

## Original Research Article

### Effect of BAP on *in vitro* propagation of *Hybanthus enneaspermus* (L.) Muell, an important medicinal plant

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#### A B S T R A C T

##### Keywords

*Hybanthus enneaspermus*; *in vitro* shooting; MS medium; BAP.

The present study was carried to study the effect of different concentrations of BAP (benylamino purine) on *in vitro* shoot induction of the *Hybanthus enneaspermus* (L.) Muell. Explants obtained from the shoot tip and nodal segments of cultivar studies were inoculated on MS medium supplemented with different concentrations of BAP (0.5, 1.0, 2.0 and 3.0mg/l) and the cultures were incubated at  $25\pm2^{\circ}\text{C}$  with a 16hrs photoperiod (2000lux) provided by cool white fluorescent tubes. The pH of medium was adjusted to 5.8 prior to autoclaving. The cultures were sub cultured at 35 days interval in same medium to produce multiple shoots. The regenerated plants of all varieties were transferred to different mixtures of compost for acclimatization. Best hardening response was obtained in Sand + Soil + Vermicompost (1:1:1) after three week of transplantation in greenhouse. These hardened plants were subsequently shifted into field. The effect of different concentration of BAP on shoot initiation and shoot proliferation were investigated.

## Introduction

*Hybanthus* is from the Greek *hypos* (hump-backed) and *anthos* (flower), referring to the spurred anterior petal; *ennaeasperus* is from the Greek *ennea* (nine) and *spermus* (seed), referring to the capsule which contains about nine seeds. *Hybanthus enneaspermus* (L.) F. Muell, belonging to family Violaceae, is a rare, perennial herb found in some of the warmer parts of the Deccan peninsula in India. Popularly called 'Ratanpurus' and in Tamil it is 'Orithaztamarai'. By the local villagers and herbalists, this ethnobotanical herb is known to have unique medicinal properties. It grows up to 15-30cm in height with many diffuse or ascending branches

and is pubescent in nature [1]. Traditionally the plant is used as an aphrodisiac, demulcent, tonic, diuretic, in urinary infection, diarrhea, leucorrhoea, dysuria and sterility [2].

The preparation made from the leaves and tender stalks of the plant are used in herbal medicine for its aphrodisiac, demulcent and tonic properties. The root is diuretic and administrated as an infusion in gonorrhea and urinary infections [3]. The fruits and leaves are used as antidotes for scorpion stings and cobra bites by the yanadi tribes [4]. It increases sexual desire. It is

aphrodisiac and a nutritive tonic. Small quantity of the herb if taken with milk, twice a day helps the body gaining strength. If it is taken for 48 days, the internal organ of the body grows healthier and stronger. Specifically, the organs of reproduction are rejuvenated, get stronger and the production of sperm increases. The peduncle of herb gives a mushy, sticky and smooth taste, like ladies finger, when chewed. Daily intake helps in curing ulcers, which occurs due to sexually transmitted diseases and even AIDS. This herb is good for regularizing the monthly periods of women. It cures problems related to menstruation. This nutritive herb cools the body. Though, *H. enneaspermus* has number of medical value.

### Tissue Culture Techniques

A plant regeneration method for 2.0mg/l benzylamino purine (BAP) within 4 weeks of incubation. Shoot differentiation was achieved from the light green compact callus. The addition of casein hydrolysate (500 mg/l) and more potassium phosphate (1.86mmol/l) to the culture medium enhanced shoot differentiation. Rooting was achieved on half-strength MS medium containing 1.0mg/l indole-3-butyric acid (IBA). The regenerated plants were morphologically uniform and exhibited a normal seed set. The highest number of shoot/explants was obtained with initial pH values between 5.5 and 6.0. Similarly, shoot differentiation occurred when light green compact callus of *H. enneaspermus* was transferred to MS medium supplemented with 2.0mg/l BAP and 0.5mg/l NAA; the highest percentage of calli-forming shoots and the highest number of shoots were achieved in this medium. Differentiated shoot buds of *H. enneaspermus* elongated to 4–5 cm within 6 weeks. This response could be improved when glucose was used as a carbon source for a larger flowered purslane,

and using higher concentrations of casein hydrolysate (500mg/l) and potassium phosphate (1.86mmol/l) for *H. enneaspermus*. It was also observed that an increase of the NAA concentration augmented shoot length up to 8cm of *H. enneaspermus* in tissue culture.

In tissue culture, plant growth regulators (PGR) are critical media components in determining the developmental pathway of the plant cells. Cytokinins such as benzylamino purine (BAP) and Kinetin are generally known to reduce the apical meristem dominance and induce both auxiliary and adventitious shoots formation from meristematic explants in *Hybanthus enneapericus*. The most established *Hybanthus enneapericus* culture system was achieved by using BAP as a supplement to basal media [6]. The effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in *Hybanthus enneapericus*. BAP has a marked effect in stimulating the growth of auxiliary and of shoot tip cultures [7]. With view of the above said things, the present investigation was mainly aimed to study the effect of different concentrations of BAP along with MS basal medium and optimize its dose level in the shoot induction and shoot proliferation of *Hybanthus enneapericus* studied.

### Materials and Methods

#### Source of Explants

For this study, the field grown *H. enneaspermus* were collected from the Agriculture Department, Annamalai University, Annamalainagar, Chidambaram and South India. Explants were collected from shoot tips and nodal segments for shoot initiation.

## **Surface sterilization**

After excision, the surface sterilization, the explants were primarily rinsed in tap water for 30 minutes followed gently rinsed with 70% ethanol for 60 seconds and with 5% sodium hypochloride solution for 10 minutes. After each step of sterilization, the explants were washed with sterile double distilled water for three times. Further sterilization procedures were carried out in laminar air flow chamber by using 0.1%  $\text{HgCl}_2$  for 5 minutes. The explants were then rinsed five times with sterile distilled water. Finally, in the laminar air flow chamber, the explants were cut into small pieces.

## **Inoculations**

After complete sterilization and slicing, the explants were inoculated in MS medium [8], supplemented with cytokinins and auxins used either singly or in combination. The pH of the medium was adjusted to 5.8 and prior to autoclaving, 0.6% agar (Himedia, Mumbai) was added to the medium. Then, autoclaving was done at 121°C and 15psi for 20 minutes.

## **Establishment of culture**

After inoculation, the cultures were maintained at a temperature of  $25\pm2^\circ\text{C}$  with a photoperiod of 16hrs per day. Lighting of  $80\mu\text{Em}^{-2}\text{s}^{-1}$  was supplied by using cool and white fluorescent tubes (Philips India Ltd.). Various types of plant growth regulator BAP were added with MS medium for better shoot induction (Table 1). The MS medium without adding of plant growth regulator was served as control. After 35-45 days of culturing, the multiple shoots were separated into pieces and the separation was done at the base of multiple shoots and they were transferred to 500ml culture bottle containing 50ml of the same kind of

medium to get a more number of shoots. For root initiation, the shoots were transferred to half strength MS medium supplemented with IAA, IBA and NAA.

## **Hardening**

Healthy plantlets with 5 to 7cm long roots were individually removed from the culture tubes. After washing their roots carefully with tap water, plantlets were transplanted into 10cm diameter plastic pots containing a mixture of sand, soil and vermicompost (1:1:1) and they were placed in the greenhouse for hardening. The plants were watered with half-strength MS salts solution every week and covered with a polythene bag for 2 weeks. Afterwards, the hardened plants were gradually transferred to 20cm pots containing pure garden soil and kept in the field for developing into mature plants.

## **Acclimatization of micro plants**

After rooting, regenerated plants were transferred to small pots containing different combinations of sand, soil and vermicompost under greenhouse conditions for 1-2 month. The hardened regenerates were then transplanted to the trial field for further growth and multiplication.

## **Statistical Analysis**

All experiments were repeated five times. The effects of different treatments were quantified and the data subjected to statistical analysis using ‘standard error of the mean’ by using SPSS software [9].

## **Results and Discussion**

In this study the effects of different concentrations of BAP on bud initiation and shoot multiplication were investigated. The

results of shoot induction and multiple shoot formation with the implementation of different concentrations of BAP along with MS basal medium presented in (Table-2). Apart from the influence of genotypes, shoot proliferation rate and elongation are affected by cytokinin types and their concentration. Adenine based cytokinins are used in several *H. enneaspermus*. For *in vitro* propagation N<sup>6</sup>- benzylamino purine (BAP) is the most commonly preferred cytokinin.

The concentration of exogenous cytokinin appears to be main factor affecting multiplication. For example, Wong (1986) stated that when 2.0mg/l BAP is

supplemented in the medium, each of the explants produces as average of 2.4 shoots, while increasing the BAP concentration to 2.0mg/l and 3.0mg/l results in 2.6, 4.3 shoots per explants respectively. However, the optimum recommended BAP concentration is 2.0mg/l for *H. enneaspermus* micropropagation [8]. In this study also it is conformed as 2.0mg/l BAP showed good results when compare to its low and high concentration in shoot induction and shoot proliferation. Hence, this BAP concentration can be recommended as optimum level for *in vitro* shoot induction and proliferation of *H. enneaspermus*.

**Table.1** Different concentrations of BAP along with MS medium

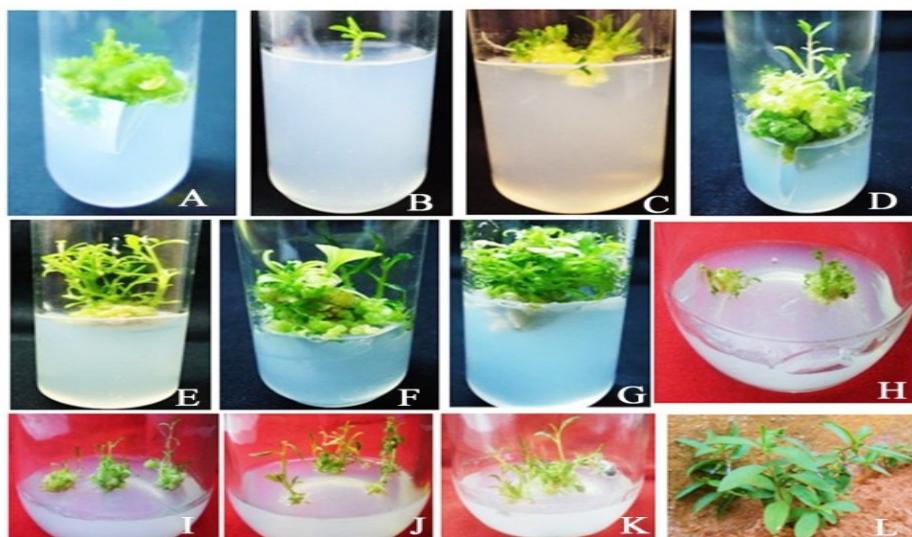
Treatments	Concentrations (mg/l)
MS+BAP	0.5mg/l
MS+BAP	1.0mg/l
MS+BAP	2.0mg/l
MS+BAP	3.0mg/l
MS+BAP	4.0mg/l

**Table.2** Results of different treatments of BAP along with MS medium in shoot proliferation

Treatments	Number of shoot (Mean ± SE)	Shoot length (cm) (Mean ± SE)
Control	0.83±0.19	1.33±0.18
MS+0.5mg/l BAP	3.17±0.22	1.98±0.19
MS+1.0mg/l BAP	8.50±0.24	3.83±0.22
MS+2.0mg/l BAP	11.28±0.37	6.68±0.37
MS+3.0mg/l BAP	10.34±0.32	6.12±31
MS+4.0mg/l BAP	4.52±0.31	5.36±0.24

**Figure.1** The successive stages of *in vitro* propagation of *H. enneaspermus*

**Sucessive stages of *Hybanthus enneaspermus***



- (a) Direct regeneration of shoot from explants
- (b) Initiation of multiple shoot formation
- (c) Development of more number of multiple shoot
- (d) Root formation from regenerated shoot
- (e) Hardening and establishment regenerated plants

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