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In Vitro Culture of Highly Valuable Medicinal Plant Bacopa Monnieri (L.) Penn. for Rapid and Mass Multiplication.

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ABSTRACT : *A protocol has been developed for micropropagation of Bacopa monnieri (L.) Penn., a medicinal plant of high commercial potential with high reputation as a memory vitalizer or enhancer. Nodal segments containing axillary buds were surface sterilized with 0.1% solution of mercuric chloride for 5 min and were inoculated aseptically on culture medium. In vitro clonal multiplication methods and the elite clones were observed that, MS basal+0.5 mg/l IAA and MS basal+ 0.5 mg/l NAA shown the best results for culture initiation and axillary shoot proliferation. For rooting MS+ Agar 7 g/l, Sugar 20g/l and MS+Agar 8g/l were found to be the best mediumin terms of shoot/root ratio and numberof shoots.Compact globular callus was best initiated and proliferated on MS+0.5 mg/l BAP+1 mg/l 2,4 D and medium with best regeneration in MS+1.0 mg/l BAP+1 mg/l IAA. The experimentation was made successful with 71% survival plantlets producing regeneration of the Bacoppa monnieri L. Penn. for mass cultivation.*

KEYWORDS: *Bacopa monnieri MS medium, Rooting, Shoot multiplication*

I. INTRODUCTION

Bacopa monnieri L. (Family Scrophulariaceae) also referred to as B. monniera, Herpestis monniera, water hyssop, and "Brahmi," has been used in the Ayurvedic system of medicine for centuries [1]. It is a genus of spreading herbs, commonly growing in damp and marshy places throughout India, ascending up to an altitude of 1320 m, and it is a small creeping, glabrous, succulent, herb rooting at nodes. It is an ancient and renowned medicinal plant with legendary reputation as memory vitalizer [2]. Traditionally, it was used as a brain tonic to enhance memory development, learning, concentration and to provide relief to patients with anxiety or epileptic disorders. The plant has also been used in India and Pakistan as a cardiac tonic, digestive aid, and to improve respiratory function in cases of bronchoconstriction. Bacopa's antioxidant properties may offer protection from free radical damage in cardiovascular disease and certain types of cancer [3]. It possesses anti-inflammatory, analgesic and antipyretic activity [4]. In very recent study, B. monnieri was placed second position in a priority list of most important Indian medicinal plants evaluated on the basis of medicinal importance, commercial value and potential candiadte for further research and development [5,6]. It is also well known to contain steroidal saponins Bacoside A and steroidal saponins Bacoside B. Some other constituents present in Brahmi are alkaloids brahmine, herpestine etc. Compounds responsible for the pharmacological effects of bacopa include alkaloids, saponins and steroids.The effects of B. monnieri have already been approved by clinical research trials. There is thus an immediate need for assessing the natural populations, developing protocols for micro propagation, regeneration and agronomical practices. The present study deals with the rapid mass scale multiplication of B. monnieri using nodal explants containing axillary bud.

II. MATERIALS AND METHODS

2.1 Collection of explants

Nodal segments were collected from the juvenile shoots of growing at Experimental Nursery, Tectona Biotech Resource Centre (TBRC). Juvenile segments containing single axillary bud shoots tips were used as source material for micro propagation.

2.2 Sterilization of explants

B. monnieri twigs with 3 - 4 nodes were collected, excised from plants maintained in the nursery, Tectona Biotech Resource Centre (TBRC) were washed thoroughly in running tap water to remove the superficial dust and mud, then explants were washed with dilute detergent (1 - 2% Labolene) solution for 15 min and then washed well in running tap water. Again these explants were dipped in to the Bavistene (3-4%) fungiside. Explants were surface sterilized with 5 min treatment with 0.1% (w/v) $HgCl_2$. B. monnieri was extremely sensitive to surface sterilizing agent, therefore, the surface sterilization procedure was optimized and this helped in preventing blackening of tissues and establishment of clean cultures. During surface sterilization treatment, it was found that treatment with 0.1% mercuric chloride leads to blackening of the explant [7,8].

Hence limited treatment of 0.1% mercuric chloride was given to plants 4 - 5 min, and it was proved to be the best sterilent. Finally, the explants were washed thoroughly (4 - 5 times) with sterilized distilled water. Throughout the experiments, MS [9] medium with 3% (w/v) sucrose and gelled with 0.7% (w/v) agar is used. The pH of all media was adjusted to 5.8 before autoclaving at 121°C (15 min). The cultures were incubated in a culture room at 25 ± 2°C under 16 hour photoperiod.

III. RESULTS AND DISCUSSION

3.1 Initiation of axillary bud

It was observed that best medium for initiation and development in terms of bud breakage (%age) and maximum shoot length were BMY4 (MS+ 0.5 mg/l IAA) with shoot length of 5.5 cm; 80% bud breakage and BMY1 (MS +0.5 mg/l NAA) with shoot length of 4.3 cm; 100% bud breakage. In the other four mediums: BMY2 (MS + 0.5 mg/l KIN.), BMY3 (MS+ 0.5mg/l BAP), BMY5 (MS+0.5mg/l BA+0.5 mg/l KIN+0.5mg/l NAA), BMY6 (MS+1.0mg/l BAP+0.4mg/l KIN+0.4mg/l NAA) and MS, shoot length was less as compared to above defined mediums "Table 1 and Fig 1".

Table I: Observation for axillary bud induction at 15 days after initiation

Medium	Contamination	Bud breakage	Avg. shoot length
MS	0.27± 0.15	1.00± 0.00	2.70 ± 0.17
BMY_1	0.22± 0.04	0.80± 0.07	4.3± 0.32
BMY_2	0.15± 0.05	1.00± 0.00	2.3± 0.12
BMY_3	0.16 ±0.04	0.70 ±0.12	2.1± 0.18
BMY_4	0.25 ±0.01	0.68± 0.10	5.5± 0.54
BMY_5	0.24 ±0.06	0.78± 0.10	2.4 ±0.44
BMY_6	0.24 ±0.02	0.77± 0.24	2.5 ±0.43

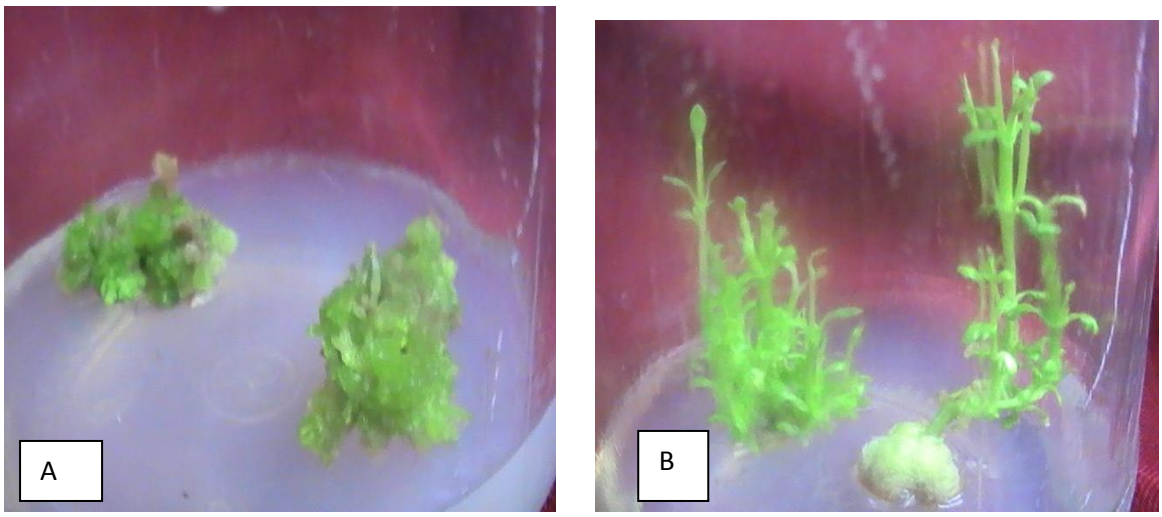

Figure 1: Base callusing & multiple shoot growth at media composition; (A) MS + .2 mg/l KIN media & (B) MS +1.5 mg/l BAP respectively.

3.2 In vitro shoot multiplication

Observations were taken for evaluating the growth of explants by taking parameters like internodal distance (inter-nodal distance was measured form the third node and measured upto sixth node from the shoot tip), average shoot length and number of nodes (15 shoots randomly selected per medium). The experiment was carried out in six mediums having different concentrations of growth regulators each with three replications; only results of best medium are given. The medium showing best results was BMY4 and BMY1 with highest average shoot length of 5.3cm and 4.6cm respectively (Fig 2). Sub culturing was carried out after 25-30 days using the same medium combinations as for initiation and establishment stages. Shoot clusters obtained in each subculture was divided in approximately 1-2cm size with 4-5 small shoots in each cluster and inoculated in each bottle. Other parameters like number of nodes, average shoot length and intermodal distance are also observed "Table 2". The texture of leaf was succulent and fleshy. Medium strength also influences the multiplication rate of shoots as observed.

Table II: Observation of multiple shoot proliferation

Medium	No. of shoots>3 cm	No. of nodes	Avg. shoot length(cm)	Inter nodal Distance(cm)
MS	3.2± 0.28	3.26± 0.00	2.42 ± 0.24	0.72± 0.01
BMY_1	10.3 ± 0.07	6.95± 0.00	4.6± 0.18	0.79± 0.00
BMY_2	7.2± 0.05	6.2± 0.02	2.3± 0.33	0.70± 0.02
BMY_3	5.5± 0.50	2.5± 0.00	2.9 ± 0.12	0.75 ± 0.06
BMY_4	18.8± 0.13	7.30 ± 0.09	5.30± 0.11	0.85 ± 0.00
BMY_5	6.5 ± 0.10	4.3 ± 0.06	3.01 ± 0.09	0.77 ± 0.08
BMY_6	6.70± 0.12	5.30± 0.07	2.90± 0.22	0.78 ± 0.07

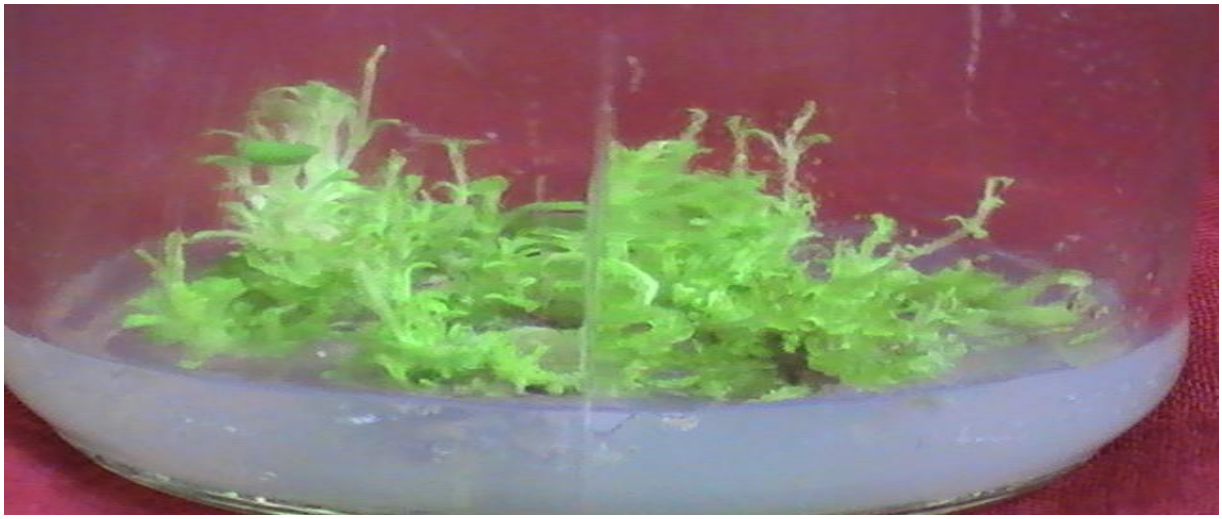

Figure 2: No of shoots produce at the media composition MS+ 0.5 mg/l IAA is 18.8

3.3 In vitro rooting

After two cycles of multiplication subculture, elongated shoots of 2-3 cm in length were excised and cultured on MS basal medium having different combinations of sugar and agar with MS basal (MS+ Sugar 30 gm/l +Agar-8 gm) as control. The experiments were conducted twice, with 3 replications (with 3 shoots per bottle). Rooted shoots were taken after 2 weeks, shoot length, root length and no of roots per explant (total 9 explants per treatment each time), fresh weight and dry weight (keeping them in an oven with 50^0C for 24 hrs) were measured. Initiation of rooting took place after 5-6 days of inoculation. Single and multiple roots were formed from the base and the nodal portions and the length of the roots were 1-2 cm within 8-10 days. It was observed during multiplication that rooting in Bacopa in vitro culture is fairly spontaneous and no addition of growth regulators is further necessary. Hence the experiment is designed to study the rooting response with different treatment combination with two variations of agar (7 and 8 gm/l) and sugar (0, 10, 20, 30 gm/l). It was postulated that highest shoot root ratio (S/R) and biomass accumulation may indicate positive responses. Minimal media (RT_1 and RT_2) i.e. having 0% sugar and lower concentration of agar also show positive results so this combination can also be used for rooting of explants "Table 3and Fig 3". Our results indicate 100% root formation in all the mediums (MS basal without any additional growth regulator).

Table III: Observations for rooting

Medium	Root length(cm)	Shoot length(cm	Shoot/ Root	No. of roots	Fresh wt. (gms)	Dry wt. (gms)
MS	2.12 ± 0.16	3.17± 0.10	2.15 ± 0.21	5.20 ± 0.04	2.38 ± 0.24	1.47 ± 0.02
RT_1	1.50 ± 0.24	2.75± 0.24	1.83 ± 0.70	3.35 ± 0.04	1.90 ± 0.02	1.16 ± 0.04
RT_2	2.40 ± 0.01	3.20± 0.09	1.33 ± 0.04	6.00 ± 0.14	1.78 ± 0.01	1.07 ± 0.07
RT_3	1.80 ± 0.19	2.60± 0.09	1.44 ± 0.19	9.30 ± 0.14	2.73 ± 0.07	1.79 ±0.02
RT_4	2.30 ± 0.09	3.60± 0.24	1.56 ± 0.54	6.10 ± 0.14	2.70 ±.0.06	1.19 ±0.08

RT_5	2.02 ± 0.01	6.00± 0.24	2.97 ± 0.04	7.20 ± 0.19	2.54 ± 0.06	1.21 ± 0.08
RT_6	2.68 ± 0.09	6.70± 0.09	2.50 ± 0.04	7.72 ± 0.01	2.58 ± 0.06	1.05 ± 0.12
RT_7	1.88 ± 0.07	2.88± 0.24	2.50 ± 0.09	5.58 ± 0.04	2.09 ± 0.06	1.18 ± 0.02

Figure 3: Growth of root from shoot at media composition: MS+20mg/l Sucrose +7 gm/l Agar

IV. DSCUSSION

From the present studies MS media proved to be the best culture medium for the establishment of shoot culture in B. monnieri. In earlier reports on Bacopa, MS medium has been successfully used for shoot initiation and culture establishment.In the present study, in vitro shoot multiplication rate gradually increased with each cycle of subculturing. Shoot multiplication was found to be the most significant when four to five shoots propagule units were used for multiplication. The significant growth of shoots multiplication in Brahmi on MS medium may be due its higher concentration. These present results are supported by the findings of other workers who have also observed and experimentally found the active influence of MS medium for optimum shoot multiplication in different Bacopa species [10-15]. Medium strength also greatly influences the rate of shoot multiplications as observed. When the explants were cultured on MS medium for multiplication, maximum shoot length was obtained on full strength medium in Bacopa species. This indicates the absolute requirement of higher concentration of salts and vitamins for multiplication.The ability to form roots by plant tissue depends on close interaction of many endogenous and exogenous factors. The role of auxin in root development was established and reviewed by [16]. As there is enough residual cytokinin present in shoots, therefore, little or no cytokinin is required in rooting medium by Hu and Wang [16].

V. CONCLUSION

Micropopagated **Bacopa monnieri (L)** may proof record increased yield at commercial rate and it is clear that if the correct strategies for the use of micro popagation of this herb are developed and used in appropriate circumstances, the technology will have a major effect in the next decades on both the nature of herbal crops that are grown internationally and also on the whole structure of horticulture and agronomy world. It is also important to analysis the bioactive compounds of the Bramhi with comparison with naturally grown plant. The Bacoppa research will give a new insight of research in medicinal components of plants through various advance techniques.

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