Effect of Different Medium on in Vitro Shooting and Regeneration of Bacopa Monnieri L. an Important Medicinal Plant

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Abstract— Bacopa monnieri L. is an important medicinal plant of Ayurveda to cure various diseases. It is also considered to have property of memory enhancer. Lots of medicinal importance of this plant attracted the scientist attention towards it. The present work is a comparative study for standardization of growth medium and growth hormone for in vitro culture of Bacopa monnieri L. Three different medium (MS, B5 and SH) was compared. These growth medium was supplemented with different concentration of auxin (NAA, IAA and IBA) and Cytokinin (BAP and KIN) alone and in combination. Best shoot bud induction was achieved on MS medium supplemented with 1.0 mg/ L BAP and 1.0 mg/ L KIN. In other medium shoot bud induction was low. Highest number of shoots and shoot length was also observed in the same medium. Highest root induction was achieved on MS medium supplemented with 0.5 mg/ L and 1.0 mg/ L IBA. Root number and root length was also highest in the same medium. Rooted plantlets were first acclimatized in green house and then successfully transferred to field. Survival rate of this plant was achieved 90%. This in vitro regeneration protocol provides a large scale production of Bacopa monnieri L. which will prevent the natural resources of this plant.

Key words: Bacopa monnieri L., Medicinal plant, in vitro regeneration

I. INTRODUCTION

Plants are one of the most important sources of medicine. The application of plants as medicine is date back to prehistoric period. The earlier civilization reveals that a considerable number of drugs used in modern medicine have figured in ancient manuscripts such as the Rigved, the Bible, the Quran, the Liad, the Odyssey & the history of Herodotus over 6000 years ago. The ancient Chinese were the first to use the natural vegetation as medicine (Khan & Khanum, 2004). Today there is an improved interest in traditional medicine and an increasing demand for more drugs from plant sources. The Bacopa monnieri L. (Scrophulariaceae) commonly known as Bramhi is an herbaceous shrub known for its valuable medicinal properties. In Ayurveda it is given to patient suffering from general debility, fever, sterility, dysmenorrhoea, elephantiasis, hoarseness, leucoderma, leprosy, skin diseases, asthma, bronchitis, constipation, dyspepsia, ulcer, tumours, inflammation and epilepsy. Its decoction is well known for improving immune system of the body (Jain et al., 1994; Elangovan et al., 1995; Tripathi et al., 1996; Vohora et al., 1997) and it shows many pharmacological activities. All its pharmacological activities have been summarized in the table number 1.

Activity	Plant Parts	Solvent	References
Anticancer activity	Whole Plant	Ethanolic extract	Peng et al., 2010; Patil et al., 2014
Antidepressant activity	Leaves part	Methanolic extract	Mannan et al., 2015
Antidiabetic activity.	Whole Plant	Ethanolic extract	Ghosh et al., 2011
Antihypertensive activity	Whole plant	Ethanolic extract	Onsa-ard et al., 2012
Anti-inflammatory activity	Whole Plant	Ethanolic extract	Channa et al., 2006
Antimicrobial activity	Whole plant	Methanolic extract	Rajashekharappa et al., 2008
Antioxidant activity	Leaves & Whole Plant	Ethanolic, Methanolic & aqueous extract	Mohan et al., 2011; Meena et al., 2012; Subashri and Pillai, 2014
Gastrointestinal protective activity	Aerial part	Hydroethanolic extract	Subhan et al., 2010

Table 1: Pharmacological activity of different extract of Bacopa monnieri L

Bacopa monnieri L. contains many medicinally important compounds such as flavonoids (Singh, 2012; Pant et al., 2015), glycosides (Sivaramakrishna et al., 2005; Tothiam et al., 2011), terpenoids, saponins (Zhou et al., 2007; Phrompittayarat et al., 2007, 2008), bacosides, bacopasides (Agrawal et al., 2006), bacopasaponins and steroids (Mahato et al., 2000). Propagation of the plant takes place mainly through by vegetative method.

Lots of pharmacological and phytochemical properties of Bacopa monnieri L. made it an important medicinal plant. Due to its high demand, its natural resources are being depleted. In vitro culture method provides an alternative technique for large scale production of Bacopa monnieri L. without damaging the natural resource.

II. MATERIAL AND METHODS

A. Plant material, Sterilization, and culture conditions

Actively growing shoots of *Bacopa monnieri* L. plant bearing nodes were collected from medicinal garden of Chhattisgarh Medicinal Plant Board, Raipur, Chhattisgarh. Apical shoot tip and nodal segments were collected and used as explants. These explants were surface sterilized with 0.2 % (W/ V) $HgCl_2$ (RFCL Ltd, India) for 2-3 min and washed with sterile double distilled water for 4-5 times. Now the explants are ready for inoculation on required medium. Shoot tips were cut from the top measuring 1-2 c.m. inside the laminar air flow chamber. P^H of the media was adjusted to 5.8 using 0.1 N NaOH and 0.1 N HCl before autoclaving at 120° C pressure for 20 min. All the cultures were transferred to fresh medium after five weeks.

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Number of shoots and shoot length were evaluated after five weeks of inoculation and observation of any morphological changes were recorded.

B. Culture initiation and shoot differentiation

After washing and sterilization the explants were inoculated into three different growth media i.e. MS (Murashige and Skoog 1962), SH (Schenk and Hildebrandt, 1972), and B5 medium (Gamborg *et al.* 1968). All the media was supplemented with different concentrations (0.5mg/l, 1.0mg/l & 2.0mg/l) of cytokinin (BAP & KIN) (6-benzyl amino purine and Kinetin) alone and in combination. For multiplication in vitro shoots were sub cultured on the same media after four weeks of inoculation. Media without any growth regulator is used as a control. All the cultures were kept in culture room at the temperature of 25 ± 2°C under 16/8 hrs light and dark period. Cool- white fluorescent tube lights were used to provide the 100µE /m^2 light intensity. All the experiments were repeated three times and ten replicates were taken for each media concentration. Observation was recorded after five weeks of inoculation.

C. Rooting of in vitro shoot

In vitro generated shoots were sub cultured on half strength MS media fortified with different concentration (0.5mg/l, 1.0mg/l & 2.0mg/l) of auxin (NAA, IAA, IBA) (1- Naphthalene acetic acid, Indole- 3- acetic acid, Indole- 3- butyric acid) and cytokinin (BAP) alone and in combination. Ten In vitro shoots of around 5-6 cm length were cut and inoculated in each media combinations and each experiment was repeated three times. Half strength MS media without any growth regulator was used as control. All the cultures were initially kept in dark for 24 hrs. and then shifted to 16/ 8 hrs. light and dark period under 25 ± 2°C temperature condition. Percentage root induction, root number and root length was recorded after five weeks.

D. Acclimatization and field transfer

Well developed plantlets were washed in sterilized water then treated with 1% bavistin and 2.5 % crystal M- 45 fungicide for ten minutes. After washing plantlets were transferred in sterilized coco peat under green house for ten days. 25 – 32 °C temperature, 70- 80 % humidity and light intensity of about 2000 lux were maintained in the green house. After acclimatization in the green house plantlets were shifted in the field.

E. Statistical analysis

SPSS (version 16) software was used to analyze the data. Mean difference of each treatments were compared by one way analysis of variance. Significant difference within each treatment was compared by Duncan's multiple range test at 5% significant level. Shoot number and root number is non parametric data so they were first subjected to log transformation for making the data parametric then analyzed by SPSS.

III. RESULTS AND DISCUSSION

Shoot buds of Bacopa monnieri L. were cultured on MS, B5 and SH medium fortified with different concentration of Cytokinin (BAP and KIN) alone and in combination. Highest shoot bud induction was observed in MS medium than in SH medium. Shoot bud induction was very low in B5 medium. Among different concentrations (0.5, 1.0 and 2.0 mg/L) of Cytokinin 1.0 mg/L is best concentration for both BAP and KIN i.e. 93.33 and 90.00% respectively (Figure 1). This result is similar to the result of Kaur et al., 2013. No shoot bud induction was observed in control medium. Shoot number and shoot length was recorded after the four weeks of inoculation. Highest number of shoots and highest shoot length was also recorded in the same medium (Table No. 2).

For rooting well developed in vitro shoots were sub cultured on half strength MS medium supplemented with different concentration (0.5, 1.0 and 2.0 mg/L) of auxins (NAA, IAA and IBA). Roots were induced directly from the shoot base without any intervening callus growth. Root induction was observed in almost all concentrations of auxins but induction is highest in 0.5 and 1.0 mg/L IBA i.e. 80% (figure 2). This result is contrary to the result of Mehta et al., 2012 and Begum & Mathur, 2014 in which root bud induction from in vitro generated shoots of Bacopa monnieri L. was highest in 1/4 strength MS medium supplemented with 2.0 mg/L IBA. This difference in the result is might be due to the difference in the environmental condition of the plant. Root number and root length was highest in the 1.0 mg/L IBA and than in 0.5 mg/L IBA (Table No. 3). Root induction was also observed in the control medium where induction was 13.33%. This result is similar with the result of Seema & Koche et al., 2015 in which 15 % of root induction was observed in the half strength MS medium without any growth regulators.

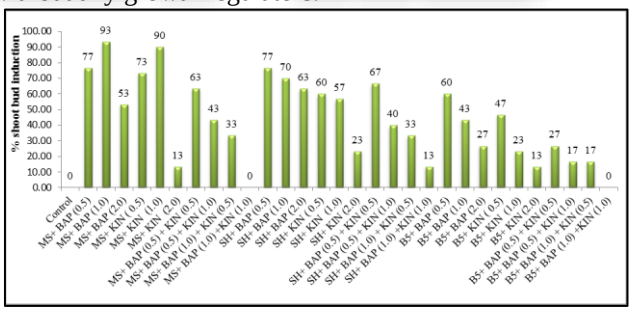

Fig. 1: Effect of different plant growth media and growth hormones on % shoot bud induction.

The rooted shoots were successfully transplanted to the plastic cups containing sterilized coco peat in the green house where humidity was maintained at approximately 90%. The plants were watered twice for about 15 days. Finally they were transferred to the field and maintained in direct sun light. The survival rate of in vitro generated plant was 90%. From this study we can conclude that MS media is best as compared to B5 and SH media for in vitro generation of Bacopa monnieri L.

Medium	MS		SH		B5	
Growth regulators mg/L	Shoot number/ explant	Shoot Length (cm)	Shoot number/ explant	Shoot Length (cm)	Shoot number/ explant	Shoot Length (cm)
BAP(0.5)	1.9 ± 0.04 bc	2.85 ± 0.31 bcd	2.06 ± 0.05 b	3.16 ± 0.34 bc	1.47 ± 0.05 bcdef	1.87 ± 0.31 efghi
BAP(1.0)	4.2 ± 0.04 a	5.04 ± 0.32 a	1.73 ± 0.05	2.49 ± 0.32	0.97 ± 0.05	1.28 ± 0.28

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			bcd	cde	efghi	hijklm
BAP(2.0)	1.1 ± 0.05 defgh	1.85 ± 0.33 efghi	2.63 ± 0.06 b	3.57 ± 0.53 b	0.63 ± 0.04 hijk	0.83 ± 0.26 klmnop
KIN(0.5)	1.9 ± 0.05 bc	3.07 ± 0.36 bc	1.47 ± 0.05 bcdef	2.05 ± 0.32 defg	0.97 ± 0.05 efghi	1.05 ± 0.22 jklmno
KIN(1.0)	4.87 ± 0.05 a	6.15 ± 0.40 a	2.63 ± 0.05 bcdef	2.00 ± 0.34 defgh	0.47 ± 0.04 ijkl	0.46 ± 0.17 lmnop
KIN(2.0)	0.27 ± 0.03 kl	0.37 ± 0.18 nop	1.20 ± 0.04 hijkl	0.68 ± 0.23 klmnop	0.23 ± 0.03 kl	0.22 ± 0.11 nop
BAP(0.5) +KIN(0.5)	1.13 ± 0.04 cdefg	1.52 ± 0.22 fghij	1.07 ± 0.05 bcde	2.22 ± 0.32 def	0.43 ± 0.03 ijkl	0.43 ± 0.14 mnop
BAP(0.5) +KIN(1.0)	0.87 ± 0.04 fghij	1.13 ± 0.25 ijklmn	0.06 ± 0.05 efghi	1.36 ± 0.32 ghijkl	0.27 ± 0.03 kl	0.03 ± 0.13 nop
BAP(1.0) +KIN(0.5)	0.63 ± 0.04 ghijk	0.95 ± 0.26 klmno	1.03 ± 0.05 ghijk	1.13 ± 0.31 ijklmn	0.03 ± 0.03 jkl	0.29 ± 0.13 nop
BAP(1.0) +KIN(1.0)	0 l	0.0 p	0 kl	0.21 ± 0.12 op	0 ± 0.00 l	0 ± 0.00 p

Table 2: Effect of different plant growth media and growth hormones on shoot number per explants and shoot length (cm)

Data shown are mean ± SE of three experiments, each experiment consist of 10 replicates, Means followed by same letter within each Column are not significantly different at p<0.05 according to Duncan's multiple range test.

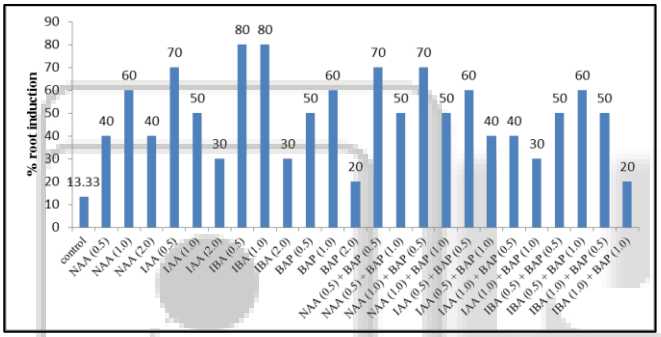

Fig. 2: Effect of different plant growth media and growth hormones on % root induction.

Hormones	**Mean root number (Mean ± SE)**	**Mean root length (Mean ± SE)**
control	1.1 ± 0.05 fgh	1.81 ± 0.42 efghi
NAA (0.5)	1.3 ± 0.06 efgh	1.87 ± 0.42 efghi
NAA (1.0)	1.8 ± 0.06 cdef	2.47 ± 0.39 defgh
NAA (2.0)	1.1 ± 0.05 fgh	1.73 ± 0.41 fghi
IAA (0.5)	2.1 ± 0.05 bcde	3.03 ± 0.39 bcdef
IAA (1.0)	1.3 ± 0.05 defgh	2.10 ± 0.41 defghi
IAA (2.0)	0.8 ± 0.05 gh	1.16 ± 0.33 hi
IBA (0.5)	3 ± 0.05 ab	4.16 ± 0.42 ab
IBA (1.0)	3.8 ± 0.06 a	05 ± 0.49 a
IBA (2.0)	0.8 ± 0.05 gh	1.2 ± 0.35 hi
BAP (0.5)	1.6 ± 0.06 defg	2.30 ± 0.44 defgh
BAP (1.0)	1.7 ± 0.05 cdef	2.6 ± 0.42 defg
BAP (2.0)	0.6 ± 0.04 h	0.84 ± 0.31 i
NAA (0.5) + BAP (0.5)	2.7 ± 0.05 bcde	3.09 ± 0.39 bcde
NAA (0.5) + BAP (1.0)	1.5 ± 0.06 defg	2.2 ± 0.05 bcd	3.21 ± 0.41 bcd
NAA (1.0) + BAP (0.5)	1.5 ± 0.06 defg	2.28 ± 0.43 defgh
NAA (1.0) + BAP (1.0)	1.5 ± 0.05 defg	2.41 ± 0.40 defgh
IAA (0.5) + BAP (0.5)	1 ± 0.05 fgh	1.47 ± 0.35 ghi
IAA (0.5) + BAP (1.0)	1.2 ± 0.05 fgh	1.64 ± 0.39 ghi
IAA (1.0) + BAP (0.5)	0.8 ± 0.05 gh	1.17 ± 0.35 hi
IAA (1.0) + BAP (1.0)	2.7 ± 0.05 abc	3.88 ± 0.41 abc
IBA (0.5) + BAP (0.5)	1.9 ± 0.06 cdef	2.76 ± 0.44 cdef
IBA (0.5) + BAP (1.0)	1.7 ± 0.06 defg	2.17 ± 0.42 defghi
IBA (1.0) + BAP (0.5)	15 ± 0.04 h	0.84 ± 0.32 i
IBA (1.0) + BAP (1.0)		

Table 3: Effect of different plant growth media and growth hormones on root number per explants and root length (cm)

Data shown are mean ± SE of 30 replicates, Means followed by same letter within each Column are not significantly different at p<0.05 according to Duncan's multiple range test.

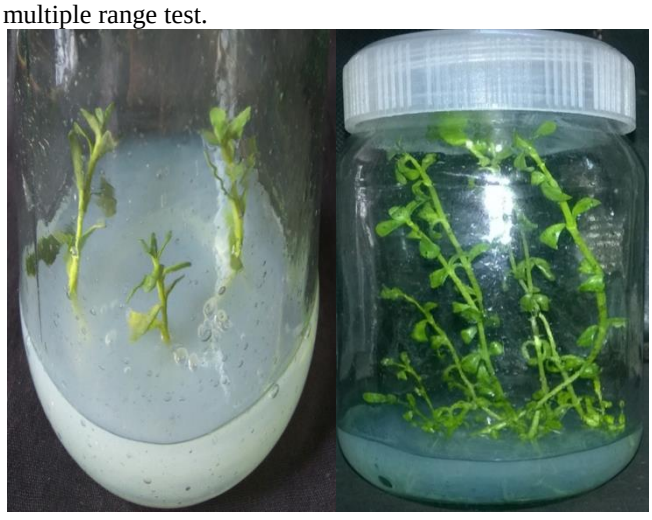

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Fig. 3: Different stages for direct In vitro regeneration of Bacopa monnieri L. from nodal culture. A) Shoot bud induction. B) Multiplication of shoot. C) Root formation from in vitro shoot. D) Complete plantlets. D) Hardening of in vitro generated plant.

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