# ผลของสูตรอาหารต่อการสร้างแคลลัสและการพัฒนาเป็นพืชต้นใหม่ของ *Bacopa monnieri* L.

# Effect of Culture Media on Callus Induction and Plant Regeneration of *Bacopa monnieri* L.

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## บทคัดย่อ

พรมมิเป็นที่รู้จักกันโดยทั่วไปในฐานะที่เป็นพืชน้ำ เป็นพืชสมุนไพรที่นิยมใช้กันอย่างกว้างขวางในกระบวนการผลิตยาในกลุ่มอายุรเวท โดยมีผลต่อระบบความจำระบบประสาทและด้านการอักเสบ จุดประสงค์หลักในการศึกษานี้คือต้องการเพิ่มปริมาณต้นพรมมิให้ได้จำนวนมากจากการเพาะเลี้ยงเนื้อเยื่อ โดยการนำชิ้นส่วนพืชมาฟอกฆ่าเชื้อและวางเลี้ยงบนอาหารแข็ง อาหารกึ่งแข็งกึ่งเหลว และอาหารเหลวสูตร MS เติม BA ความเข้มข้น 2 มิลลิกรัม/ลิตร ร่วมกับการเติมหรือไม่เติมผงถ่านความเข้มข้น 0.2% วางเลี้ยงในห้องที่มีการให้แสง 14 ชั่วโมง อุณหภูมิ 27±1 องศาเซลเซียสเป็นเวลา 3 เดือน เพื่อชักนำการสร้างแคลลัสและการพัฒนาเป็นพืชต้นใหม่ จากการศึกษาพบว่า อาหารแข็ง อาหารกึ่งแข็งกึ่งเหลว และอาหารเหลวสูตร MS เติม BA ความเข้มข้น 2 มิลลิกรัม/ลิตร ไม่เติมผงถ่านให้เปอร์เซ็นต์การสร้างแคลลัสสูงถึงร้อยละ 100 โดยอาหารแข็งสูตร MS เติม BA ความเข้มข้น 2 มิลลิกรัม/ลิตร ไม่เติมผงถ่านให้ขนาดแคลลัสสูงสุด 1.86 เซนติเมตร และให้การพัฒนาเป็นพืชต้นใหม่สูงสุด 27.03 ยอด/ชิ้นส่วน โดยมีความสูงยอด 11.81 เซนติเมตร

**คำสำคัญ:** พรมมิ, อาหารเพาะเลี้ยง, การชักนำแคลลัส, การพัฒนาเป็นพืชต้นใหม่

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## ABSTRACT

*Bacopa monnieri* L., commonly known as aquatic plant is an herb often used in Ayurveda. That has been used for centuries as a memory enhancer and anti-inflammatory. The aim of this study was to increase the number of fresh weight of traditional plant by tissue culture. *Ex vitro* plant was surface sterilized and cultured on solid, liquid and semi-solid Murashige and Skoog (MS) medium supplemented with 2 mg/l BA ($N_6$-benzyl adenine) with or without 0.2% activated charcoal. The cultures were placed under light conditions at 14 hphotoperiod, 27±1°C to initiate callus induction and plant regeneration for 3 months. The result revealed that Solid, semi-solid and liquid MS medium supplemented with 2 mg/l BA without activated charcoal gave the highest percentage of callus induction (100%). Solid MS medium supplemented with 2 mg/l BA without activated charcoal gave the highest of plant regeneration (27.03 shoots/explant), shoot length (11.81 centimeter) and size of callus (1.86 centimeter).


**Key words:** *Bacopa monnieri* L., culture media, callus induction, plant regeneration

## INTRODUCTION

*Bacopa monnieri* L., commonly used in Ayurvedic medicine, has an age-old reputation for being an effective and powerful herb helpful for memory and combating stress. *B. monnieri* L., known as Brahmi, acts as an adaptogen which helps the body adapt to new or stressful situations. The following 9 facts show the power and versatility of this therapeutic plant including, supports the brain, promotes liver health, protects against neonatal hypoglycemia, positively impacts opioid dependence, fights systemic redness and swelling, encourages normal blood pressure, promotes strong antioxidant activity, produces organic compounds and its fresh. It is characterized by its green leaves and flowers. Mention earlier for considerable healing properties, especially in relation to mental health. It is relatively inexpensive to buy from health food stores, and is usually consumed in tablet or capsule form (although some people like to drink the leaves in a tea).

The requirement of brahmi is met solely from the natural populations, leading to their gradual depletion. Seeds of *B. monniera* L. are poor propagule due to their short viability and frequent seedling death, which makes it difficult to raise plants from seeds. Vegetative propagation is also slow (Tiwari and Singh, 2010; Sharma *et al.*, 2012) and is further hampered by specific habitat requirements and by poor performance of propagates. *B. monniera* L. seems to be a poor competitor so that it can colonise open spaces only (Shah, 1965). Further increased demand for plant material and loss of habitat, will put this medicinal species under

more pressure and may result in adulteration of plant material, which may endanger human health or undermine product efficacy. This in turn may lead to loss of consumer confidence in herbal medicines (Tiwari *et al.*, 2001)

Plant tissue culture remains one of the most basic biotechnological techniques with its varied and vast applications. The rapidity of multiplication of true-to-type plants and efficient transplantation of *B. monnieri* L. can be useful in conservation and propagation of elite plants for commercial exploitation. Protocols for *in vitro* clonal propagation and conservation have also been conducted in *B. monnieri* L. by several researchers (Sharma *et al.*, 2007; Joshi *et al.*, 2010; Jain *et al.*, 2013). In present study different cytokinins evaluated for its multiple shoot induction so that a suitable concentration of 6-benzyladenine (BA) recommended for high yield of biomass and continuous propagation of this medicinal plant. It has been the focus of many research groups to develop strategies for its conservation, *in vitro* regeneration, micropropagation and shoot regeneration using different explants like leaves, nodal, internodal segments and shoot tips (Bhusari *et al.*, 2013; Kumari *et al.*, 2010). Activated charcoal is often used in tissue culture to improve cell growth and development. The promotary effects of AC on morphogenesis may be mainly due to its irreversible adsorption of inhibitory compounds in the culture medium and substancially decreasing the toxic metabolites, phenolic exudation and brown exudate accumulation (Thomas, 2008). The present study deals with the rapid mass scale multiplication of *B. monnieri* L. using nodal explants containing axillary bud when culture on different types of culture media with or without AC.

## MATERIALS AND MATHODS

Plant material was washed under running tap water for 10 minutes and they were rinsed with 70% alcohol for 30 sec, followed by 20% (w/v) sodium hypochlorite together with 1-2 drops of Tween-20 for further 5 min, 10% (w/v) sodium hypochlorite together with 1-2 drops of Tween-20 for further 10 min, 5% (w/v) sodium hypochlorite together with 1-2 drops of Tween-20 for further 5 min. The final step of sterilization was carried out in a laminar air flow chamber by rinsing the plant material 3 times in sterile distilled water. Nodal explants (consisted of 2 buds) were excised with sterile scalpel blade, and then they were inoculated on 6 types of culture media for shoot bud induction. The media were consisted of solid, semi-solid and liquid MS medium supplemented with 2 mg/l BA, 3% (w/v) sucrose with or without 0.2% AC. The pH was adjusted to 5.7 with 1 N NaOH or HCl and autoclaved at 121°C with 15 p.s.i. (1.04 kg $cmi^2$) pressure for 15 min. The cultures were maintained at 27±1°C under a 14-h photoperiod of 50 μmol $m^{-2}$ $s^{-1}$ irradiance provided by cool white fluorescent. All explants were placed horizontally on the medium in contact with the medium. Completely randomized design with 10 replicates (each replicate consists of 10

nodules) was performed. Callus induction and plant regeneration were recorded every month. Data were analysed by ANOVA. Means were compared with Duncan's multiple range tests (DMRT) at the 0.01 of probability. The F-test showed significant differences among means.

## RESULT AND DISCUSSION

After 1 week of culture, growth response of explants in different types of culture medium was observed. The axillary buds of nodal explants began to grow within 3 days after culturing (Fig. 1). Initiated callus was occurred on several media after culture for 1 week. Solid, semi-solid and liquid MS medium supplemented with 2 mg/l BA without AC gave 100% callus induction (Table 1). While, Solid MS medium supplemented with 2 mg/l BA gave the highest size of callus at 1.86 cm (Table 1), followed by semi-solid MS medium supplemented with

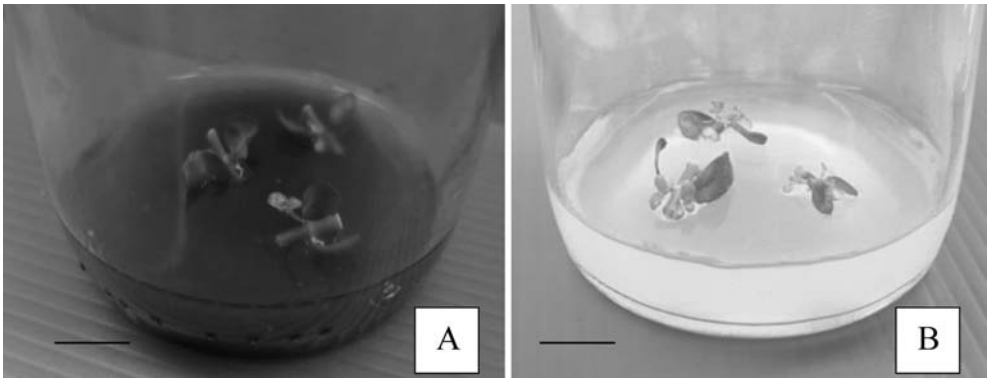


**Figure 1** The axillary buds of nodal explants began to grow within 3 days of culture on MS medium supplemented with 2 mg/l BA with 0.2%AC (A) or without 0.2%AC (B) (bar = 0.5 cm).

**Table 1** Effect of culture media on callus formation and average size of callus after 3 months of culturing.

| Culture media | Callus induction (%) | Size of callus (cm.)[1/] |
|---|---|---|
| 1. solid MS medium+0.2% AC | 0 | 0.00c |
| 2. solid MS medium | 100 | 1.86a |
| 3. semi-solid MS medium+0.2% AC | 0 | 0.00c |
| 4. semi-solid MS medium | 100 | 1.73a |
| 5. liquid MS medium+0.2% AC | 0 | 0.00c |
| 6. liquid MS medium | 100 | 1.09b |
| CV. | - | 19.54 |
| F-test | - | ** |

** = Significant difference at p≤0.01 level.

- = Data not shown.

1/ = Value followed by different letter are significantly different according to DMRT.

2 mg/l BA (1.73 cm) and liquid MS medium supplemented with 2 mg/l BA (1.09 cm), respectively that there were significant difference with MS medium supplemented with 2 mg/l BA with 0.2%AC. In this study, the significant influence of AC was found. Solid MS medium supplemented with 2 mg/l BA without 0.2% AC gave the highest number of shoots at 27.03/ explant and shoot length at 11.81 cm after 3 months of culture (Table 2) followed by semi-solid MS medium supplemented with 2 mg/l BA without AC (19.77 shoot/explant). Solid MS medium gave the best result on callus induction, number of shoots and plant regeneration which are similar results of the authors that selected solid MS medium (Subashri and Pillai, 2014)

This present study showed that AC may have either beneficial or harmful effects on the culture, depending upon the medium, and tissue used (Pan and Staden, 1999). AC is improper for callus induction of *B. monnieri* L. and different types of culture media gave the different response on number of shoots and plant regeneration. Weatherhead *et al.* (1979) reported that adsorptive capacities of AC have also been shown to affect the composition of the media in a selective manner; thiamine HCl and nicotinic acid were removed from media by AC, whereas inositol and sucrose were not and AC gave the effected on callus growth and shoot organogenesis in tobacco (Constantin *et al.*, 1977). However, culture media with AC gave the *in vitro* generated healthy shoots greater than culture media without AC (Fig. 2).

Cytokinin is the major phytohormones to regulate plant growth and development. A classic phytohormone involved in cell division, growth, and organogenesis. There is enough residual cytokinin present in shoots therefore, little or no cytokinin is required in rooting medium (Hu and Wang, 1983). Several researchers have reported multiple shoot induction with cytokinins in the growth medium (Stamp *et al.*, 1990; Tiwari *et al.*, 2001) same as our investigation. The characteristics of callus induction (Fig. 3) and plant regeneration (Fig. 4) of *B. monnieri* L. were observed on different culture media.

**Table 2** Effect of culture media on No. of shoot/explant and shoot length (cm.) after 3 months of culturing.

| Culture media | No. of shoot/explant[1/] | Shoot length (cm.) |
|---|---|---|
| 1. solid MS medium +0.2% AC | 5.60c | 9.64a |
| 2. solid MS medium | 27.03a | 11.81a |
| 3. semi-solid MS medium+0.2% AC | 5.23c | 7.39b |
| 4. semi-solid MS medium | 19.77b | 9.99a |
| 5. liquid MS medium+0.2% AC | 15.97b | 10.22a |
| 6. liquid MS medium | 17.40b | 6.19bc |
| CV. | 20.92 | 24.32 |
| F-test | ** | ** |

** = Significant difference at p≤0.01 level.

[1/] = Value followed by different letter are significantly different according to DMRT.

## CONCLUSION

MS medium supplemented with 2 mg/l BA without 0.2%AC gave 100% of callus induction. greater than MS medium supplemented with 2 mg/l BA with 0.2%AC. Solid MS medium supplemented with 2 mg/l BA without 0.2%AC gave the number of shoots at 27.03/explant, shoot length (11.81 centimeter) and size of callus (1.86 centimeter). AC plays the role in the different response to callus induction of *B. monnieri* L.

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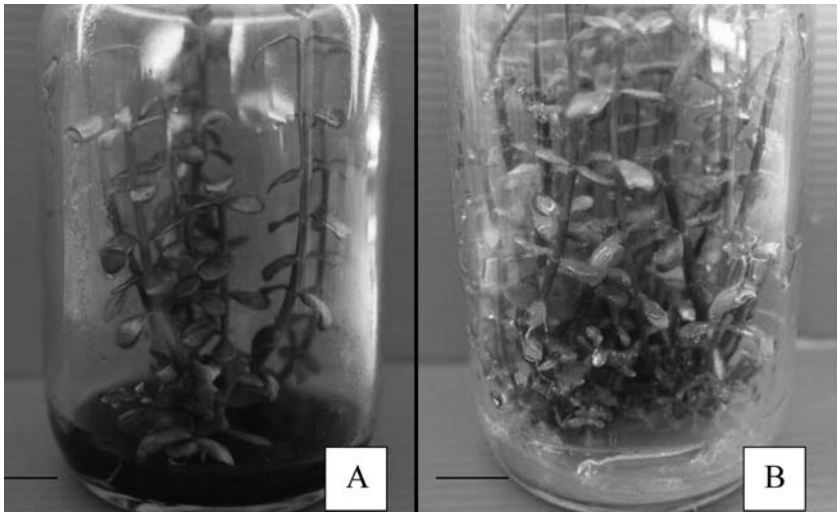


**Figure 2** Characteristics of plant regeneration of *B. monnieri* L. after culture on MS medium supplemented with 2 mg/l BA with 0.2% AC (A) or without 0.2% AC (B) (bar = 0.5 cm).

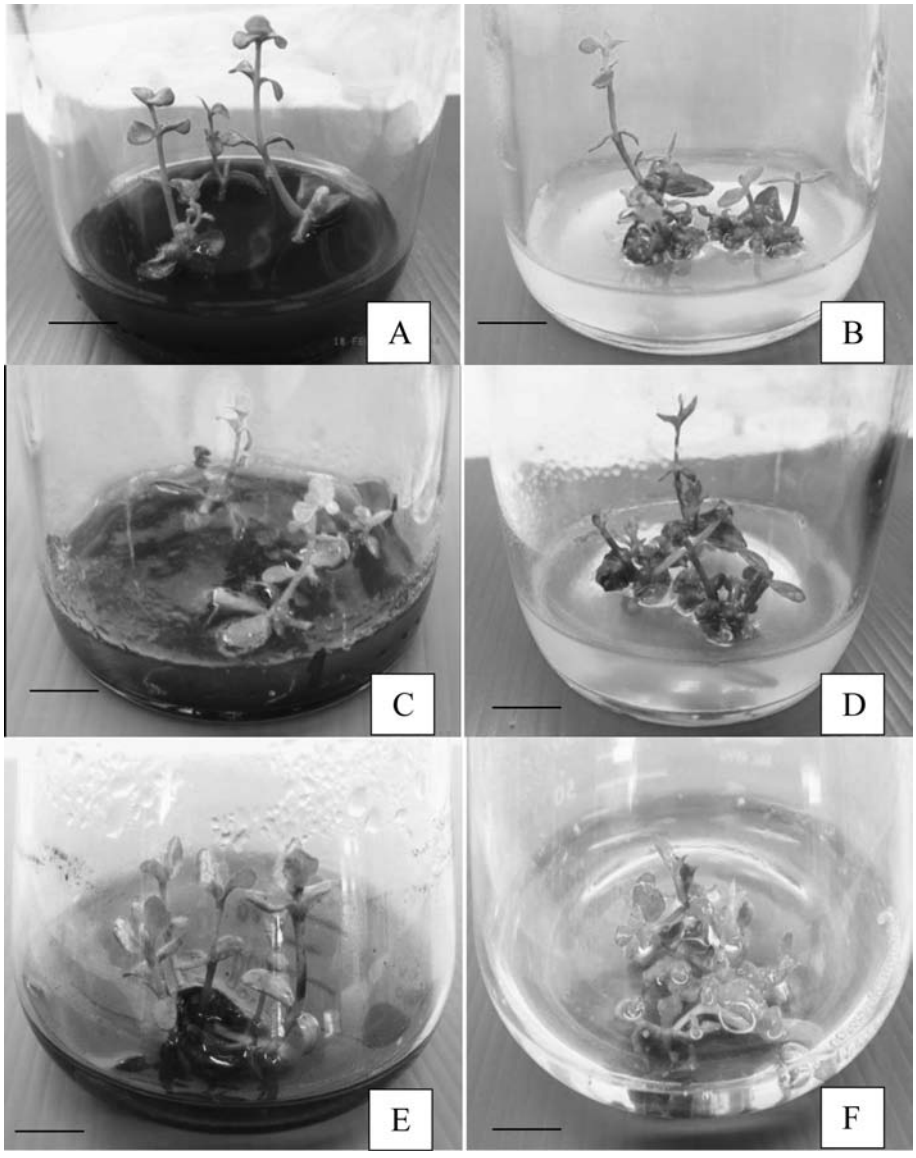


**Figure 3** Initiated callus of *B. monnieri* L. was cultured on different media for 1 week. (bar = 0.5 cm).

A: Solid MS medium supplemented with 2 mg/l BA and with 0.2%AC.

B: Solid MS medium supplemented with 2 mg/l BA and without 0.2%AC

C: Semi-solid MS medium supplemented with 2 mg/l BA and with 0.2%AC.

D: Semi-solid MS medium supplemented with 2 mg/l BA and without 0.2%AC.

E: Liquid MS medium supplemented with 2 mg/l BA and with 0.2%AC.

F: Liquid MS medium supplemented with 2 mg/l BA and without 0.2%AC.

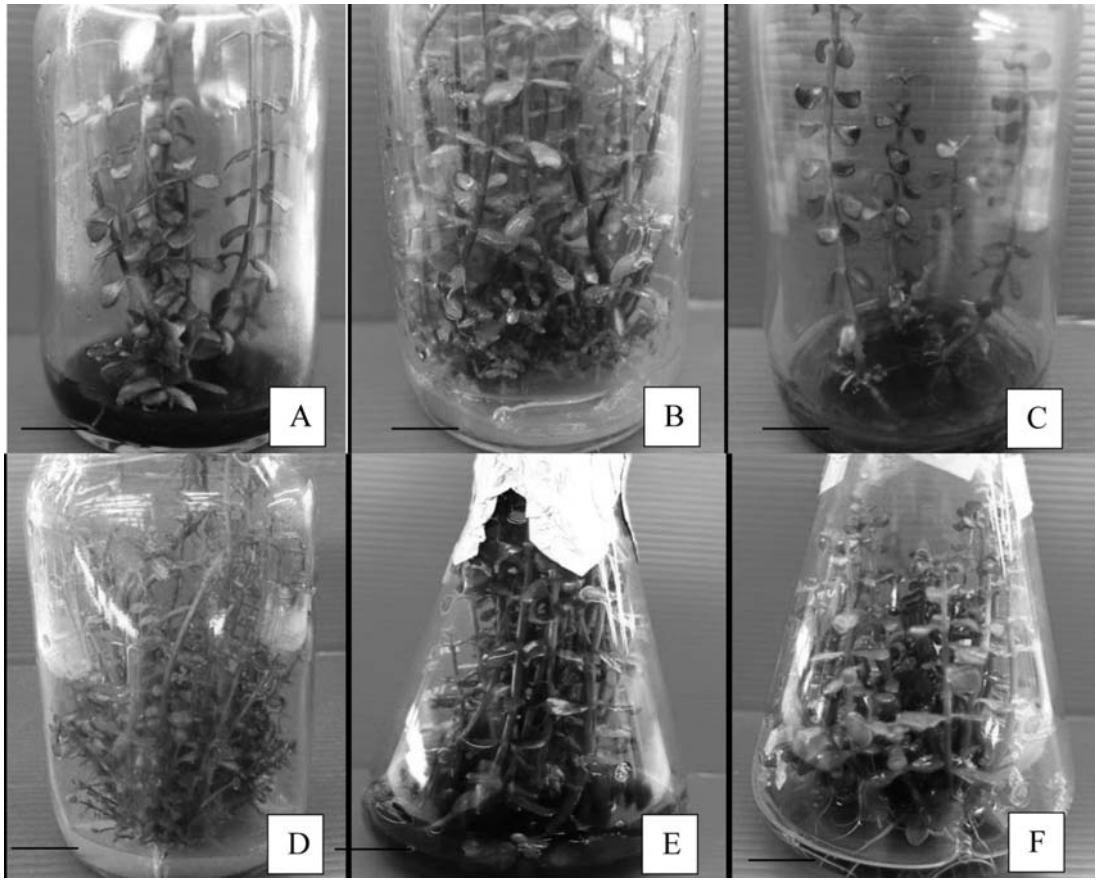


**Figure 4** Plant regeneration of *B. monnieri* L. on different culture media after 3 months of culture (bar = 0.5 cm).

A: Solid MS medium supplemented with 2 mg/l BA and with 0.2%AC.

B: Solid MS medium supplemented with 2 mg/l BA and without 0.2%AC

C: Semi-solid MS medium supplemented with 2 mg/l BA and with 0.2%AC.

D: Semi-solid MS medium supplemented with 2 mg/l BA and without 0.2%AC.

E: Liquid MS medium supplemented with 2 mg/l BA and with 0.2%AC.

F: Liquid MS medium supplemented with 2 mg/l BA and without 0.2%AC.

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