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Conservation Efforts through *In Vitro* Seed Germination of a Hepatoprotective Plant - *Phyllanthus beddomie* (Gamble) Mohanan

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Introduction

Whole Plants and plant parts used in ethnomedicine contain curative agents that are used in many modern medicines. The *Phyllanthus* genus contains over 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical regions of both hemispheres (Figueira *et al*., 2006), and have long been used in traditional medicine to treat chronic liver diseases (Dhiman *et al.*, 2005). *Phyllanthus* appears to be promising in patients with chronic hepatitis B virus (HBV) infection (Thyagarajan *et al*.1988; 1990;1999). Bioactive principles like alkaloids, tannins, flavonoids, lignans, phenols and terpenes have been isolated from various species of *Phyllanthus* and their compounds showed antinociceptive activity (Cechinel Filho et al.1996). Liu *et al.* (2001) published a meta-analysis of the effect on and safety of genus *Phyllanthus* for chronic HBV infection.

In - vitro propagation of endangered plants can offer considerable benefits for the rapid cultivation of species that are at risk, that have limited reproductive capacity and exist in threatened habitats (Fay, 1992). *In vitro* propagation methods are essential components of plant genetic resources management and they are becoming increasingly important for conservation of rare and endangered plant species (Sudha *et al.* 1998; Benson et al. 2000; Iankova et al. 2001; Bhatia et al. 2002). The successful use of plant tissue culture techniques for the micropropagation of members of the genus *Phyllanthus* has been reported for species such as *P. emblica*, *P. urinaria*, *P. amarus*, *P. abnormis*, *P. caroliniensis*, *P. tenellus*, and *P. niruri* (Unander, 1991; Ishimaru *et al.*, 1992; Santos *et al.*, 1994). *Phyllanthus beddomie* (Gamble) Mohanan is a sub-shrub found in evergreen forest and the flowering and fruiting season is April - May. Viswanathan *et al*., (2002) reported the existence of this species (rediscovered) after a lapse of about 73 years in 1998 *(*collected from Kalakad Mundanthurai Tiger Reserve, Tirunelveli District), and they have treated this species as critically endemic one. Thus, there is an urgent need for the conservation of this species. There are no reports on the *in-vitro* micropropagation of *P. beddomie*. The present study represents a preliminary approach for the development of a rapid and reproducible protocol for*in vitro* multiplication of *P. beddomie*, which may be helpful in the conservation of this medicinal plant. Moreover, this type of approach may be a vital tool to have a continuous supply of raw materials for the pharmaceutical industry throughout the year to formulate antihepatitic medicines.

Materials and Methods

In vitro culture of *Phyllanthus beddomie*

Phyllanthus beddomie seeds were collected from Kalakad Mundanthurai Tiger Reserve forest area (Kodhaiyar) and sterilized by immersion in 70% ethanol for 5sec, followed by immersion in 0.5% aqueous solution of sodium hypochlorite with one drop of Tween 20 per 500ml for 20min, and rinsed three times in sterile double distilled water. Surface sterilized seeds were sowed for germination in MS medium. Ten duplicate sets were maintained (one in light and one in complete dark condition). Prior to this, an attempt was made to test the viability of the seeds by sowing them in fertile soil. But there was no appreciable rate of germination.

Sowed seeds in MS medium (Murashige and Skoog, 1962) were incubated for 21 days in darkness in a growth cabinet at 24 ^{o} C. Varying levels cytokinins like BAP and Kinetin (0.10 – 4.00 µM) and auxins like NAA, IAA, IBA (Sigma Chemicals, USA) supplemented with MS medium. For all the combinations, 10 duplicates were maintained. During the course of incubation, parameters like shooting, rooting and callusing were observed, average values were calculated and tabulated (Table 1).

Results and Conclusion

Among the tested concentrations and combinations of PGRs, auxins were able to facilitate the rooting phenomenon than that of shooting (Benson, 2000). Even in case of the duplicates supplemented with cytokinins there was no notable percentage of shooting. This may be due to the improper placement of plumule during the initial stage of germination. Among the auxins tested, IBA was able to enhance the rooting ability of the implants at the maximum level (90%) in the concentration of 4µM. These observations were indicating the indigenous level of hormones especially the auxins in the seeds specifically during the initial phases of germination (Table.1)

Regarding the nature of emerged roots, sturdy nature of the roots was proportional to the increasing concentration of auxins like IBA and NAA (Fig.2). In case of the roots developed from other sets i.e. media combinations supplemented with cytokinins and in low levels of auxins, there appeared fragile and less sturdy ones (Fig.1).

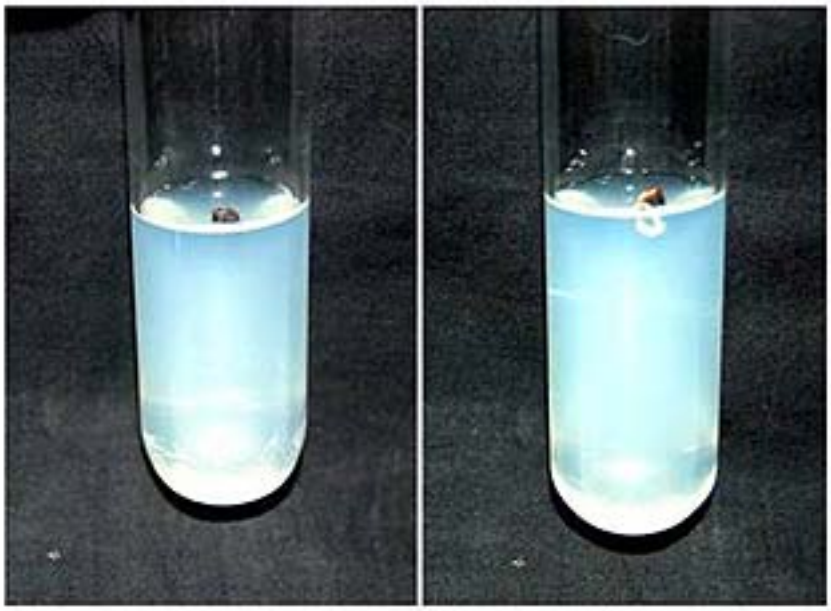
No responses in root and shoot formation of MS medium containing BA, and Kinetin in low concentrations (0.10 – 2.00 µM). The findings of the present study may be helpful for the development of micropropagation of *P. beddomie*. Further studies are ongoing for the further establishment and ex *situ* conservation of *P. beddomie* and such type of medicinal plants are in need to fulfill the present need of the pharmaceutical industry (Fay, 2002).

Table 1. The frequency (%) of seed germination responses of *P. beddomie* after 21 days on MS medium supplemented with PGRs in different concentrations.

PGRs used	Concentration µM	Shooting (%)	Rooting (%)	Callusing (%)
BAP	0.10	-	-	-
	0.50	-	-	-
	1.00	-	-	-
	2.00	-	-	-
	3.00	-	-	-
	4.00	-	-	-

Kinetin	0.10 0.50 1.00 2.00 3.00 4.00	- - - - - -	0 20 40 50 60 60	- - - - - -
IAA	0.10 0.50 1.00 2.00 3.00 4.00	- - - - - -	- - - - - -	- - - - - -
IBA	0.10 0.50 1.00 2.00 3.00 4.00	- - - - - -	20 35 40 60 80 90	- - - - - -
NAA	0.10 0.50 1.00 2.00 3.00 4.00	- - - - - -	10 10 20 20 25 40	- - - - - -

Live endemic medicinal plant *Phyllanthus beddomei*



Phyllanthus beddomei seed dormancy of ms medium

Phyllanthus beddomei regenerated from seed

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