ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 4, Issue, 05, pp.095-097, May, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

In vitro propagation of *Chlorophytum borivilianum* (Safed Musli) and its root regeneration

A.M. Jakkulwar and V. S. Wadhai

Department of Biotechnology, Sardar Patel Mahavidyalaya, Chandrapur-442402 (India)

ARTICLE INFO

Article History:
Received 20th February, 2012
Received in revised form
25th March, 2012
Accepted 18th April, 2012
Published online 30th May, 2012

Key words:
Chlorophytum borivilianum,
Tissue culture,
Plant growth regulators,
Micropropagation.

ABSTRACT

Roots of *Chlorophytum borivilianum* (safed musli) were regenerated by taking explants from isolated field grow mature plants. Two types explants stem disc and shoot base were used in the micropropagation. This was achieved by culturing on MS solid medium with and without harmones. Both leaf base and tuber explants gave very poor response. MS medium containing 3mg/l NAA gave more number of roots. The root proliferation was observed on full strength of MS medium after inoculation with the increasing concentration (1 to 3 mg/ml) for Indole acetic acid (IAA) at 1mg/l and Napthaline acetic acid (NAA) at 3mg/l. Micropropagation is to be the effective method compared with other methods of multiplication. This protocol can be used to generate cost-effective protocol for large scale *in vitro* cultivation of safed musli.

INTRODUCTION

Medicinal plants are of great interest to the researchers in the field of biotechnology as most of drug industries depend, in part, on plants for the production of pharmaceutical compounds (Chand *et al.,*1997). Roots are the economic parts and having aphrodisiac properties due to presence of steroidal saponins, viz., neotigogenin, stegmasterol, tokoregenin. Conventional method of propagation of this plant is through root tubers, since poor seed germination restricts its use. Tissue culture methods have been successfully used for multiplication of many medicinal plants and several others members of *Liliaceae.* Indian herbal industry is at a blooming stage now-a-days. There is an increasing awareness towards consumption of herbal medicines. Large numbers of plants have medicinal properties like Aloe, Jatropa, Satavari, Piper methystium, Ginkgo etc. One of such important medicinal plant is *Chlorophytum borivilianum* Sant and Fern. The genus includes about 300 species, which are distributed throughout the tropical and subtropical parts of the world (Canter *et al.,*2005)

Safed musli, one of the finest Indian herbs,is a traditional medicinal plant. Its tuberous root are used to prepare herbal products and ayurvedic medicine,espscially a nutritive tonic used for curing general sexual weaknass.It is a rich source of over 25 alkaloids, vitamins, mineral, proteins, carbohydrates, steroids and carbohydrates. The demand of safed musli is not only in India but all over world which has made it famous by such names as 'Indian Viagra','Roots of gold','Herbal Viagra", "The wonder crop etc. Safed Musli a unique gift of nature to mankind since the time immemorial.Safed musli 'a golden root' is a unique gift of nature to mankind since time immemorial as an alternative to chemical aphrodisiac. Safed musli botanically known as *Chlorophytum borivilianum* belongs to family *Liliaceae.* The genus *Chlorophytum* comprises of about 250 other species which are distributed in tropical and subtropical parts of the world. Seventeen among them are found in India, the most common and commercial being *Chlorophytum borivilianum* which is diploid with basic chromosome number 2n=16 (Kumar and Subramanium,1986). It has exorbitant market demand in domestic and global market as aphrodisiac.

There is a lot of demand for its processed products. But the production is far behind the demand. The estimated global market demand is approximately35000 tonnes/annum. Presentaly estimated production is 5000 tonnes/annum which is not even 5 % of the estimated demand. Current global trade in medicinal plant is more than $ 60 billion which is expected to rise to 222 billion by 2050.India earn more than $ 3000 billion per year as foreign exchange from products of medicinal and aromatic plants (Biradar, 2005).

MATERIALS AND METHODS

Safed musli (*Chlorophytum borivilianum* Sant and Fern) plants were collected from Shri Shale Medifarm, Nagpur (M.S.) in month of October 2011. Stem disc and shoot base portion were used for culturing. Vegetative propagated plants raised under moist house condition were carefully brought to laboratory and washed 3-4 times under running water and then different explants were treated and soaked with detergents like teepol or tween-20 + systematic fungicide

Corresponding author: spmicro1747@rediffmail.com

Table No.1 Contamination, survival and days taken for ro

Medium Code	Contamination	Survival	Days tak root initiat
IT1	+	–	
IT2	–	+	23
IT3	–	+	25
IT4	–	+	28

bavistin (0.1%) + 0.2 per cent streptomycin sulphate. Further surface sterilization of these explants was done with mercuric chloride ($HgCl_2$) at concentration of 0.1 per cent for 5 minutes duration except tuber (10-12 minutes) and finally these explants were washed with double sterilized water for 3-4 times before inoculation. Auxins are used to induce cell division and root differentiation. E.g. IAA, NAA.

Research, Vol. 4, Issue, 05, pp.095-097, May, 2012

vitro propagation of roots of safed musli (*Chlorophytum borivilianum)* Murashige and Skoog (1962) medium was used for the present investigation. The stock solutions of macronutrients (31.25 ml), vitamins (5ml) were mixed in the required proportion and three per cent sucrose per litre was added and after dissolving sucrose, growth regulators (as per treatment requirements) were added and volume was made up to 250 ml by adding distilled water. The pH of the medium was adjusted between 5.6 to 5.8 by using either 0.1 N HCl or NaOH with the help of a digital pH meter. Finally the volume was adjusted (250 ml) and required amount of agar (1.5 g/250 ml) was then added and boiled to clear it. The medium was then immediately

dispensed into culture tubes or bottles, the media was autoclaved at 121°C at 15 lbs/square inch pressure for 20 minutes. Inoculation was carried out under aseptic conditions inside laminar air flow chamber, The cultures were incubated in an air conditioned room at a temperature of 25±2°C Under 16:8 hour light: dark photoperiods. All cultures were examined daily and the observations were recorded.

RESULTS AND DISCUSSION

Maximum contamination percentage (80%) was recorded in case of tuber explants followed by leaf base explants (27.4%).Maximum survival percentage (80%) was noticed in case of stem disc and shoot base explants (77.5%).But survival percentage was absolutely nil in case of leaf base and tuber explants. Significant differences were noticed among the growth regulator levels with respect to root initiation. Cultures on MS medium containing 3mg/l NAA (NT6) and 1 mg/l IAA (IT2) took minimum number of days 22 and 23 days for root initiation respectively. On the other hand the cultures on MS medium with other concentration of NAA and IAA required more number of day's i.e up to 30 days. Traditionally, safed musli could be multiplied by planting seed tubers, with an annual multiplication rate of six to eight folds. Like seed, seven to eight months dormancy is recorded in the fleshy roots of *Chlorophytum borivilianum* (Jat and Bordia, 1990). Due to the fact of long tuber dormancy period, only one crop per year can be grown.

In general, various explants viz., meristems, shoot tips, hypocotyl, epicotyl, seeds, singal nodal cuttings, leaf disc, tuber disc, roots, etc., can be used for micropropagation. In the present investigation, two different explants from grown up safed musli plants raised on earthen pots through vegetatively viz., shoot base and stem disc were used for standardization of better explants to achieve rapid multiplication of safed musli plantlets. From the results following out comes could be observed. As the first step in tissue culture of any plant involves the establishment of aseptic cultures and the sterilization of the explants is very important Therefore, explants must be free from all the microbial contaminants when they are placed on a nutrient medium. To achieve this objective in the present study the explants were treated with various chemical solutions. From results, it could be observed that above treatment found to be better for shoot base and stem disc explant to generate a root resulting least (10%) contamination percentage and highest (90%) survival percentage. Present results are in agreement with (Suri *et al.,*1999; Patil *et al.,*2002; Mishra *et al.,*2004).

In the present study, significant differences observed for root induction from shoot base and stem disc on the MS media both full strength and ¾ strength used in the study. Significant differences were also observed by using 1, 2, 3, 4 mg/lit NAA and 0.5, 1, 1.5, 2, mg/lit IAA. This result is in conformity with the findings of Pudake and Dhumale (2003).The MS media containing high concentration of salt (full strength of MS) have produce more number of roots (++++, intense) with NAA concentrations ranging 2 to 3mg/lit (T6 to T14). This result is supported by Ranjan (1998). Plantlets on MS media having low concentrations of salts (3/4 of MS) produced the highest root length (4.21 cm) at 3mg/lit NAA (T7) .Similar type of result observed for IAA of 1mg/lit. This result is in agreement with findings of Ranjan (1998), Pudake and Dhumale (2003). By the combination of 3mg/lit NAA and 1mg/lit IAA with full strength of MS shown maximum root yield within 19 days, whereas with same concentration on ¾ strength of MS shown maximum root length within 29 days.

CONCLUSION

Two different explants viz., stem disc, shoot base were used for micropropagation and it was observed that stem discs gave the quickest response and maximum survival percentages (80%). Growth regulators Auxin like, IAA and NAA at different concentrations and combinations were used for the study. Survival percentage of leaf base and tuber explants was nil. The growth regulator combinations of IAA (1 mg/l) and NAA (from 2 to 3 mg/l) resulted in intense root proliferation. Maximum root length (4.21 cm) was observed on ¾ strength of MS medium with 3 mg/l NAA+/1mg/lit IAA. Maximum root yield was observed on full strength of MS medium with 3 mg/l NAA+/1mg/lit IAA.

REFERENCES

Biradar, M.S. 2005. Tissue Culture Studies in Safed Musli (*Chlorophytum borivilianum*), M.Sc (Agri) Thesis, University of Agriculture Science, Dharwad.

Canter H.P.,Thomas, H.and Ernst, E. 2005. Bringing Medicinal Plants into Cultivation: Opportunities and Challenges for Biotechnology. Trends in Biotechnology, Vol 23, No 4.

Chand,S., Sahrawat, A.K. and Prakash, DVSSR 1997. In Vitro Culture of *Pimpinella anisum L. (Anise)* Journal of Plant Biochemistry Biotechnology 6:1-5.

Jat, R.D. and Bordia, P.C.1990. Comparative Performance of Transplanted Seedlings of Safed Musli (*Chlorophytum* species) from Sexual and Asexual Means, Crop Breeding Research Newsletter 1(1-2): 14-15

Kumar, V. and Subramanium, B. 1986. Chromosome Atlas of Flowering Plants of Indian Subcontinent(B.S.I.,Calcutta), Vol.2. Monocot.

Mishra, A., Pandey R. K. and Gupta, R.K. 2004. Micropropagation of Tuberose (*Pollianthus tuberosa* L.) cv. Culcuttia Double. Progressive Horticulture, 37(1):226-230.

Murashige, T. and Skoog, F. 1962. A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Culture. Physiological Planetarium, 15:423-497

Patil, G.D., Pudake, R.N. and Dhumale, D.B. 2002. Response of Indian Aloe Barbadensis Miller to In Vitro Culture. *Journal of Maharashtra Agricultural Universities*, 28 (3): 267-268.

Pudake, R.N. and Dhumale,V 2003. In Vitro Multiplication of *Chlorophytum borivilianum* Sant and Fern. *Journal of Maharashtra Agricultural Universities*, 28 (3): 265-267.

Ranjan, M.P. 1998. In Vitro Propagation of Cucumber (*Cucumis sativus* L.) M.Sc (Agri)Thesis. University of Agricultural Sciences, Bangalore.

Suri, S.S., Jain, S., Arora, D. K. and Ramavat, K.G. 1999. In Vitro High Frequency Regeneration of Plantlets and Tuberous Root Formation in *Chlorophytum borivilianum.* Gartenbau Wissenschaften, 64 (3): 106-110.