MICROPROPAGATION OF *PHYLLANTHUS AMARUS* SCHUM. & THOM. BY MERISTEM CULTURE

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ABSTRACT

A novel protocol for production of multiple shoot tip clumps of Keelar nelli (Phyllanthus amarus Schum. & Thom.) were developed in vitro from shoot tip explants cultured on Murashige and Skoog (MS) medium containing only BAP or BAP with NAA, 3% sucrose and 0.8% agar. The addition of the following combination of growth substances 0.1mgL^{-1}BAP and 0.05mgL^{-1}NAA, caused an extensive proliferation of multiple shoot primordia. Subculture of these on the same medium was successful for the multiplication. High frequency rooting was observed in MS medium containing IBA (1.0mgL^{-1}). The plants were successfully transplanted to glasshouse and grown to maturity with a survival rate of 95%.

INTRODUCTION

Today the medical world is posed with complex challenges. Thus time demands an integrated and pluralistic approach towards health care to cope effectively with this situation. There has been a growing interest in scientific approaches for ayurveda in the past one decade.

Phyllanthus amarus (Family Euphorbiaceae), a medicinal herb, is widely used against various liver disorders

(Rajasri Bhattacharyya and Sabita Bhattacharya, 2001). A way of obtaining genuine crude drug is by large-scale destruction of natural habitat due to population pressure and overexploitation, which have become a major threat to important bioresources (Sangeeta Nath and Alak K. Buragohain, 2005). Moreover, availability of the plant is subjected to seasonal variations leading to uncertainty in stable supply throughout the year. Development of viable micropropagation protocol will be important for *ex situ* conservation and sustainable utilization of selected species. Therefore, this paper describes rapid and efficient propagation of *Phyllanthus amarus* using shoot tip culture for providing a better source for continuous supply of plants in manufacturing of drugs.

MATERIALS AND METHODS

Plants of *Phyllanthus amarus* were collected from the campus of Loyola College, Chennai, India. Shoot tips were dissected out and washed thoroughly under running tap water for half an hour. The explants were surface sterilized by treating sequentially with 70% alcohol for 2 minutes and 0.1% (w/v) bavistin and 0.1% mercuric chloride for 3 minutes each. Each treatment was followed by 3-4 times rinsing in sterile double distilled water.

CULTURE MEDIA AND CONDITION

The culture medium was MS (Murashige and Skoog, 1962) basal composition containing 3% sucrose (w/v), supplemented with various concentrations of growth hormones (Table 1) and 0.8% (w/v) agar. All media were adjusted to pH 5.7 before autoclaving at 121^{o}C for 15 minutes. Cultures were incubated at a temperature of 25$\pm$ 2^{o}

C and illuminated for 16 hours per day at 3,000 Lux. Subcultures of established multiple shoots were carried out in 4 weeks period. Rooting of elongated shoots was assessed by transferring them to MS medium containing IBA (Table 2). After 4 weeks, the rooted shoots were hardened and transferred to pots.

RESULTS AND DISCUSSION

The shoot tips were initially cultured on MS medium with different concentrations of BAP alone and in combination with NAA. After two weeks average number of shoots (11) were found to be more in the medium supplemented with $0.1mgL^{-1}$BAP and $0.05mgL^{-1}$NAA (Table1). MS medium with BAP alone showed poor response. All shoots of the size upto 3 cm were separated from the bud clusters and transferred into rooting medium. The MS medium supplemented with IBA was suitable for rooting. Nearly 80% of the shoots formed 3-6 healthy roots within 3 weeks (Table 2) at a concentration of $1.0mgL^{-1}$ IBA (Figure 1). The results of the present study are in conformity with the findings of Rajasri Bhattacharya and Sabita Bhattacharya, 2001; Syamala and Prathiba Devi, 2003; Sangeetha Nath and Alak K. Burahohain, 2005. After four weeks, the rooted micro shoots were maintained for one week in full strength MS liquid medium, followed by transfer for another one week to half strength MS liquid medium for acclimatization. The plantlets were finally transplanted to pots containing sterilized mixture of sand and soil (3:1). About 95 % of the plantlets survived in the glass house and produced normal phenotypic plants. This simple protocol outlined for micropropagation of *Phyllanthus amarus* offers a potential alternative system for conserving medicinal plants.

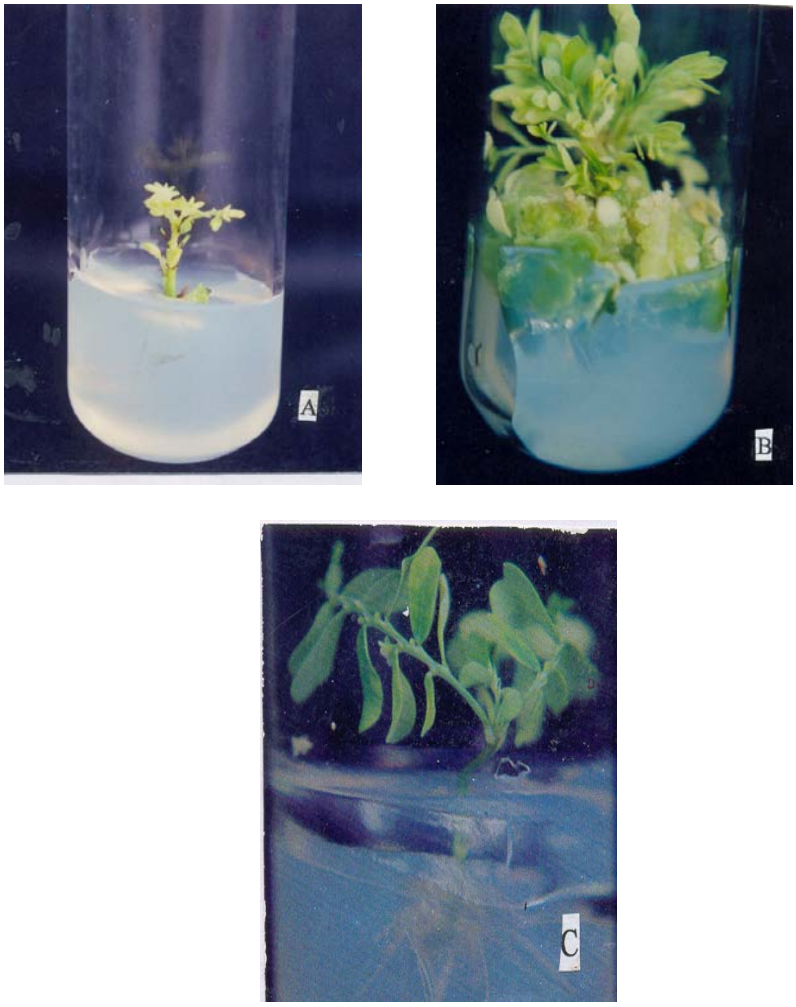
Table 1 : Influence of BAP and BAP + NAA on multiple shoot induction in *Phyllanthus amarus*

Plant Growth Regulators mgL^{-1}		% of responded explants	No. of regenerated shoots/explants Mean ± S.D
BAP	**NAA**		
0.05	0.05	60	2.1 ± 0.06
0.1	0.05	74	10.5 ± 0.07
1.0	0.05	65	3.7 ± 0.03
2.0	0.05	45	1.50 ± 0.03
4.0	0.05	25	1.0 ± 0.02
0.05	0.0	40	1.91 ± 0.23
0.1	0.0	50	4.60 ± 0.02
1.0	0.0	40	2.00 ± 0.07
2.0	0.0	10	1.00 ± 0.09
4.0	0.0	-	-

Table 2: Influence of IBA on rooting of micropropagated shoots of *Phyllanthus amarus*

Plant Growth Regulators IBA mgL^{-1}	% of responded explants	No. of roots /shoot Mean ± S.D
0.0	-	-
0.5	30	2.2 ± 1.29
1.0	75	5.1 ± 1.5
2.0	40	3.5 ± 0.03
3.0	35	2.1 ± 0.38
4.0	-	-

Figure 1 : Micropropagation of *Phyllanthus amarus* throught shoot tip explants



a. *Phyllanthus* (shoot tip) explant at initial stage

b. Multiple shoot proliferation

c. Rooted plantlet of *Phyllanthus.*

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