INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

MICROPROPAGATION AND FIELD PERFORMANCE OF *CHLOROPHYTUM BORIVILIANUM*

Garima V and Shruthi SD*

P.G. Department of Biotechnology, The Oxford College of Science, Bangalore 560102, Karnataka, India

Article Received on: 22/05/12 Revised on: 30/06/12 Approved for publication: 02/08/12

*Email: sdshruthi@gmail.com

ABSTRACT

Medicinal plant *Chlorophytum borivilianum* also called Safed musli, is an endogenous medicinal plant of India and distributed in certain parts of India like Assam, Eastern Ghats, Eastern Himalayas, Bihar and Andhra Pradesh. It is valued for its tuberous roots having aphrodisiac properties. Farmers in India cultivate this medicinal herb on a commercial scale because of its high economic value. To cater the growing demand for planting material, a highly responsible field-tested and cost-effective micropropagation scheme has been developed. Best shoot multiplication was achieved on agar- gelled MS medium containing 22.2mM6-benzylaminopurine (BAP) and 3% sucrose. With the optimized conditions, more than 15,000 plantlets could be produced in 20 week. Plantlets subjected to hardening under agro-shadenet conditions during the monsoon months of high humidity showed better survival rate and growth compared to plantlets hardened *in vitro* and subsequently transferred to the greenhouse for acclimatization. Rate of plantlet survival was 87% and 90% under open field and agro-shadenet conditions, respectively. Plantlets grown *ex vitro* under agro-shadenet and field conditions produced tuberous roots which could be grown in the next season as a secondary propagule. *In vitro* production of safed musli was cost effective compared to conventional propagation and holds great potential for commercial production.

Key words: aphrodisiac herb, callus culture, *Chlorophytum borivilianum,* shoot culture, micropropagation, root tubers

INTRODUCTION

Plant tissue culture is one area of biotechnology that had a dramatic impact on agriculture. Plant tissue culture is the only way to increase the number of plant within a short time period. Teoretically, a single cell or piece of plant tissue can produce an infinite number of new plants. The main industrial goal of plant tissue culture is to produce a large number of plants in a short period of time. *Chlorophytum borivilianum* belongs to family Liliaceace, commonly known as safed musli is one of the endangered species valued for the dried fasciculated storage roots[1]. These are reputed to have aphrodisiac properties and form an important ingredient of herbal tonics prescribed in the Ayurvedic system of medicine in India[2]. The plant is of economic importance in India since its dried roots are currently sold in the market at a price of Rs. 1500/- (US$30) per kilogram. Due to large scale and indiscriminate collection of wild material and insufficient attempts either to allow its replenishment or its cultivation, *C. borivilianum* is rapidly disappearing. The natural regeneration of this herb is through tuberous roots that have become scarce in nature. And the rate of seed germination is only 14 – 16%. *C. borivilianum* has a very short life cycle of 90 - 100 days. The tuberous root of this plant is the only propagule which can either be sold in the market for economic gains or saved for commercial cultivation year after year. This has created a severe shortage of planting material for large scale cultivation. To fill the gap of demand and supply and to provide genetically uniform planting material from a known source, micropropagation is the best alternative. Micropropagation technology has been widely applied for commercial cultivation of many horticultural, ornamental and forest plants[3,4]. But the reports on large-scale multiplication of medicinal herbs are few. Protocol for micropropagation of *C. borivilianum* and strategies for scale-up production alongwith evaluation of field performance of micropropagated safed musli is to be followed at laboratory level. The method of micro-propagation goes through several stages-establishment, multiplication, pretransplantation and finally transfer from culture. In the final stage, the processed plantlets are removed from the plant media and transferred to soil or more commonly to potting compost for continued growth by conventional methods. The major advantage of micro-propagation is the production of many plants that are clones of each other. It can also be used to produce disease free plants. It produces rooted plantlets ready for growth, saving the time for the grower when seeds or cuttings are slow to establish or grow. Also, the production is higher than those produced by conventional methods. A large number of plants produced on a comparatively smaller area.

Safed musli is a traditional medicinal plant found is natural forest right from east Assam to Andhra Pradesh. It is a pretty herb of 1.5 feet high with erect lanceolate herbed leaves; erect dense flowered racemoses of white colour. In India about eight species of safed musli are reported out of them only *Chlorophytum borivillianum, Chlorophytum arundinaceam* and *Chlorophytum tuberosum* are commercially collected by our tribes from the forest. *C. borivillianum* is the only species which is under commercial cultivation.

Its tubers are used in Ayurvedic medicines; it contains about 27 alkaloids, steroid saponin (2-17%), polysaccaroids (40-45%), carbohydrates, proteins (7-10%), minerals, vitamins etc. White musli or Dhauli musli is used for the preparation of health tonic used in general and sexual weekness. It contains spermametogic properties, decoction of safed musli for curing impotency as they are rich in glycosides.

The aim of the present study was to increase the production and field performance of safed musli through micropropagation method. The work supports the commercial importance of the medicinal plant.

MATERIALS AND METHODS

Culture establishment:

C. borivilianum plants collected from natural populations along with root tubers were selected on the basis of their yield performance and maintained in the University Botanical Garden for obtaining explants. For culture initiation and establishment, conical flasks (100 ml capacity, Borosil) were used. For large-scale multiplication of shoots and rooting, neutral glass pickle-jars (400 ml) with autoclavable polypropylene screw caps were used. Cultures were grown

under 16 hour photoperiod under 45 $molm^{-2}s^{-1}$ (cool white light provided by Philips fluorescent tubes) in a growth room at 28°c and 40-50% humidity.

Explant preparation and inoculation:

C. borivilianum starts its life cycle in nature by proliferation of shoot buds from perennating root tubers in soil in the month of June. Young shoot buds proliferating from tuberous roots attached to stem discs of the identified nursery grown plants served as the source of explants. More than 500 explants were treated with sodium hypochlorite (1% v/v active chlorine) and two or three drops of Tween-20 (Loba, India) for 5 – 20 mins followed by immersion in 0.1% (w/v) aqueous mercuric chloride for 7 mins, then rinsed with autoclaved distilled water several times. Surface sterilized explants were inoculated on MS medium containing 22.2 mM benzylaminopurine (BAP). Proliferated shoots were subsequently transferred to the same or modified fresh medium every 3 week for multiplication[5].

Shoot multiplication:

For scale-up production, experiments were conducted to improve the method of shoot multiplication. Different nutrient media, having different carbon sources (sugar cubes, commercial sugar, maltose, jaggery, glucose and fructose) were tested to optimize the nutritional requirements for multiplying shoots[6,7].

Rooting:

For induction of rooting the shoots were inoculated on the standard rooting medium (MS salts and vitamins reduced to three quarters) which contained 0.8% agar, 3% sucrose and 9.8 mM indole-3-butyric acid (IBA). The culture-room conditions remained the same[8].

Hardening, acclimatization and field transfer:

Three week old rooted plantlets were removed from the culture vessel and washed carefully with distilled water to remove agar without damaging the delicate root system. Plantlets were transferred to culture bottles containing autoclaved Soilrite and irrigated with 1/4MS salt solution. In the greenhouse, the caps of culture bottles were gradually opened and 15 days plants were transferred to polybags containing sand and farmyard manure in a 1:1 ratio. The temperature in the greenhouse was maintained at 25- 28°c while a gradient of reducing relative humidity in the range of 65-55% was created using a fan-pad evaporative cooling system. Twenty five day old tissue culture plants (involving 10 days of *in vitro* and 15 days of *ex vitro* hardening and acclimatization) of approximately uniform size were transplanted into the field on different dates where the soils had been previously amended with organic manure. Plantations were done on 30 cm raised beds, where the plant to plant distance was kept at 20 cm while the row to row distance was 30 cm. Parallel rows of tuber raised plants were also maintained for comparison[9].

All the experiments were conducted under natural rainfed conditions. Leaf characteristics (number and length) were recorded at maturity. Data in respect of number, length and fresh weight of root tubers were recorded at the end of the season. A large number of plants (~5000) maintained in the polytrays were allowed to mature in the nursery shade. Root tubers produced either in the open field or under nursery shade conditions were buried under soil during the off season and were subsequently used as secondary propagules to test their performance in comparison to vegetatively propagated plants, under greenhouse conditions.

RESULTS AND DISCUSSION

The best response in terms of shoot multiplication was achieved on MS medium containing 22.2 mM BAP at 3% level, sucrose compared to any other carbon source used. More than 3.5 fold multiplication was obtained with sucrose, followed by commercial sugar (3), fructose (2.5), glucose (2.5), sugar cubes (2.0), maltose (1.5) and jaggery (1.2) during every subculture period of 21 days. 3 to 3.5 cm long shoots were found to be most suitable for the purpose of rooting. On the standard rooting medium more than 80% of the shoots were successfully rooted. The fibrous adventitious roots originating from the basal shoot part were more than 16 in number and measured 6.4 cm on average. Root induction could be uniformly obtained after 7 days of inoculation of shoots while roots suitable for soil transfer developed in 21 days. Rooted plants on agar solidified medium were removed and subjected to different modes of hardening and acclimatization[10]. The results of which are shown in **table 1**.

Plantlets removed from culture vessels were directly transferred in pots under natural environmental conditions could not withstand the initial transplantation shock and died within 2 - 3 days. Subsequently, more than 10,000 plantlets were subjected to *in vitro* hardening in culture bottles containing autoclaved soilrite and moistened with a nutrient solution containing reduced concentration (one-quarter) of MS salts and vitamins. Such plantlets grew vigorously, had bright green and healthy leaves; attained an average height of 10 cm in 15 days at culture room conditions. More than 80% of the plants survived in the greenhouse and continued to grow either under agro-shadenet or in the open field conditions. The growth of such plants was compared with the plants grown conventionally from root tubers[11].

The root tubers produced by tissue culture raised plants, either in the open field or nursery shade conditions, showed more than 90% sprouting during the next planting season both in the open field and in polybags. The growth performance of such plants is shown in **fig 1** and **fig 2**. Generally a single tuberous root propagule of an average length of 6.0 cm showed about 70% sprouting, while a cluster propagule comprising two or three roots produced vigorously growing plants with better foliage growth in more than 90% of cases. The plants rose from the tuberous roots developed from tissue culture and used as a secondary propagule produced on average 14 tuberous roots measuring an average length of 11 cm.

CONCLUSION

Worldwide, there have been numerous efforts to effectively commercialize the large-scale propagation of plants by tissue culture and some of these efforts have been more successful than others[12]. In the present investigation a highly reproducible and commercially viable micropropagation system has been developed for a rare medicinal herb, safed musli. The econimic yield per plant in terms of tuberous roots of this herb is very high. Therefore the tissue culture technology seems to be very promising in providing genetically uniform planting material of known source to cater the growing needs of the herbal drug industry[13]. During the present studies a crop of 15,000 tissue culture plantlets could be produced using the standard protocol and production schedule spread over 20 week[14]. The tissue culture plantlets showed competitive advantage over seed grown or vegetatively propagated plants both in terms of yield and cost. The micropropagation method described above ensures a regular supply of planting material with an assurance of genetic uniformity and performance of the required planting material for commercial cultivation. Efforts are being made to explore the possibility of round the year tuberization in

plants of *C. borivilianum* produced in culture, under greenhouse conditions.

REFERENCES

1. Nayar MP, Sastry ARK. *Chlorophytum borivilianum*. In: Nayar MP, Sastry ARK, Eds. Red data book of Indian plants, Vol. 2. Calcutta: Botanical Survey of India; 1988. p. 142.
2. Kirtikar KR, Basu BD. Indian medicinal plants, vol 1, 2nd Ed, B. Singh and MP Singh publishers, India; 1980. p. 345.
3. Debergh PC, Maene LJ. A scheme for commercial propagation of ornamental plants by tissue culture. Sci Hort 1981; 14: 335-345.
4. Anonymous. Popular Plant tissue culture from research to commercialization- a decade of support. A report by the Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi; 2000. p. 74-75.
5. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 1962; 15: 473–497.
6. Gamborg OL, Miller RA, Ojima K. Nutrient requirements of suspension cultures of soyabean root cells. Exp Cell Res 1968; 50: 151–158.
7. Gupta PK, Mascarenhas AF. Eucalyptus In: Bonga JM, Durzan DJ, Eds. Cell and tissue culture in forestry, Vol. 3. Dordrecht: Martinus Nijhoff; 1987. p. 385-399.
8. Purohit SD, Dave A, Kukda G. Micropropagation of safed musli (*Chlorophytum borivilianum*) -a rare Indian medicinal herb. Plant Cell Tiss Organ Cult 1994a; 39: 93–96.
9. Purohit SD, Kukda G, Sharma P, Tak K. *In vitro* propagation of adult tree *Wrightia tomentosa* through enhanced axillary branching. Plant Sci 1994b; 103: 67–72.
10. Purohit SD, Singhvi A. Micropropagation of *Achras sapota* through enhanced axillary branching. Scientia Horticulturae 1998; 76: 219-29.
11. Jat RD, Bordia PC. Propagation studies in safed musli (Chlorophytum species). In: Chaudhary, B. L.; Aery, N. C.; Katewa, S. S., eds.Proceedings of the national symposium on advances in plant sciences: current status and emerging challenges. Udaipur Department of Botany, M. L. Sukhadia University; 1990. p. 46.
12. Chu IYE, Kurtz SL. Commercialization of plant micropropagation. In: Ammirato PV; Evans DA; Sharp WR; Bajaj YPS, eds. Handbook of plant cell culture, Vol. 5. New York: McGraw Hill; 1990. p. 126–164.
13. Gupta PK, Shaw D, Durzan DJ. Loblolly pine: Micropropagation, somatic embryogenesis and encapsulation. In: Bonga JM; Durzan DJ, eds. Cell and tissue culture in forestry, Vol. 3. Dordrecht: Martinus Nijhoff; 1987. p. 101–108.
14. Prakash V. Indian medicinal plants; current status. In: Himalayan medicinal plants potential and prospects (Editor SS Samanth, uU Dhar, LMS Palni). Publisher Gyan Odaya Prakashan, Nainital; 2001. p. 45-63.

Table 1: Effect of various carbon sources on shoot multiplication of *C. borivilianum* grown on MS medium containing 22.2

	Subculture (After 42 days of initial culture)		**Subculture (After 63 days of initial culture)**	
Type of carbon source	**No. of shoots**	**Length of shoots (cm)**	**No. of shoots**	**Length of shoots (cm)**
Sucrose (control)	15.0	3.3	58.0	3.4
Sugar cubes	11.5	3.1	18.0	3.1
Commercial sugar	15.0	2.9	39.0	3.0
Jaggery	8.0	2.5	8.0	3.5
Glucose	13.0	2.9	21.0	2.5
Fructose	14.0	3.4	27.0	3.8
Maltose	9.0	1.7	12.0	1.5

Fig 1: *In vitro* shoot growth and multiplication in *C.borivilianum*

Fig 2: Micropropagated plantlets of *C.borivilianum* transferred to nursery

Source of support: Nil, Conflict of interest: None Declared