RESEARCH ARTICLE

Effect of Medium Composition on Callus Morphology from different explants of the Medicinally Important Pantropical weed *Phyllanthus amarus* Schum and Thonn

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ABSTRACT

Phyllanthus amarus is a widely distributed pan-tropical herb under an angiosperm family Phyllanthaceae. It is an erect annual herb10 to 50cm high with a smooth cylindrical stem 1.5 to 2mm thick. The leaves are alternate on petioles 0.3 to 0.5mm long elliptic, oblong or obovate. The phytochemicals of value present in this herb are: lignans, flavonoids, hydrolysable tannins, polyphenols, triterpenes, sterols and alkaloids.The extracts and compounds isolated from *P.amarus* show a wide spectrum of pharmacological activities including antiviral, antibacterial, antiplasmodial, anti-inflammatory anti-malarial, antimicrobial, anticancer, anti-diabetic, antioxidant, hepatoprotective, nephroprotective and diuretic properties. It is an excellent medicine for gallstones and jaundice. It is also an excellent shampoo. During the second half of 20th Century there was a rapid, parallel and synergistic development of the Allopathic and Ayurvedic systems of medicine in India. It generated commercial demand for pharmaceutical drugs of plant origin. Using advanced biotechnological methods for culturing plant cell and tissue would provide new means of conserving a rapidly propagating valuable, rare and endangered medicinal plant. Besides preventing the depletion of stocks of wild plants due to indiscriminate harvesting, tissue culture can be helpful in increasing the quality of the product extracted from plant. Tissue culture is one of the ways by which plant material in pure form can be supplied continuously.

Keywords:- *Phyllanthus amarus*, Tissue Culture, Callus, Cell Biomass

INTRODUCTION

Phyllanthus amarus is a common pantropical weed that grows well in moist, shady and sunny places (Cabieses, 1993; Nanden, 1998; Morton, 1981). The secondary metabolites present in *Phyllanthus amarus* are alkaloids, flavanoids, hydrolysable tannins, major lignans and polyphenols. Foo and Wong (1992) report that in India this plant is used in traditional medicine to treat liver diseases, asthma and bronchial infections. Chevallier (2000) notes that *P. amarus* is also used traditionally in India to treat cardiovascular problems.

This popular medicinal herb is also a remedy around the world for influenza, dropsy, diabetes and jaundice (Foo, 1993). . It is also shown to work as an antifungal, antibacterial and antiviral agent (Houghton et al., 1996). The above applications exemplify a considerable demand of this plant in India and this demand can be met from the cell culture and selection of high-yield cell line for that particular secondary metabolite. Plant tissue and cell culture system are being exploited for the accumulation of the variety of natural products (Komaraiah. P, 2003).The tissue culture systems for a number of medicinal plants have been established, and this enables the analysis of callus and suspension for the presence of the various secondary metabolites (Rao, SM *et al*, 2002) Before we select a medicinal plant for any biotechnological study it Is necessary to find out the details of its usage. The medicinal plants are normally consumed by Ayurvedic or Siddha industries and Pharmaceutical industry. Most of the herbs are available in wild. However some plants are cultivated due to its shortage in wild. One such plant is *Phyllanthus amarus* which is valued for its multidrug potential. (Pusalkar, 2001).

MATERIALS AND METHOD

Whole plants of *P. amarus* were collected in the month of July and August from L.A.D.College, Shankarnagar Campus, Nagpur, Maharashtra, India and got Identified from P.G.T.D Botany, R.T.M.Nagpur University, Nagpur.

Tissue Culture:

Different media compositions were validated for induction of callus and optimization for high yield of Biomass. MS(Murashige and Skoog) medium, N6 medium White's medium and Gamborg's B5 medium with varying hormonal concentrations were experimented for the above purpose.

Initiation of Agar Gel Cultures of *P. amarus* explants

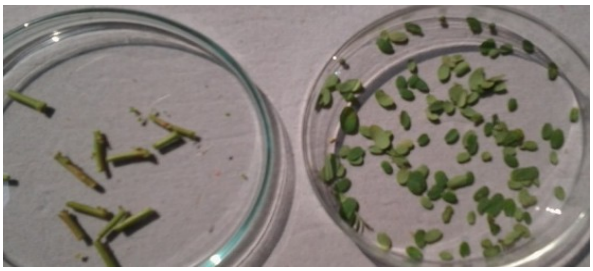
Preparation of explants for inoculation (Surface sterilization)

Explants used for tissue culture studies are shown in Fig.1. The plant material (explants) was washed with running tap water for 30 minutes followed by washings with sterile distilled water thrice. Further sterilization was done under aseptic conditions in a laminar air flow cabinet. The explants, leaf, Rachis, Epicotyl, Hypocotyl and internodes (stem) were prepared and surface-sterilized with 70% Alcohol for 3 minutes and with mercuric chloride (0.1% w/v) for 10 minutes. This was followed by washings with sterile distilled water several times to remove the traces of mercuric chloride. Good Laminar Airflow Table practices were followed for inoculating the explants into suitable medium. All cultures were maintained under cool white, fluorescent light with a 16 h photoperiod, the intensity of light was maintained at 1500 lux, at 25 ± 2ºC temperature. The response of these inoculated explants was observed for callusing.

Induction of Callus

Attempts were made to induce callus using various explants. The explants were cultured in various media supplemented with different hormone combinations. The explants used were leaf, stem, epicotyl, hypocotyl and rachis.

***Fig.1. Phyllanthus amarus* herb**



Stem and leaf explants

Table 1 : Callus induction response in the explants of *P.amarus*

Type of Explant	No. of Explants inoculated	% Response	Time duration for response (Days)
Leaf	20	82.5	7
Stem	20	90	7
Rachis	20	45	8
Epicotyl	20	22.5	12
Hypocotyl	20	17.5	15

Table 2: Nature of callus in MS medium with different hormone combinations

Medium	Hormone Combination	Colour and Texture of Calli
MSDK-1	(MS+0.1mg/L kinetin+3.0mg/L 2, 4-D)	green friable stem callus
MSDK-2	(MS+0.2mg/L kinetin+4.0mg/L 2, 4-D)	yellow friable stem callus
MSDK-3	(MS+0.3mg/L kinetin +4.0mg/L 2, 4-D)	yellowish green stem callus
MSDK-4	(0.1mg/L kinetin+4.0mg/L 2, 4-D)	yellow friable leaf callus
MSDK-5	**(**MS+0.1mg/L kinetin+3.0mg/L 2, 4-D)	green nodular leaf callus
MSDK-6	**(**MS+0.3mg/L kinetin and 5.0mg/L 2, 4-D)	yellowish green friable leaf callus
MSNP-1	**(**MS+0.1mg/L BAP+2.0mg/L NAA)	yellow compact stem callus
MSNP-2	**(**MS+0.2mg/L BAP+4.0mg/L NAA)	yellow compact stem callus
MSNP-3	(MS+0.1mg/L BAP+5.0mg/L NAA)	yellow compact leaf callus
MSNP-4	(MS+0.2mgBAP+4.0mg/L NAA)	yellow compact leaf callus
MSDP-1	(MS+0.2mg/L BAP+3.0mg/L 2, 4-D)	green compact stem callus
MSDP-2	(MS+0.1mg/L(MS+5.0mg/L 2, 4-D)	green friable stem callus
MSDP-3	(MS+0.2mg/L BAP+4.0mg/L 2, 4-D)	green compact leaf callus
MSDP-4	(MS+0.1mg/L BAP+5.0mg/L 2, 4-D)	greenish yellow friable leaf callus

The explants were inoculated on MS basal medium fortified with 3% sucrose and supplemented with various combinations and concentrations of auxins 2, 4-D or NAA and cytokinin, kinetin or BAP . pH of the media was adjusted to 5.8 by using 0.1N NaOH and 0.1N HCl before gelling the medium with agar- agar (Himedia). MS medium,Gamborg's B5 medium, N6 medium and Whites medium were used for inoculation of explants with varying hormone concentrations. The cultures were incubated at 25 ± 1°C with a photoperiod of 16 h at 1500 lux light intensity of cool white fluorescent light. Data were collected by visual observation of the culture. To determine the callus induction by the explants of *P.amarus*, the different explants (Leaf, stem, rachis, hypocotyl and epicotyl were inoculated in Murashige and Skoog (MS) medium fortified with 2,4-D, since 2,4-D supports callus induction. It was observed that stem and leaf explants showed maximum response as compared to other explants like rachis, hypocotyl and epicotyl and hence stem and leaf explants were used in further studies. (Table 1).

Effect of hormonal combinations on the induction of callus on different media viz:- MS, B5, N6 and Whites

The best responding explants (leaf and stem) were treated with different hormone combinations (auxin + cytokinin) in different media viz. MS, B5, N6 and Whites, to determine the best combination for induction and proliferation of biomass. In this study 20 explants of leaf and 20 explants of stem cut into small pieces aseptically were inoculated in 100 mL of agar gelled MS, B5,N6 and whites medium. In total four hormones were used in the study. Two auxins (2,4-D and NAA) and two cytokinins (Kinetin and BAP) were tested in different combinations to assess the response of the explants for inducing callus on four different media viz:- MS, B5,N6 and Whites medium. Five leaf and stem explants were inoculated for each combination in separate flasks and the experiment was repeated thrice.

The explants were surface sterilized as per the protocol given in Materials and Methods and inoculated into the respective medium aseptically under laminar airflow and incubated at 25±2.0^{0}C under cool white fluorescent light (1500 lux) and visual observations were recorded every day for callus induction, growth of biomass and nature of callus in terms of color and texture.

Response to callus induction was observed in terms of percent of explants responding to callus induction and also the amount of callus formed by visual observation

RESULTS AND DISCUSSION

Of the four media tested, MS medium was found to be more suitable for callusing. Combinations of 2,4-D and Kinetin and combinations of BAP and 2,4-D & BAP and NAA supported callusing in MS medium**.** A moderate response was seen in B5 medium, poor response in Whites medium and no response observed in N6 medium.

A. Green friable leaf callus | B. Green friable stem callus | C. Yellow friable leaf callus

D. Yellow friable stem callus | E. Green nodular leaf callus | F. Green compact leaf callus

G. Yellowish green compact leaf callus | H. Yellow compact stem callus | I. Yellow compact leaf callus

J. Greenish yellow friable leaf callus | K. Green friable stem callus | L. Yellow compact leaf callus

Fig. 1: Callus of Different Morphology obtained from Stem and Leaf explants of *Phyllanthus amarus*

A combination of 0.2mg/L kinetin and 4.0mg/L 2,4-D in MS medium showed maximum percentage of callusing(86%) by the stem explants whereas a combination of 0.2mg/L Kinetin and 4.0mg/L 2,4-D showed maximum callusing percentage (93%) by the leaf explants. The morphology of the calli was quite varied ranging from green friable, green compact, green nodular, to yellow compact, yellow friable and yellowish green friable.

Kinetin and NAA combination also showed a good response in callusing with varied callus morphology by both leaf and stem explants on MS medium but as compared to kinetin and 2,4-D combination the response was quite low. The maximum callusing percentage by the stem explants was 29% and by the leaf explants 31%.

Combinations of BAP and 2,4-D also showed moderately good response. A combination of 0.2mg/L BAP and3.0mg/L 2,4-D yielded 35% callusing by the stem explants and a combination of 0.2mg/L BAP and 4.0 mg/L 2,4-D yielded 40% callusing by the leaf explants. The morphology of the callus varied from greenish compact, greenish friable to yellowish compact.

Similarly 0.1mg/L BAP and 2.0mg/L NAA showed 55% callusing by the stem explants and 65% by the leaf explants. BAP and NAA combinations produced yellow compact callus.

CONCLUSIONS

In the Callus Induction study, Of the five explants tried for callus induction viz:- Leaf, Stem, Rachis, hypocotyl and epicotyl, the stem was found to be the most responsive (90%) explant for callusing followed by leaf (82.5%), Epicotyl (62.5%) Rachis (45%) followed by Hypocotyl (17.5%) Hence stem and leaf explants were used in inducing callus for further studies. Also callus induction was found to be optimum in MS medium supplemented with varied concentrations of different auxins and cytokinins

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