant regeneration of Cicer arietium from callus culture via organogenesis, Plant Cell reports, 13:510- 513.

Blakesley, D., and Chaldecott, M.A. (1993). The role of endogenous auxins in root initiation. II Sensitivity and evidence from studies on transgenic plant species. Plant Growth Regul. 13:77-84.

Bhojwani, S.S. (1981). A tissue culture method for propagation and low tempeature storage of Trifolium repens genotypes, physiol. Plant, 52:187-190 Cellular and Development Biology Plant, 33: 44-46.

Dave, A., Purohit, S.D., Bilochi, and G., Joshi, N. (2002). Scaling-up production and field performance of Micro propagated medicinal Herb. 'Safed Musli' (chlorophytum borivilianum). In vitro cellular and Developmental Biology- Plant, volume 39, Number 4, July 2003, pp. 419-425 (7).

Dave, A., Purohit, S.D., Bilochi, G., and Joshi, N. (2003). Factor & influencing In vitro culture of Chlorphytum borivilianum oikoassay 15 (1 and 2): 19-27.8.

Dave, A., Sharma, P., and Purohit, S.D. (2004). In vitro propagation of Chlorophytum borivilianum using encapsulated shoot buds. Euro. J. Hort., SCi. 69(1): 37-42.

Deberg, P.C. (1983). Affect of agar brand and concentration on the tissue culture medium. Physiologia Plantarum 59:270-6.

Garland, P., and Stoltz, L.P. (1981). Micro propagation of Pissrdi Plum, Ann. Bot., 48:387-389.

Joshi, N., Purohit, S.D. Dave, A. and Bilochi G., (2003). Factors influencing In vitro culture of chlorophytum borivillianum oikoassay 15 (1 and 2) : 19-27.8.

Kothari, S.K., and Singh, K. (2003). Production technique for the cultivation of safed musli (chlorophytum borivilianum). J. Horticulture Sci. Biotech. 78(2) 261-264.

Kukda, G., Purohit, S.D., and Dave, A. (1994). Somatic embryogenesis and plantlet regeneration in safed musli. Indian journal Plant Genetic Resources 7 (1) : 65-71.

Lane, W.D. (1979). In vitro propagation of spirea bumalda and prunus cistena from shoot apics, Can. J. plant Sci. 59: 1025-1029.

Maiti, S., and Geetha, K.A. (2005). Characterization, genetic diversity and cultiation of Chlorophytum borivilianum - an important medicinal plant of India. Plant genetic Resources Characterization and utilization. 3(2) 264- 272(9).

Murashige; T., and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tabacco tissue cultures, Physiol. Plant 115: 493-497.

McComb, J.A. (1978). Clonal propagation of woody plant species with special reference to apples, proc. Int. Plant Prop. Sc. 28 : 413-426.

Murashige, T. (1977). Clonal Crops through tissue culture. In:W. Barz et al. (Editors), Plant Tissue Culture and its use in Biotechnological

Application, Springer-verlag, Berlin 392-403.

Naim B. Furneaux, R.H., and Stevenson, T.T. (1995). Identification of an agar constituent responsible for hydric control in micropropagation in radiata pine. Plant cell, Tissue and Organ Culture 43: 1-11.

Oudhia, P. (2001). Problem perceived by safed musli (Chlorophytum borivilianum) growers of Chhattisgarh (India) region: A study Journal of Medicinal and Aromatic Plant sciences. 22/4 A and 23/1A : 396-399.

Oudhia, P. (2001 b). Improved cultivation practices for medicinal crops: Glimpses of research at farmer's fields in Chhattisgarh (India). In souvernier cum abstracts. (Editor: P. OUDHIA) National Research Seminar on Herbal conservation, cultivation, marketing, and utilization with special emphasis on Chhattisgarh, "The Herbal State" Srishti Herbal Academy and Research Institute (SHARI) AND Chhattisgarh Minor Forest Produce (trading and dev) Co-operative Federation Ltd., Raipur (India), 13-14 December, 2001, pp. 44.

Oudhia, P. (1996). Parthenium : A curse for the bio-diversity of Chhattisgarh plains. National Reen. R.D. Govt. P.G. College, Mandla,

Indian, 30 July p. 26

Purohit, S.D., Dave, A., and Kukda, G., (1994). Micro propagation of safed musli (Chlorophytum borivilianum), a rare Indian medicinal herb. Plant cell Tiss. Org. Cult. 39: 93-96.

Purohit, S.D., Dave, A., Bilochi, G., and Joshi, N. (2003). Factors influencing In vitro culture of Chlorophytum borivilanum Oikoassay 15 (1 and 2) : 19-27

Ramawat, K.G., Sharma, R., Jain S. and Suri, S.S., (1996): Problem of propagation of desert plants : seed Biology and Biotechnological

Approaches. In : seed Technol., G.S. Paliwal (ed.), Srinagar, pp. 98-100.

Oudhia, P. (2001 b). Improved cultivation practices for medicinal crops: Glimpses of research at farmer's fields in Chhattisgarh (India). In souvernier cum abstracts. (Editor: P. OUDHIA) National Research Seminar on Herbal conservation, cultivation, marketing, and utilization with special emphasis on Chhattisgarh, "The Herbal State" Srishti Herbal Academy and Research Institute (SHARI) AND Chhattisgarh Minor Forest Produce (trading and dev) Co-operative Federation Ltd., Raipur (India), 13-14 December, 2001, pp. 44.

Oudhia, P. (1996). Parthenium : A curse for the bio-diversity of Chhattisgarh plains. National Reen. R.D. Govt. P.G. College, Mandla,

Indian, 30 July p. 26.

Purohit, S.D., Dave, A., Bilochi, G., and Joshi, N. (2003). Factors influencing In vitro culture of Chlorophytum borivilanum Oikoassay 15 (1 and 2) : 19-27.

Ramawat, K.G., Sharma, R., Jain S. and Suri, S.S., (1996): Problem of propagation of desert plants : seed Biology and Biotechnological

Approaches. In : seed Technol., G.S. Paliwal (ed.), Srinagar, pp. 98-100.

Rines, H.W., and McCoy, T.J. (1981). Tissue culture initiation and plant regeneration in hexaploid species of Oats, Crop Science, 21: 837-842.

Rizvi, M.Z., and Kukreja, A.K. (2006). Current science, Vol. 92, No. 1. 10 January.

Romberger, J.A., and Tabor, C.A. (1971). The Picea abies shoot meristem in culture. 1. Agar and auto claving effects. American Journal of Botany 58:131-140.

Suri, S.S. Jain, S., Arora, D.K. and Ramawat, K.G. (1998). In vitro high frequency regeneration of plantlets and tuberous root formation in Chlorophytum borivillanum. Gartenbau, 64(3) : 106-110.

Stoltz, L.P. (1979). In vitro propogation of Acalypha wilkesiana, Hort science, 14 : 702-703.

Zimmerman, R.H., and Broome, O.C. (1980). Blue berry micropropagation. In: proceedings of the conference on Nursery production

of fruit plants through Tissue culture - Application and Feasibility. Agric. Res. Sci. Educ. Admin., U.S.D.A., Beltsville 44-47.

Zimmerman R.H., and Broome, O.C. (1981). Phloroglucinol and In vitro rooting of apple cultivar cuttings, J. Am. Soc. Hortic. Sci., 106 : 648-652.