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IN VITRO MASS MULTIPLICATION OF *Bacopa monnieri* (L.) AN ENDANGERED AND VALUABLE MEDICINAL HERB

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ABSTRACT

***Bacopa monneiri* (L.) an important medicinal herb, commonly used as memory vitalizer has been used for mass multiplication through culture of nodal and apical shoot apex in MS (Murashige and Skoog, 1962) basal medium supplemented with various concentrations of 6-Benzyle amino purine (BAP), Kinetin, either alone or in combinations with IBA. Both BAP and KN at 0.5 mg/l concentration were found most suitable Cytokinin with respect to multiple shoot induction. At this concentration, the mean number of shoots on nodal explants was 18.0 where as it was 15.0 in shoot tip explants on subculture in the same composition of the medium. However, in case of KN,at the same concentration the mean number was 16 in the nodal explants and 12 in the shoot tip explants.When both the Cytokinin were used together, no promising result was obtained. This was also true for the supplementation of IBA. Well-developed shoots raised *in vitro* were excised and used for rooting in MS basal medium supplemented with various concentration of IBA.95% explants rooted in the medium supplemented with 0.25 mg/l IBA where the mean number of roots was 12.0 and average length 5.6 cm after 4 weeks. Well rooted plants were carefully taken out from the medium and washed properly with glass distilled water to remove the agar adhere with the roots. They were planted in the rooting pot containing 1:1:1 autoclaved vermiculite sand and soil. Humidity of the chamber (polybag) was maintained through water soaked sponge platform. Half strength liquid MS medium was used for irrigation on alternate day.82% plantlets survived in this condition while survival rate in the field was 72%.**

KEYWORDS: *Bacopa monneiri* L., mass multiplication, nodal explants, shoot tips, *in vitro* rooting, Poly Bag

Bacopa monneiri L. belongs to the family Scrophulariaceae is an important medicinal herb that grows in wild conditions. Well grown plants can be seen from May to July, however, few plants with poor growth may be seen in other months also. The plant is commonly known as “Brahmi” and found as spreading herbs. Multiple branches form a mat like structure if they are growing on hard surface. Small roots are produced from each node. They may be found in acute dry places or even in moist one also. However, plants growing in moist shady places contain larger leaves than the plants growing in dry places .White flowers can be seen in a well grown plants during rainy season .Seeds are small and they germinate in the month of February to March.

Bacopa monneri (L.) has been mentioned in Ayurveda as Medha Rasayana i.e., brain tonic. We now know that this plant is used to reduce mental stress, improves memory and also used in case of anxiety .It possesses anti-inflammatory, analgesic and antipyretic properties (Vohra et al., 1997). Brahmi is also used to treat epilepsy, enlargement of spleen, leprosy, eczema and other skin diseases, rheumatism, insanity asthma and snake bite, appetitive and cardiotonic (Basu and Walia,1994). *B. monneiri* has been placed second in priority list of most important Indian medicinal plants evaluated on the basis of medicinal plants evaluated on the basis of medicinal importance, commercial value and potential for further research and development (Mohapatra and Rath, 2005; Sharma et al.,2007). Active ingredients in this plants are steroidal saponins Bacoside A and steroidal saponins Bacoside BAP alkaloid Brahmine, herpestine, saponins –d-mannitol, hersaponin acid A and monnerin etc. In addition, butulic acid, stigmasterol, beta tistosterol and bacosides have been reported from this plant (Shrma et al., 2010). When the capsule memory plus was released in the market the species faced over exploitation by the agents of pharmaceutical companies. The actual demand as per National Medicinal Plant Board was 6621.8 tons in 2004-2005.It has 7% growth rate per year. Therefore, one can imagine the actual requirement to meet the need inside the country or for export. Inspite of all, even today, we depend on the population growing in the wild habitat. Natural propagation is through the seeds. But their numbers are not sufficient to be used for large scale plantation. Micropropagation through tissue culture is the only way for the production of uniform planting materials.

Plant tissue culture technique are being used for the conservation of germplasm of important and threatened medicinal plants. *Bacopa monnieri* L. needs also immediate conservation. Micro propagation technique provides new possibilities for *in vitro* propagation and multiplication of plants and also recognised as an efficient tool for rapid clonal propagation (Negrutiu *etal.,*1984). This technique have been utilised by (Singh et al., 1999; Sehrawert et al.,

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2001; Nagaraza et al., 2003; Anil Kumar et al., 2005; Das et al., 2005; Jain and Chatruvedi , 2005; Mahendra et al., 2005; Ahmad et al., 2007; Zia et al., 2007; Vijaya Kumar, 2010). Keeping this idea in mind the present work was done in the Plant Biotechnology Laboratory of University Department of Botany,B.R.Ambedkar Bihar University Muzaffarpur.

MATERIALS AND METHODS

Murashige and Skoog's basal medium was used for the in vitro culture of different explants of *Bacopa monneri* L. Stock solutions of different constituents were prepared separately .Now required amount for 1L of culture was taken from these stock solution. Final volume was obtained by adding glass distilled water .MS basal medium supplemented with 3% sucrose and gelled with 0.8% Agar(Hi Media). Growth regulators were added in different concentrations & combinations. The pH was adjusted to 5.8 adding few drops of 1N NaOH/KCl.40 ml of the above medium was dispensed in the 250 ml culture bottles. Above bottles were autoclaved at 15 lb pressure for 20 min. The temperature was expected to be at 121^0C.The culture bottles were allowed to cool at room temperature and were stored in freeze for further experiments

Preparation of explants

On 3rd day, plants of *Bacopa monnieri* were collected from its wild habitat from the botanical garden of University Department Muzaffarpur. Plants were brought in the laboratory in a beaker containing fresh water. Plants were washed in running tap water for 45 min. to remove the soil particles other extraneous fine particles attached with the plants. Nodal segments, the shoot tips were cut from the healthy branches. They were again washed in running tap water and then soaked in 0.2-0.5 % bavistin and 0.04 % streptomycin aqueous solution for 8-10 minutes. This was followed by washing with sterile distilled water. This was reapeted thrice. Above explants were put in the 1.5 % solution of savlon for 8-10 minutes. After this these explants were washed thoroughly with sterile glass distilled water. This was repeated thrice. Finally the explants were treated with 0.01 % $HgCl_2$aqueous solution for 1 minute and again rinsed with sterile distilled water shaking manually so that even a trace of the chemical was removed completely. Above explants were wrapped in pre-sterilised cloths soaked in sterile distilled water. It was stored in freeze and inoculation was done just after it.

Inoculation

To avoid any contamination during inoculation, all the procedures of inoculation were performed in the aseptic chamber of laminar flow. All the cultures were incubated in the culture room at 26 ± 1^0C temperature with a photoperiod of 16 hours day light and 8 hours dark under the cool white fluorescent light. For rooting well grown in vitro, plantlets were inoculated in the same way in the cultures bottles containing different concentrations of auxins. They were also incubated in the similar conditions as mentioned above

RESULTS AND DISCUSSION

Plant tissue culture technique arte being used to produced large number of uniform planting materials. They are being used to colon the woody trees having important role in forestry, as cash crops, ornamentals as well as in medicinal plants. *In vitro* propagation techniques are the powerful tool for germplasm conservation and therefore, they are being used for rapid multiplication of endangered or threatened species. The ability to generate plants directly from, the explants is the fundamental basis for clonal propagation of elite germplasm via micro propagation (Ignacimuthu, 1997). Plants biotechnology is consider in a wide sense which comprises the various culture of plant organ and explants to facilitate experimental approaches with a large objectives of developmental biology in grain legumes for crop modification (Ramawat, 2003). In the present work, attempts have been made to raise multiple shoots in the nodal and shoot tip explants of *B. monnieri*. In this study above explants were inoculated in MS Basal medium supplemented with various concentrations of (BAP 0.1-1.0) KN alone and in combinations. It was also supplemented with IBA. The data were collected and have presented ion the table 1 & table 2. From the table 1, it is apparent that axillary bud break was achieved almost in the all concentration of BAP, KN used alone or in combinations. However, BAP at 0.5 mg/l concentration gave the best result where 92% of the explants responded and the mean number of axillary shoots in the nodal explants was 16.2, and on the shoot tips 12.3 respectively. At the similar concentration Kinetin induced shoot buds among 91% of the explants and the mean number of shoots was 12.4 in nodal explants whereas 10.2 in the apical shoot.

From the table 1 it is also clear that BAP(0.5 mg/l) +KN and NAA(0.2 mg/l each) could initiate axillary buds among 96% of the explants and

BAP+KN+NAA(0.5 mg/l+0.5mg/l+0.2mg/l) induced axillary shoots among 98% of the explants. Here, the mean number of axillary branches was 18.8 and 14.6 in nodal and shoot tip explants respectively. The maximum mean length of the axillary shoot was 3.48 cm while the lowest 1.22 cm. From the table it is clear that increasing concentration of BAP and KN upto 0.5 mg/l had pronounced impact on the axillary shoot bud initiation. Similarly, BAP+KN+NAA had synergistic action on shoot bud initiation due to which there was increase in the mean number of axillary shoot buds. Likewise, among the explants the nodal explants were more suitable than the shoot tips and among the growth regulators.BAP was superior over KN. Above findings corroborate with the findings of Shrivastava,1999;Tiwary *etal*.,2000;Tiwary *etal*.,2007; Sharma *etal*.,2010.

In vitro raised plants do not contain functional roots. Initiation of roots among such plants depends on several endogenous and exogenous factors. The role of auxins in the root development has been established and reviewed by Torrey,1965,1976.Hu and Wang (1983) reported that because the shoot contain enough cytokinin therefore, it is usually not supplemented in the medium used for rooting. In the present work, experiments were performed for rooting in tissue culture raised plantlets of *B.monneiri* L.

From the table 2, it is clear that 0.2 mg/l IBA and 0.3 mg/l NAA induced rooting among 86% and 84%of the plantlets cultured.Here the mean number of roots was 18.6 and 12.4 respectively. It was further noted that 0.2mg/l each of IBA +NAA had no promising impact on the percent response, however the mean number of roots were slightly increased.

MS medium supplemented with different Cytokinin and one Auxins was found suitable for multiple shoot initiation. Above findings are in agreement with the findings of other worker viz; (Tiwary et al, 1998 & 2000; Tejavathi et al, 2001; George, 2004; Binita et al, 2005; Escandon et al, 2006; Sharma, 2007; Sharma et al, 2010).These workers expressed their ideas that for *Bacopa*, high concentrations of salts and Vitamins of MS medium is required.

Hardening of the in vitro rooted plants was done in the cabin in which the temperature and humidity were maintained artificially. 82% plantlets survived in these condition while 72% in the field conditions.

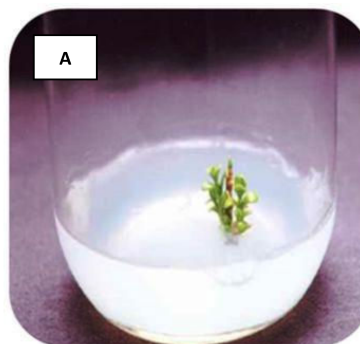

Figure A: Initiation of axillary buds on the nodal explants

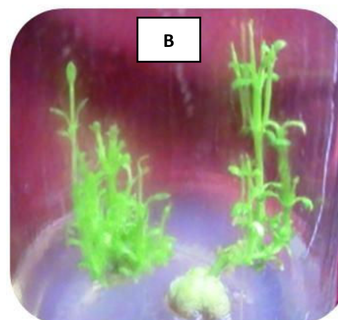

Figure B: Multiple shoots in MS+0.5 mg/l BAP+0.5mg/l KN+0.2 mg/l NAA

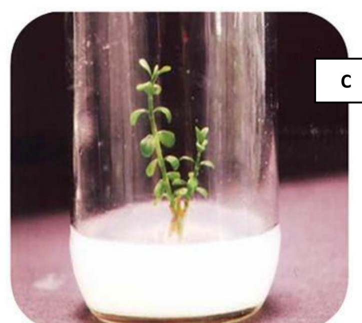

Figure C: Seperated plants cultured in the same medium

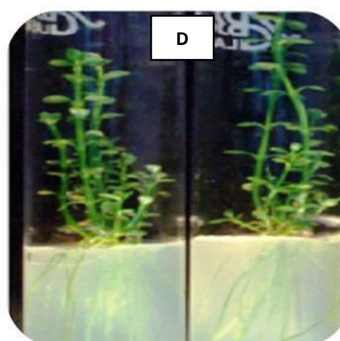

Figure D: Multiple shoots after 4 weeks used for rooting

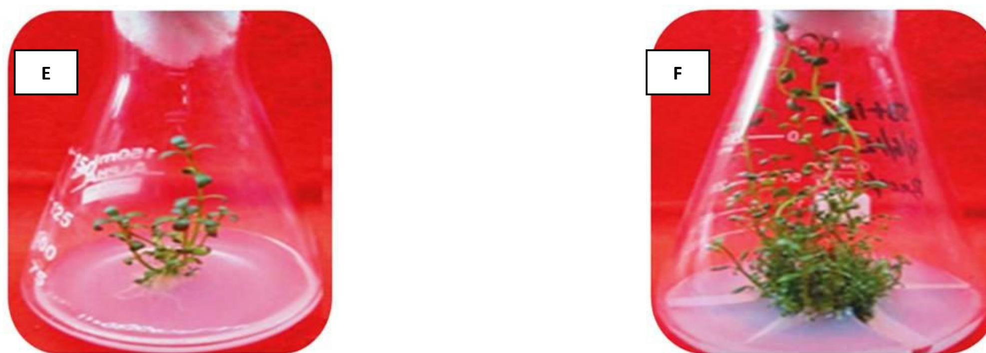

Figure E&F: Plantlets showing rooting

Table 1: Effect of Cytokinins (BAP and KN) and auxins IBA supplemented in MS medium on the initiation of multiple shoots on nodal and apical shoot explants of *Bacopa monnieri* L.

Growth Hormones			Explant- Nodal Segment			Explant-Apical Shoot		
BAP	KN	NAA	% Response	Mean Number of Shoots	Mean length in cm	% Response	Mean Number of Shoots	Mean length in cm
0.10	-	-	32%	6.6±.20	1.22±.15	26%	4.2±.15	0.8±.18
0.20	-	-	46%	7.4±.22	1.56±.18	38%	5.8±.20	0.8±.18
0.30	-	-	66%	7.8±.26	2.40±.22	58%	6.4±.26	1.20±.24
0.40	-	-	84%	12.4±.30	2.62±.24	76%	8.6±.30	1.68±.26
0.50	-	-	92%	16.2±.38	3.16±.28	84%	12.3±.32	2.4±.36
-	0.10	-	28%	4.2±.18	0.6±.15	22%	2.8±.18	0.52±.16
-	0.20	-	41%	4.8±.20	0.72±.20	36%	3.6±.22	0.64±.18
-	0.30	-	62%	6.5±.24	1.10±.24	59%	5.4±.30	0.88±.22
-	0.40	-	83%	10.8±.34	1.86±.30	71%	8.5±.34	1.22±.26
-	0.50	-	91%	12.4±.36	2.48±.40	78%	10.2±.36	1.68±.30
0.50	0.20	0.20	96%	16.6±.38	3.18±.26	85%	12.3±.15	2.4±.36
0.50	0.30	0.20	96%	16.8±.38	3.18±.26	85%	12.6±.18	2.4±.36
0.50	0.40	0.20	94%	18.2±.36	3.40±.30	88%	12.8±.20	2.66±.32
0.50	0.50	0.20	98%	18.8±.40	3.48±.42	90%	14.6±.26	2.80±.36
0.20	0.50	0.20	91%	12.4±.36	2.48±.44	78%	10.2±.36	1.68±.30
0.30	0.50	0.20	91%	12.8±.38	2.50±.46	78%	10.38±.36	1.68±.30
0.40	0.50	0.20	92%	12.6±.32	2.60±.48	81%	10.82±.40	1.76±.36
0.50	0.50	0.20	96%	10.0±.40	3.48±.50	90%	14.6±.42	2.76±.36

Table 2: Effect of two auxins IBA and NAA at five different concentrations used alone or in combinations on rooting of plantlets raised through tissue culture

Growth regulators (mg/l)		Percent response	Mean number of roots	Mean root length	Remark
IBA	NAA				
0.15	-	68%	8.4± 1.32	6.4±.76	Slow growth,
0.20	-	86%	18.6±1.42	7.6±.80	thin root
0.30	-	82%	16.2±1.36	7.2±.80	Healthy root
0.40	-	78%	12.4±1.32	5.4±.74	Healthy root
0.50	-	72%	11.8±1.38	5.4±.74	Slow growth
1.00	-	20%	4.4±1.26	3.6±.66	Slow growth
-	0.15	52%	6.6±1.24	5.5±.74	Thin root
-	0.20	66%	6.8±1.24	5.8±.76	Poor growth
-	0.30	84%	12.4±1.32	6.2±.84	Poor growth
-	0.40	76%	9.6±1.28	4.8±.68	Healthy
-	0.50	70%	7.2±1.16	4.2±.62	
0.20	1.00	18%	3.6±1.12	2.8±.66	
0.20	0.10	66%	18.6±1.42	7.6±.80	
0.20	0.20	86%	18.8±1.42	7.4±.74	
0.20	0.30	86%	18.8±1.42	7.8±.82	
0.20	0.40	76%	12.5±1.22	6.2±1.16	
0.20	0.50	72%	10.6±1.14	6.6±1.24	
0.20	1.00	20%	3.8±1.12	5.2±1.26	

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