Callus Induction in *Bacopa monnieri* (Linn.) Pennell by Nodal, Internodal, Young and Mature Leaf Explants

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Abstract:

Bacopa monnieri (L.) Pennell, is an annual herb of the family scrophulariaceae. *In vitro* callus induction protocol through young leaf, mature leaf, nodal and internodal segment culture was achieved when Murashige and Skoog medium supplemented with different combinations of phytoharmones. Of all the explants mature and young leaf were found to be best explant for callus induction. The MS-medium supplemented with 0.8mg/1 2,4-D and 0.3 mg/1 kinetin was the best medium for callus induction in mature leaf. The very simple and effective protocol developed can be used for the large scale cultivation of this very important medicinal herb.

Keywords:- *Bacopa monnieri*, Brahmi, Callus induction, Tissue Culture, *In vitro* callus Culture.

Introduction:

Medicinal plants are of great demand in modern civilization, India is known for the raw repository of medicinal plants, which are largely collected as raw materials for manufacture of drugs.

Ayurveda, the Indian Indigenous system of medicine, dating back to the vedic ages (1500-800 BC), has been an integral part of Indian culture (Weiss, 1987). *Bacopa monnieri* (L.) Pennell, commonly known in India as "Brahmi' is an important ancient Ayurvedic medicinal plant in the Scrophulariaceae family. It is also known as Jala-Brahmi. It is one of the sources of the 'Medhya Rasayana' in Ayurveda as it increases mental clarity and brain stimulating action (Bhattacharya *et al.*, 1998). It occurs throughout India in wet, damp and marshy areas (Anonymous, 1988). However it is frequently planted in freshwater aquaria. *Bacopa monnieri*, an herbaceous plant commonly found in temperate regions (Zuloaga *et al.*, 1999), is one of the 12 native species of the genus *Bacopa* present in Argentiana. This species has economical relevance as a medicinal plant, a pot plant and as an ornamental in aquatic garden (Tiwari *et al.*, 2001),

Bacopa monnieri (L.) Pennell, was placed second in a priority list of the most important Indian medicinal plants evaluated on the basis of their medicinal importance; commercial value and potential for further research and development (Shrivastava *et al.*, 1999). *Bacopa monnieri* works as an antioxidant that is reported to stimulate brain circulation and also enhances the seratonin level in brain (Tripathi *et al.*, 1996). It has also been used to treat blood and kidney disorders, reducing stress and also reported to activate an antistress response in animals (Chowdhuri *et al.*, 2002). It is also used to treat asthma, insanity, epilepsy, hoarseness, enlargement of the spleen, snake bite, rheumatism, leprosy, eczema and ringworm and as a diuretic asperitive and cardiotonic (Basu *et al.*,1944). It also possess anti-inflammatory, analgesic, antipyretic, anticancerous

and antioxidant activities (Satyavati *et.al.*1976; Jain *et.al.*1994; Elangovan *et al.*1995 and Vohora *et al.*1997). and diseases of the nervous system. Two plants *Centella asiatica* and *Bacopa monnieri*, are refered to as Brahmi.

Material and Methods:

Collection of plant materials:

In this investigation *Bacopa monnieri* belongs to the family scrophulariaceae, were selected as an experimental material. Explants used for in vitro regeneration were collected from wet land (stagnant water), water logged areas around Laxmi Narayan Institute of Technology Campus Nagpur. Few plants were also grown in experimental field of the Botany Department, Rashtrasant Tukdoji Maharaj Nagpur University campus, Nagpur, in order to keep the easy availability of the material whenever required for the experiments.

In Vitro Studies:

The success in cell, tissue and organ culture technology is related to the selection or development of the culture medium. As no single medium will support the growth of cell tissue cultures, moidifications in the nutritional component including growth regulators are often necessary for different types of growth responses in a single explant material.

Media preparation:

In this investigation a modified **Murashige and Skoog's** (1962) medium was selected as nutrient medium. Henceforth it will be referred to as MS-medium. A nutrient medium generally contains inorganic salts, vitamins, growth regulators, a carbon source and gelling agent. Other component added for specific purposes include organic nitrogen compounds, hexitols, amino acids, antibiotics.

In this investigation, MS-medium with Sucrose 3% (w/v) was used as carbon source. The medium was also supplemented with different combinations of 2,4-D, NAA, BAP, IBA, Kinetin a various concentrations (Table-A). The Stocks for the MS medium were prepared by dissolving one compound at a time in distilled water and precipitation was avoided by dissolving the inorganic nitrogen source first. Auxins were dissolved in ethanol and cytokinin in 0.5N HCL and final volume was made up with distilled water. vitamins and hormone stocks were preserved at –20^{0}C whereas, other stocks were preserved at 4^{0}C in freezer. In all the experiments, the analytical grade chemicals (Himedia, Merk and Sigma make) and double glass distilled water were used for the preparation of media. The PH of the medium (supplemented with respective growth regulators) was adjusted to 5.8 with 1N NaOH or 1N HCl. Then the medium was gelled with 0.8% w/v (8gm) Difco-Bacto Agar (Himedia, India) and kept in a microwave for (2 min.) dissolving the agar. Fifteen to twenty ml. of hot medium was then dispensed into culture vessel (Borosil, India) *i.e.* in conical flask (100 ml & 150 ml), test tubes (150 x 25 mm). Mouth of the culture tubes and flasks were plugged tightly with non-absorbent cotton. The tubes and flasks were autoclaved at 121^{0}C for 20 min. After autoclaving the tubes were transferred to culture room and placed in horizontal position for making a slant.

Explant selection and sterilization:

In present study, different vegetative explants used were node, internode, young and mature leaves. Explants were taken from disease free fresh plants.

Surface sterilization and Inoculation:

The explants, excised from plants were first washed thoroughly with distilled water. The washed explants were then surface sterilized with 70% ethanol for 1 minute and washed thrice with sterilized distilled water. The ethanol treated explants were surface sterilized with 0.01% $HgCl_2$ for 1 minute. Finally, explants were washed 5-6 times with sterilized distilled water. These surface sterilized explants were then inoculated in culture tubes or flasks containing MS medium supplemented with various concentrations of auxins and cytokinins in different combinations (Table-A). The inoculated culture tubes and flasks were then transferred to culture room, maintained at 25 ± 2°C temperature and 16h photoperiod with the irradiance of 45-50 µEM of fluorescent white light. The light intensity was 2400 lux with the humidity of 60%. All the operations were carried out in laminar air flow bench.

Induction of callus :

In the *Bacopa monnieri* (L.) Pennell, attempts were made to induce the callusing. The surface sterilized explants as mentioned earlier were inoculated on callusing medium (Table-A). When the callus grew to 1-2 cm. in diameter it was subcultured on same medium to maintain it and then used for inducing the organogenesis.

Observation and Result:

Effect of medium on callus induction and its growth

In *Bacopa monnieri* MS medium was found to be suitable for callus induction, multiple shooting and rooting.

The auxin 2,4-D and cytokinin Kinetin were tested in combination at various concentrations for callus induction. The MS medium supplemented with 2,4-D and Kinetin was found to be effective in all explants (mature and young leaves, node, internode) for induction of callus, except roots.

The concentrations of 2,4-D and Kinetin were observed to influence the initiation and nature of callus from different explants. The leaves inoculated on MS medium supplemented with 2,4-D (0.8mg/l) and kinetin (0.3mg/l) were observed to be profusely swollen from abaxial side of the margin and then showed callus induction on all over the surface on 8th day. Nodes after inoculation also showed sign of swelling and after 9th day epidermis was observed to be ruptured and it was followed by the transformation of entire nodal region into callus mass. After 15-20 days, a vigorously growing callus was obtained. In case of internodal explant the callus induction was seen exclusively at the cut ends on 9th day of inoculation (Table-1).

The increase in the concentration of 2,4-D to 1mg/lit was found to induce the early response for callusing in all the explants. In case of leaf explants, when

MS medium was supplied with 2,4-D (1mg/l) and Kinetin (0.3mg/l) callus initiation occurred on 5th day and then the whole leaf was transformed into white, soft, shining callus. Similarly, nodal and internodal explants responded on 7th day of inoculation for callus initiation. Callus obtained was also compact, soft shining. (Table -2).

The further increase in the concentrations of 2,4-D to 2mg/lit (MS medium supplemented with 2,4-D (2mg/l) and Kinetin (0.3mg/l)) initiated callus formation on 4-5th day in leaf and on 6-7th day in nodal and internodal explants (Table -3).

Similar response was found on the MS-medium with 2,4-D (2mg/l) and Kinetin (0.4mg/l), 2,4-D (2mg/l) and Kinetin (0.5 mg/l) (Table- 4 and 5).

In the present study, MS medium with 2,4-D (0.8mg/l) and kinetin (0.3mg/l) was found to be best medium for callus induction in all the explants. (Table -1)

Response of explants to Media

In the present investigation attempts have been made to generate callus using different parts of the plant. The parts used were mature leaf, young leaf, node, internode and root. All the explants used, except root, produced callus on the MS medium supplemented with various concentrations of 2,4-D and Kinetin. The mature leaf was found to be best the explant for callus generation, the percentage of callus formation being 99.10. The minimum percentage of callusing was observed when node was used as an explant. It varied between 65 to 85.36 depending on the concentrations of 2,4-D and Kinetin in MS medium. Young leaf and internode produced calli in little more than 96% of the explants (Table-1 to Table 5), (figure-I to III).

Discussion:

Callus induction usually requires the presence of auxins and cytokinins or both in the nutrient media. In this investigation, various types of explants such as leaf segments, nodal and internodal region, epicotyl, hypocotyl, roots were used for callus induction. These were inoculated on the media having different combinations and concentration of 2,4-D, Kinetin, BAP, NAA. In present study attempts were made for the development of protocol for callus induction.

In *Bacopa monnieri*, five different explants were used for callus induction. The response of all explants, except root, was found to be nearly same for callusing.. Mature and young leaves were the best responding explants, as compared to node and internode. Patra *et al.* (1998), reported the positive response of callus formation from stem and leaf explants of *Centella asiatica.* Similarly, Matkowaski (2004) established the callus formation from leaf, stem and root explants of *Nigella sativa*, *Azadirachta indica* and *Pueraria lobata* respectively. Similar reports were made by Youssef *et al.*, (1998) and Lei *et al.*(1998) in these plants. Sreedhar *et al.*(1999), found that explants like petiole and leaf segments were good for callus induction in case of *Pelargonium graveolens*. Manickam *et al.*(2000) used stem explants, in *Withania somnifera*, for obtaining callus. Reddy *et al.*(2001) used mature leaves of *Coleus forskohlli* for callusing. Zafar and Mujeeb (2002) reported that in *Tephrosia purpurea* stem and roots were the best seedling

parts for the callus induction than leaf. Toker *et al.*(2003) reported the formation of callus using different types of explants like stem, leaf and seed of *Ecballium elaterium* where, stem, nodes and leaf formed the callus to a lesser extent while, seed and root did not yield callus at all. Various types of explants such as leaf segments, leaf segments with petiole and internodal segments were used for callus initiation in *Centella asiatica*, by Sharma, (2004). Zheng *et al.*(2001) reported the induction of callus from leaf sections of *Trichosanthes kirilowii*. Callus induced from leaf and stem explants was reported by Srivastava and Rajani (1999) in *Bacopa monnieri*.

Nature of the callus

Calli derived from all explants were subcultured periodically on the medium used for inoculation of the explant. In *Bacopa monnieri* all the explants produced White, Soft and Compact callus. The variation in concentration of 2,4-D and Kinetin did not affect the nature of callus (Table-6) Effect of MS media on induction and nature of callus in different species (**Figure-IV and V)**.

Table. 1- Different concentrations of harmone used with MS medium

Medium for (mg/l)	Harmone concentrations (mg/l)
Callus induction	2,4-D(0.8) + Kin (0.3). 2,4-D(1) + Kin (0.3). 2,4-D(2) + Kin (0.3). 2,4-D(2) + Kin (0.4). 2,4-D(2) + Kin (0.5).

Table. 2- Effect of **MS**+ (0.8mg/l) 2,4-D and 0.3mg/l) Kinetin in combination on callus induction in different explants

Type of explant	No. of explant inoculated	% Response	Time duration for response
Mature leaf	10	99.10±0.52	8
Young leaf	10	96.66±3.28	8
Node	10	65.99±0.50	9
Internode	10	96.66±3.28	9
Root	10	00.00±0.00	0

Table. 3- Effect of MS+ (1mg/l) 2,4-D and (0.3mg/l) Kinetin in combination on callus induction in different explants

Type of explant	No. of explant inoculated	% Response	Time duration for response
Mature leaf	10	99.10±0.52	6
Young leaf	10	96.66±3.28	6
Node	10	81.93±0.90	7
Internode	10	96.66±3.28	7
Roots	10	00.00±0.00	0

Table. 4- Effect of **MS+** (2mg/l) 2,4-D and (0.3mg/l) Kinetin in combination on callus induction in different explants

Type of explant	No. of explant inoculated	% Response	Time duration for response
Mature leaf	10	99.10±0.52	5
Young leaf	10	96.58±0.25	5
Node	10	85.36±0.31	6
Internode	10	96.58±0.25	6
Root	10	00.00±0.00	0

Table. 5- Effect of **MS+** (2mg/l) 2,4-D and (0.4 mg/l) Kinetin in combination on callus induction in different explants

Type of explant	No. of explant inoculated	% Response	Time duration for response
Mature leaf	10	99.10±0.52	5
Young leaf	10	96.66±3.28	5
Node	10	85.36±0.31	6
Internode	10	96.66±3.28	6
Root	10	00.00±0.00	0

Table. 6- Effect of MS+ (2mg/l) 2,4-D + (0.5 mg/l) Kinetin in combination on callus induction in different explants

Type of explant	No. of explant inoculated	% Response	Time duration for response
Mature leaf	10	99.10±0.52	5
Young leaf	10	96.66±3.28	5
Node	10	85.36±0.31	6
Internode	10	96.66±3.28	6
Root	10	00.00±0.00	0

Table. 7- Effect of MS media on nature of callus in Bacopa monnieri (L.) Pennell

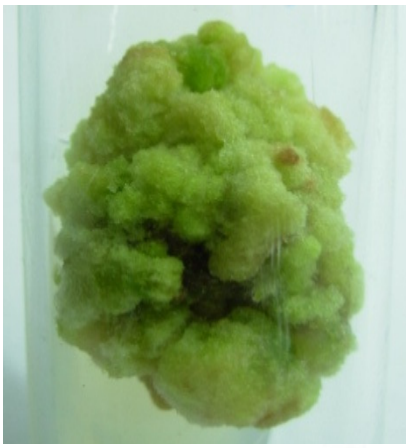
MS+Harmone conc . (mg/l)		Nature of callus
2,4-D	Kinetin	
0.8	0.3	White, Soft, Compact
1	0.3	White, Soft, Compact
2	0.3	White,Soft,Compact
2	0.5	White, Soft, Compact
2	0.5	White, Soft, Compact

(1)

(2)

(3)

Figure. 1- Callus proliferating from mature leaf explant
Figure. 2-Callus initiation from internodal explant
Figure. 3- Callus initiation from nodal explants



(4)

(5)

Figure. 4- Well developed callus from internodal region
Figure. 5- Subcultured callus from leaf explant

References:

Anonymous (1988) CF: Srivastava N, Rajani M (1999) Multiple shoot regeneration and tissue culture studies on *Bacopa monnieri* (L.) Pennell, *Plant Cell Reports* 18:919- 923.

Basu NK, Walia JS (1944) The Chemical investigation of the leaves of *Herpestis monniera. Indian J. Pharm* 4:84-91.

Bhattacharya SK and Ghosal S (1998) Anxiolytic activity of standardized extracts of *Bacopa monniera.* An experimental study. *Phytomed* 5:77-82.

Chowdhuri DK, Parmar D, Kakkar P, Shukla R, Seth PK and Srimal RC (2002) Antistress effects of bacosides of *Bacopa monniera* modulation of Hsp 70 expression, superoxide dismutase and cytochrome P 450. activity in rat brain. *Phytotherapy Research* 16(7):639-645.

Elangovan V, Govindasamy S, Ramamoorthy N and Balasubramanian K (1995) *In Vitro* Studies on the anticancer activity of *Bacopa monnieri. Fitoterapia* 66:211-215.

Jain P, Khanna NK, Trehan N, Pendse VK and Godhwani JL (1994) Anti-inflammatory effects of an Ayurvedic preparations Brahmi Rasayana, in rodents. *Ind. J. Exp. Biol*. 32:633-636.

Manickam VS, Mathavan RE & Antonisamy R (2000) Regeneration of Indian ginseng plantlets from stem callus. *Plant Cell Tissue and organ Culture* 62(3):181-185.

Matkowaski A (2004) *In vitro* isoflavonoid production in callus from different organs of *Purearia labata* (wild) ohwi. *J. Plant Physiol* 16(3):343-346.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tabacco tissue cultures. *Physiol plant* 15: 473-497.

Patra A, Rai B, Rout GR & Das P (1998) Successful regeneration from callus cultures of *Centella asiatica* (Linn.) Urban. *Plant Growth Regulation* 24:13-16.

Reddy PS, Rosalin R, Rajasekharan R (2001) Shoot organogenesis and mass propagation of *Coleus forskohlii* from leaf derived callus. *Plant cell Tissue and Organ Culture* 66:183-188. Satyavati GV, Raina MK and Sharma M (1976) Indian medicinal Plants. *Indian Council of Medical Research*. New Delhi 1:20-35.

Sharma S (2004) Micropropagation studies on *Centella asiatica* Linn. an important medicinal plant. A dissertation RTM Nagpur University, Nagpur .

Sreedhar D (1999) Tissue culture of *Pelargonium graveolens* L Her. Ex. A it (Geranium). In Khan IA and Khanum A (eds) A Role of Biotechnology in Medicinal and Aromatic. Plants. Ukaaz Publication, Hyderabad. 2:177-184.

Srivastava N, Rajani M (1999) Multiple shoot regeneration and tissue culture studies on *Bacopa monnieri* (L.) Pennell, *Plant Cell Reports* 18: 919-923.

Tiwari V, Tiwari KN and Singh BD (2001) Comparative. Studies of cytokinins on *in vitro* propagation of *Bacopa monniera. Plant Cell Tissue and Organ Culture* 66(1):9-16.

Toker G, lu Memio M, Toker MC and Yeilada E (2003) Callus formation and Cucurbitacin B accumulation *Ecballium elaterium* callus cultures. *Fitoterapia* 74 (7-8):618-623.

Tripathi YB, Chaurasia S Tripathi E, Upadhyay A. Dubey GP (1996) *Bacopa monniera* Linn. as an antioxidant : mechanism of action. *Indian J. Exp.* 34:523-526.

Vohora SB, Khanna T. Athar M and Ahmad B (1997) Analgesic activity of bacosine, a new triterpene isolated from *Bacopa monnieri. Fitoterapia* 68:361-365.

Weiss MG (1987) CF: Narayanaswamy S (1994) Plant Cell and Tissue Culture published by Tata Mc Graw Hill Publishing Compa ny Limited. New Delhi.

Youssef AA, Radi MR, Ghanem SA (1998) Growth and some primary Products in callus cultures of *Nigella sativa* as influenced by various cultural conditions and salt stress. *Fitoterapia* 69(4):329-326.

Zafar R, Mujeeb M (2002) Rotenoids and Rutin in callus cultures of *Tephrosia purpurea* (L) Pers. *Indian Jour Pharma Sci.* 64(3):217-221.

Zheng SS, Yuan HY, Wang LJ, An CC, Chen ZL. (2001) The tissue culture of medicinal plant *Trichosanthes Kirilowii* and its protein analysis. *Sheng Wu Gang Cheng Zue Bao* 17: 420-422.

Zuloaga FO and Morrone O (1999) Catalogode plants Vasculares de la *Republica argentina*. In: Monographs in systematic Botany From the Missouri Botanical Garden. *Missouri Botanical Garden Press* 74:1037-1038.