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In-vitro Approach of Medicinal Herb: Bacopa monnieri

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Abstract: Bacopa (Brahmi) is a medicinal plant of high commercial potential with legendary reputation as a memory vitalizer. The plant is used in traditional Ayurvedic medicine in India for thousands of years to treat asthma, epilepsy and mental illness. Many different studies have showed that *Bacopa* is useful in treating Alzheimer's disease, anxiety, and age-related cognitive decline. It is also used to improve mental function and memory, treat respiratory ailments, and heal stomach ulcers. In this study Nodal segments of *Bacopa* have been taken which contained axillary buds. These buds were surface sterilized and were inoculated aseptically on culture medium. Axillary bud break was achieved in semisolid MS medium. These proliferated axillary shoots were excised and sub cultured on MS medium supplemented with 1.5 mg/l BAP for shoot multiplication. After shoot multiplication 1.0 mg/l IBA was used for rooting (90% rooting plantlets) and ¼ strength of liquid MS medium having 2% sucrose for hardening and acclimatized in the field. This showed better mass production of *Bacopa monnieri*.

Keywords: *Bacopa monnieri*, Memory vitalizer, Axillary shoots, MS medium.

I. INTRODUCTION

Bacopa monnieri is a small, annual, creeping herb with numerous branches, succulent, rooting at the nodes, with numerous prostrate branches, each 10-30 cm long. Brahmi leaves are oblong, sessile and fleshy. Flowers are purple in color; axillary, solitary with peduncles. Brahmi is found throughout India including the North Eastern region. Flowers and fruit appear in summer and the entire plant is used medicinally. It is an important Ayurvedic drug and traditionally it is reported to be used in skin diseases, fever, inflammation, anaemia, urinary disorder, and psychiatric disorders. It is also considered to be cardiotonic, a potent nervine tonic, and useful for asthma, hoarseness, insanity, and epilepsy. The leaves of *Bacopa* are used in speech disorders[1], in premature ejaculation[2], flatulence[3], abdominal pain[4], Aging, , Antioxidant, Stress, cough and cold[5,6,7] and leaf juice is used in rheumatism[8,9]. Revitalizer of intellectual faculty[10] . The major therapeutically important chemical constituents of this plant are triterpenoid saponins bacosides. Plant Tissue Culture is the culture and maintenance of plant cells or organs in sterile, nutritionally and environmentally supportive conditions (in- vitro). Plant cell and tissue culture has developed to a specialized branch in plant science through the establishment of tissue culture technique, demonstrating plant cell totipotency including micropropagation.The technique owes its origin to the German plant physiologist who described that plant cells have the ability to regenerate into a whole plant, the phenomenon known as totipotency. An additional advantage of tissue culture propagation of plants is known as "MICROPROPAGATION" which is economy of time and free from seasonal constraints

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Bacopa monnieri

Systematic Position and Habit of *Bacopa monnieri*

Kingdom	Plantae
Order	Lamiales
Family	Scrophulariaceae
Genus	*Bacopa*
Species	*monnieri*
Common names	Brahmi, *Bacopa*
English Name	Thyme leaved graticula
Sanskrit Name	Brahmi, Nira-brahmi
Synonyms	*Herpestris monnieri, Monnieri euneifolia.*

Picture of *Bacopa monnieri*

II. MATERIALS AND METHODS

Young and juvenile nodes of *Bacopa monnieri* were collected from natural source at Lucknow Utter Pradesh India. Nodal segments were used as explants material.

Surface sterilization of explants

For standardization of the technique of surface sterilization of the explants mercuric chloride was tried in various concentrations. The best concentration of sterilant was selected for sterilization of explants due to its supremacy over the others. Environmentally toxic $HgCl_2$ is a widely accepted surface sterilant in plant tissue culture. Best results are achieved when used judicially and carefully[11].

Procedure

1. Nodal explants were cut and washed in running tap water to remove the dust particles adhering to its surface.
2. Explants were washed in liquid detergent citramide (5-10 drops/100ml) in a vial by gentle agitating conditions.
3. Explants were again washed with distilled water to remove excess of detergent.
4. Explants were transferred to sterile empty flasks under aseptic conditions and given a quick dip in 70% alcohol and subsequently they were washed in distilled water.
5. The explants so washed were surface sterilized with different concentration of sterilant ($HgCl_2$) for different duration as per the treatment to find out the best treatment for sterilization of explants. To remove the traces of sterilant explants were washed in sterilized distilled water at least 5-6 times

Aseptic inoculations of Explants or establishment of Explants

Procedure:

1. The explants so sterilized were inoculated on to MS media supplemented with different concentration of cytokinin and auxins in a laminar flow cabinet under aseptic conditions using flame sterilized forceps.
2. Prior to the inoculation proximal and distal ends of explants were trimmed and finally 5mm-8mm long explants were inoculated on to the nutrient medium supplemented with varying concentration of cytokinin and auxins.
3. Mark each test tube with glass marking pencil.
4. In all the experiments 20 replicates were maintained.
5. Tubes were plugged with light fitting cotton plugs made of non-absorbent cotton wrapped in muslin cloth. All the cultures were incubated in a culture room at 23^{o}C for 16 hours in light illuminated by 40 watt. Fluorescent tubes, 1200 lux and for 8 hrs in dark.

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Vol. 3, Issue 5, May 2014

Multiplication of culture

Procedure

1. For optimum shoot multiplication best hormone's concentration were selected on the basis of number of shoots per explants. The shoots obtained on this concentration were sub cultured on medium having same composition for further multiplication.
2. Several shoots were transferred from a culture tubes to sterile petriplate using flame-sterilized forceps. The shoot was subdivided into small individual nodal segments using flame-sterilized scalpel.
3. These shoots were transferred to a new individual culture flasks containing best hormonal concentration for multiplication.
4. Sub-culturing was done at 21 days interval.
5. Process is repeated 3-4 times & effect of subsequent sub culturing was observed on the multiplication of shoots.
6. Incubate the flasks at 23^0C ±1 under cool white fluorescent light

Rooting of cultures

Procedure

1. Multiplied shoot mass was transferred to the Petri plate using flame sterilized forceps

Long shoots were cut with the help of flame-sterilized scalpel and transferred to rooting medium.

2. For this the salt concentration of MS basal medium was reduced to half and supplemented with the different concentration of auxins. Lower side of shoots should remain in the contact with media and shoots remain straight.
3. The mouths of tubes were heated and they were tightly plugged.
4. Each tube was marked with marker. In all experiments 20 replicates were maintained for each treatment.
5. Tubes were incubated at 23^oC under the cool white fluorescent light.
6. The following observations were recorded
    i) Percentage of rooted shoots
    ii) Average root length
    iii) Average number of roots
    iv) Number of days taken for rooting

Hardening & Acclimatization of Cultures

Before transferring the *in vitro* grown plantlets to pots it is advisable to harden them *in vitro*.

Procedure

Two methods were used:

1. Rooted shoots of *'Bacopa* 'were carefully taken out from the solid media.
2. (a) These plantlets were shifted to absorbent cotton. This is saturated with hormones free liquid ¼ strength MS medium having 2% sucrose. In this step absorbent cotton just acted as a substrate to support the root system of the plantlet.
   (b) Plantlets were shifted to the test tubes filled with quartz sand, saturated with hormones free liquid, ¼ strength MS medium having 2% sucrose.
3. These were placed in culture room for 2 weeks.
4. When rooted shoots had attained a height of about 2-3 cm, plantlets were taken in petriplate and media was removed from it carefully.
5. They were transferred to poly bags containing a mixture of soil and sand (1:1) in *ex vitro* conditions and covered with perforated polythene bags. The polythene bags were periodically withdrawn to acclimatize the plantlets and when new leaves started emerging the polybags were completely removed.

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6. Plants were transferred to field conditions.

III. EXPERIMENTAL RESULTS

Sterilization of explants

It was observed that when $HgCl_2$ was used with different time duration, 0.1% $HgCl_2$ treatment for 10 minutes gave fruitful results and at this concentration up to 90% nodal explants remained green and uncontaminated. On the basis of this finding 0.1% $HgCl_2$ for 10 minutes treatment was used for surface sterilization of nodal explants.

Table No. 1: Different percentages of Sterilant and Survival % of Explants

S.No	Explant	Sterilant	Conc.	Time	Survival %	Condition of explant
1	Node	$HgCl_2$	0.1%	10 min	90%	Green & Healthy
2	Node	$HgCl_2$	0.1%	5 min	80%	Green & Healthy

Initial Establishment of Explant (Shoot Induction / Bud break)

Bud break was achieved in 20 days in different media combinations. Maximum percentage of bud break was achieved MS medium supplemented with BAP (0.5 mg/l-2.0mg/l).

Table No. 2: Effect of plant growth regulator (BAP) on number of shoots Buds per explants formed.

BAP (mg/l)	Response %	Mean shoot number	Mean shoot length (cm)
0.0	15.88	1.12	0.57
0.5	48.97	2.15	2.52
1.0	57.53	2.64	2.60
1.5	29.90	1.49	1.29
2.0	24.10	1.38	1.05

Shoot Elongation

It was observed that shoots multiplied in this way showed very poor elongation and buds were in form of rosette clumps. Although repeated sub culturing of pre-established cultures showed slight increase in shoot length even then shoots required elongation prior to *in vitro* rooting. To facilitate elongation different treatments were tried for duration of 15-20 days. Proper elongation was observed in ½ strength MS medium devoid of plant growth regulators.

Table No. 3: Effect of different treatment on Elongation of shoots after 20 days of Inoculation.

S. No.	Media combination (mg/l)	No. of shoot bud per culture (approx.)	*Remark*
1	MS+1.0 mg/l BAP	30-35	Good elongation with weak elongated shoots
2	MS +1.5 mg/l	30-35	Proper elongation with healthy shoots.

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***In vitro* Rooting**

After elongation individual shoots were transferred to ½ strength MS medium supplemented with different concentration of IBA. Rooting was achieved within 10 days.

Table No.4: *In vitro* Rooting on Shoots

S. No.	Medium/Treatment (Conc. In mg/l)	No. of shoots kept for rooting	No. of shoots rooted	Rooting percentage	Remarks
1.	½ MS+ 1.0 mg/l IBA	10	9	90%	Good rooting
2.	½ MS + 1.5mg/l IBA	10	5	50%	Good rooting

***In vitro* Hardening**

Hardening of tissue culture desired plantlets are the most crucial step in the entire micropropagation process. The rooted shoots after attaining a height of 2.0-2.5 cm were hardened *in vitro* by placing them on liquid ¼ strength MS medium devoid of plant growth regulators and having 2% sucrose. Absorbent cotton soaked in this liquid medium was used for supporting root system of *in vitro* raised plantlets. Plantlets were maintained in this step for 7 days.

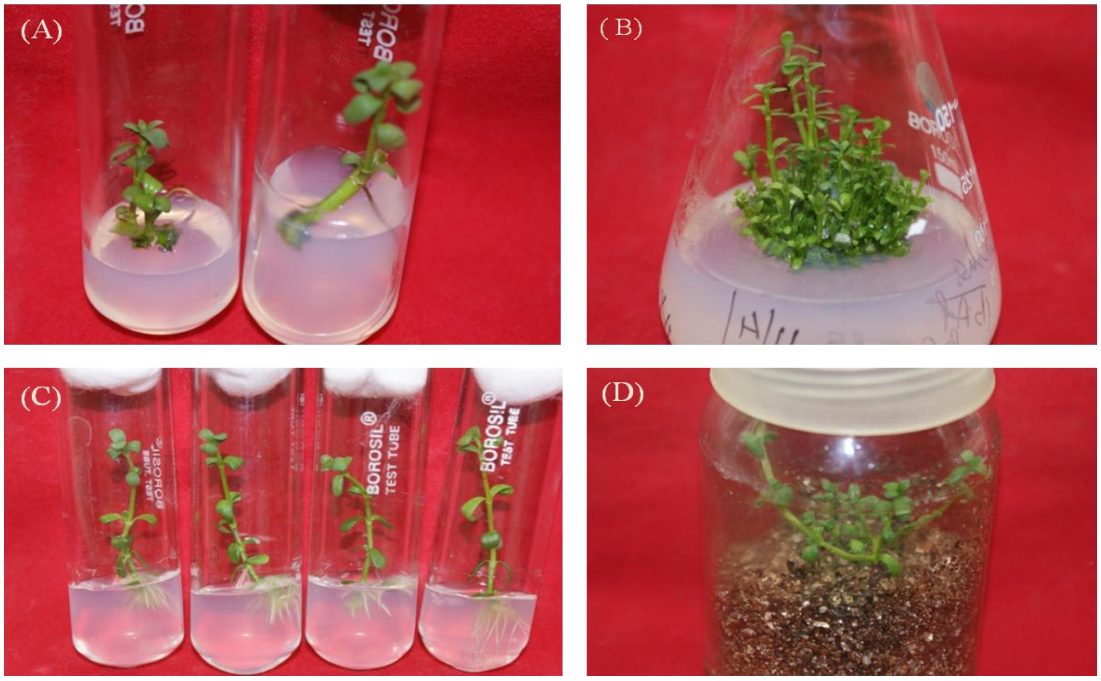

Figure 1: (A- D) *In-vitro* propagation of *Bacopa monnieri* (A) Bud breaks in nodal Explant(B) Shoot Multiplication(C) in vitro rooting (D)Hardening

IV. DISCUSSION

Bacopa monnieri is a medicinal plant used in Ayurvedic medicine for thousands of years to treat asthma, epilepsy and mental illness, Alzheimer's disease, anxiety, and age-related cognitive decline. It is also used to improve speech disorders[1], premature ejaculation[2], flatulence[3], abdominal pain[4], Aging, , Antioxidant, Stress, cough and cold[5,6,7] and

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leaf juice is used in rheumatism[8,9], Revitalizer of intellectual faculty[10]. Previous studies have shown the in vitro mass production of *Bacopa monnieri* by taking different combinations of sugar and agar with MS basal[12] and 0.15 mg/l IBA[13] for rooting. This showed the mass production in limited amount.and rooted plantlet are not hardened to the field. But in our study 1.0 mg/l IBA was used for rooting (90% rooting plantlets) and ¼ strength of liquid MS medium having 2% sucrose for hardening and acclimatized in the field. This showed better mass production of Bacopa monnieri compared to earlier study. This work was undertaken in order to learn the tissue culture technique for medicinally important plant *Bacopa which* is the mostly used for memory enhancing purposes. In the present study leaf & Nodal explants were taken from mature plant of *Bacopa* as Explant material for *in vitro* propagation. Leaf & Nodal segments were inoculated in MS media with different hormonal concentrations under aseptic conditions. The bud break was observed after two weeks &Axillary bud break was found to be best in MS medium. These axillary shoots were excised and cultivated in MS media supplemented with different hormonal concentrations which showed very high rate of shoots multiplication. The shoots were rooted in MS media supplemented with different concentration of IBA Old culture on rooting medium developed healthy roots in mist chamber and there after transferred in shade house for acclimatization in Field. So this technology is effective as it produces thousands of plants in a short span of time.

V. CONCLUSION

Bacopa monnieri is an important Ayurvedic drug useful against skin diseases, fever, inflammation, anaemia, urinary disorder, psychiatric disorders, asthma, hoarseness, insanity, and epilepsy. From the above study, it is concluded that in-*vitro* approach of B*acopa monnieri* is used to produce, maintain, multiply and transport pathogen-free plants safely and economically. **.** Such plants are usually free from bacterial and fungal disease, Shoot culture multiplication cycle is shortened (2-6 weeks) - each cycle resulting in a logarithmic increase in the number of shoots. *In-vitro* approach of B*acopa* also showed many useful functions, like a small piece of this plant tissue can grow millions of same plants within 2 months. Apart from this in vitro micropropagation of B*acopa monnieri* showed a highest rate of multiplication which cannot be seen in naturally found species of *Bacopa*. That is why *in vitro* approach showed better production of *Bacopa monnieri.*

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