Micro propagation and evaluation of growth parameters of Bacopa monnieri (L)

Vicky1, Ikbal1, Vivek Srivastava2
1Department of Biotechnology, Om Sterling Global University, Hisar
2Department of Botany, Dayanand College, Hisar

Abstract
Bacopa monnieri is an important medicinal and economically useful plant. Its extracts are of primary ingredient in many pharmaceutical goods and are utilized for therapeutic purposes. Due to indiscriminate pharmaceutical demand, it has been obliterated from its natural environment. Therefore, an efficient protocol was developed for rapid micropropagation of Bacopa monnieri. In vitro multiplication method was used for the regeneration of this extremely endangered germplasm. Nodal and shoot tips were explanted on MS media with 3% sucrose, 0.8% agar, and different plant growth regulators. Shoot tip explants on MS media with 1.0 mg/l BAP + 0.25 mg/l Kinetin had the best responses. The best rooting response was on MS media with 1.0 mg/l IAA + 0.2 mg/l NAA.The regenerated and rooted plantlets were acclimatized and successfully transferred to soil for normal growth under field conditions with a 75-83% success rate after primary and secondary hardening. This procedure could be used to preserve and massively multiply economically significant and commercially valuable plants.

Keywords: Bacopa monnieri, In-vitro culture, Micropropagation, Plant regeneration

Introduction

Plant tissue culture is a multi-skilled technique. It is frequently used in the development of novel, higher-value varieties of economically significant plant species as well as in vitro genetic improvement. In vitro propagation now forms the basis of a growing industry by supplying quality planting material in vegetatively propagated species. There is a wide variety of medicinal plants in India and a significant progress has been made on various medicinal plant species in last decade (Deepika et al. 2015). Ayurvedic, Unani, and other remedies, as well as a number of pharmaceutical products, utilize these medicinal plant extracts for therapeutic purposes (Anand et al, 2022). The plant Bacopa monnieri (L.) is an essential medicinal and commercial resource. It belongs to the Scrophulariaceae family and is frequently referred to as Brahmi or Nirbrahmi. Bacopa monnieri is another name for Brahmi. (L) (Pothiaraj et al., 2022). It is a highly popular intellect promoter, antihypertensive, and neuropathic antidepressant (Alkahtani et al., 2022). It is a genus of spreading herbs that can grow up to 1320 m above sea level and is frequently found in marshy, wet areas throughout India (Shahid et al., 2017). It is a tiny, glabrous, succulent herb with nodes (Sharma et al., 2010). It is a recognized and old medicinal herb with a legendary reputation for boosting memory (Fatima et al., 2022). Bacopa monnieri contains a wide range of chemical substances, including alkaloids (including nicotine, herpestine, and brahmin), flavonoids, saponins, betulinic acid, glycosides, and phytosterols (Sanyal et al., 2022).

Due to its numerous active chemical components, it has anti-inflammatory, immunomodulatory, antioxidant, anti-cancer, and antipyretic properties (Sulaiman et al., 2022). Asthma, hoarseness, spleen enlargement, insanity, leprosy, epilepsy, ringworm, eczema, rheumatism, and snake bite are among the conditions it is used to cure (Sudheer et al., 2023). It has the potential for further research and development due to its medical benefits and market value. B. monnieri was named number two among the most important medicinal herbs in India (Lala, 2020). Additionally, it has diuretic, appetite-stimulating, and cardiotonic properties (Hu et al., 1983). It is crucial to preserve this therapeutic plant using micropropagation of shoot apical meristem and nodal segments. With its numerous and extensive uses, plant tissue culture continues to be one of the most fundamental biotechnological processes.

The efficient transplantation of B. monnieri and the rapid multiplication of true-to-type plants can help preserve and propagate superior plants for commercial use (Aggarwal et al., 2011). Numerous researchers have also used in vitro clonal propagation and conservation protocols with B. monnieri (Mokat and Jadhav, 2003). Researchers studied how benzyl amino purine affected the regeneration of shoot buds from isolated. We present a method for micro-propagating Bacopa monnieri using explants taken from the nodal and apical meristem regions of the stem.

Materials and Methods

Source of explants

The specimens of Bacopa monnieri were acquired from the Department of Molecular Biology and Biotechnology Chaudhary Charan Singh Haryana Agricultural University, Hisar. Following the gathering of plants, they were then kept in pots within the greenhouse located at Om Sterling Global University Hisar. Various plant components were utilized as explants in order to facilitate in-vitro regeneration. The initial investigations involved

the utilization of the nodal and shoot tip of Bacopa monnieri as explant material.

Sterilization of explants
The nodal and shoot tip samples of the explants were put through a cleaning technique that began with a wash in running water for eight minutes and was then followed by treatment with a liquid detergent known as teepol for five minutes. Explants were cleaned in laminar air flow using 70% alcohol for one minute, 4% sodium hypochlorite for five minutes, and then four to five times washed with water.

Inoculation of Explants
The excision of the explants was performed under sterile conditions in a Laminar Air Flow cabinet. Subsequently, the excised explants were placed in a culture medium known as M.S., which included 3% sucrose, 0.8% agar, and had a pH of 5.8. Prior to the addition of agar, the pH of the medium was adjusted using a solution of either 1 N NaOH or 1 N HCl. The media was thereafter transferred into culture tubes and subjected to autoclaving at a temperature of 121°C and a pressure of 15 pounds per square inch for 20 minutes. The explant was subjected to incubation at a temperature of 25°C. In each experimental trial, a total of ten different media variations were utilized for the purpose of culturing. Following a four-week period of initial culture, the explants were subsequently sub-cultured using the same media. The investigation focused on analyzing the initial locations and dispersal patterns of shoot and root regeneration, with a particular emphasis on understanding the growth responses of the explants on a weekly basis.

Shoot regeneration via nodal segments
Single node segments taken from an adult plant of B. monnieri were cultured on both full and half-strength MS Media. The nodal explants were also cultured on MS medium (full strength) supplemented with various combinations of concentration (M.S +1.0 BAP + 0.25 Kn, M.S +1.0 BAP + 0.2 NAA, M.S +1.0 BAP + 0.5 Kn, M.S +1.0 BAP + 0.5 NAA, M.S +1.5 BAP) in mg/l of auxin with BAP and NAA as well as BAP, Kinetin and NAA in combinations. So, their effects are monitored on shoot induction growth and multiplication.

Shoot regeneration via shoot tip explants
Leaf explants were taken from an adult plant of B. monnieri cultured on both full and half strength of MS basal medium. The explants were also cultured on MS medium supplemented with BAP and NAA as well as BAP, Kinetin, and NAA in combinations (M.S +1.0 BAP + 0.25 Kn, M.S +1.0 BAP + 0.2 NAA, M.S +1.0 BAP + 0.5 Kn, M.S +1.0 BAP + 0.5 NAA, M.S +1.5 BAP) in mg/l.

Rooting of regenerated plants
Elongated shoots regenerated from both the explants were transferred to both half and full strength. MS basal medium with growth regulators, like IAA, IBA, 2,4-D and NAA added in different concentrations (M.S +1.0 IAA + 0.25 IBA, M.S +1.0 IAA + 0.5 NAA, M.S +1.0 IAA + 0.2 NAA, M.S +1.5 IAA, M.S +1.0 IAA + 0.5 (2,4 D) in mg/l.

Hardening
The plantlets were transferred to autoclaved vermiculite for the purpose of hardening, subsequent to a thorough washing procedure aimed at eliminating any residual agar. The plantlets were cultivated in a controlled tissue culture setting and were provided with a nutrient solution consisting of half-strength MS medium (devoid of organic components) on a biweekly basis for duration of two weeks. After a period of two weeks, the bottles were moved to a mist chamber that had a temperature of 30º and a relative humidity that ranged from eighty percent to ninety percent. Prior to being transferred into polybags, the caps on the bottles were removed and the plantlets were retained within the bottles for 3-4 days. The polybags contained a mixture of sand, farmyard manure, and soil in equal proportions (1:1:1). In the beginning, the humidity levels were kept elevated through periodic water spraying. Following this, the plantlets underwent a hardening process for duration of 10 days before being transplanted into the field located at Om Sterling Global University Hisar.

Evaluation of in vitro raised plants under field condition:

a) Photosynthetic rate: The estimation was conducted using the Infra-Red Gas Analyzer (IRGA) method, as described by Kumari et al. (2018). The measurement of trace gases with an infrared gas analyzer involves the assessment of the absorption of infrared light emitted by a certain light source, as it passes through a given air sample.

b) Estimation of chlorophyll content: The chlorophyll pigments were estimated by using dimethyl sulphoxide (DMSO) (Kumari et al., 2018). The extraction is based on soaking tissue fragments in a predetermined amount of DMSO and incubating them at a temperature of 60–65° C. After incubation, the solvent is removed, and the absorbance at the proper wavelengths that penetrate the photosynthetic pigments was measured.

c) Nitrogen: The estimation of nitrogen concentration in various plant sections was conducted using Kjeldahl's steam distillation method, as described by AOAC (Association of Analytical Chemists) in 1975. The

digestion of the sample occurs at an estimated temperature of 420 °C, utilizing concentrated sulphuric acid (Werner et al., 1975). The presence of potassium sulfate, which helps to raise the boiling point, as well as a catalyst, in the form of selenium, which helps to speed up the digestive process, both contribute to the ease with which this process can be carried out. Consequently, the nitrogen present in the sample undergoes a conversion process, resulting in the formation of nonvolatile ammonium sulfate. Following the process of cooling and dilution of the digest, the ammonium sulphate undergoes conversion into volatile ammonia gas with the application of heat in the presence of sodium hydroxide. The process involves the steam distillation of ammonia into a surplus of boric acid solution, resulting in the formation of ammonium borate when it becomes trapped. The procedure of titration, which makes use of hydrochloric acid (HCl), is what is used to determine the amount of borate that is created. The employment of a color indicator allows for the achievement of the goal of titration, which is the determination of the endpoint.

d) Net assimilation rate

Net assimilation rate is the rate of accumulation of dry mass expressed on the leaf area.

$$NAR = \frac{1}{LA} \cdot \frac{dww2 - w1}{dtt2 - t1} \text{ or } \frac{\log e\, LA2 - \log e\, LA1}{LA2 - LA1} \times \frac{}{}$$

Unit is weight leaf area-1time-1 log e is natural or neparian leg. This formula is used when the ratio between plant weight and leaf area is one.

Change in RGR can be better explained in terms of NAR and LAR (Watson, 1958).

e) Protein content

The Bradford Method was used to measure the protein content of tissue-cultured plants by comparing the findings to Bonjoch and Tamayo's 2001 BSA standard curve. During the experimental process, the whole nitrogen content of the sample is liberated through the use of elevated temperatures. The optimal wavelength for quantifying the blue coloration resulting from the Coomassie dye-protein complex is 595 nm, as this particular wavelength exhibits the greatest disparity between the two dye forms. The blue coloration can be perceived within the wavelength range of 575 nm to 615 nm, as needed. The underlying principle of this assay is predicated on the interaction between protein molecules and Coomassie dye in an acidic environment, which subsequently induces a perceptible alteration in color from brown to blue. The ultimate volume of the mixture was 1 liter after it was generated by combining 5 mg of Coomassie Blue with 100 mL of orthophosphoric acid. This produced a solution that had a total volume of 1 liter. The substance in question was employed as a Bradford reagent.

Results

Tissue culture is the sole quick method for bulk plant replication and a potent tool for conserving plant germplasm. The ability to produce seedlings directly for explants is essential for the clonal micropropagation of elite germplasm. Shoot tip and node segments were used as the material for explants. But shoot tip explants showed a quick and positive response after 15 days of culture. These explants were injected into MS media that contained various growth hormones in combinations (M.S +1.0 BAP + 0.25 Km, M.S +1.0 BAP + 0.2 NAA, M.S +1.0 BAP + 0.5 Km, M.S +1.0 BAP + 0.5 NAA, M.S +1.5 BAP) in mg/l. Within 12–14 days of inoculation, the explants had grown; however, callus development did not begin until 20 to 25 days. In MS, media supplemented with growth regulators M.S +1.0 mg/l BAP + 0.25 mg/l Kn promote callus growth to a 90% success rate. However, after 21 days of culture, the optimal conditions for callus formation obtained from the tip of the shoot as explant were discovered on the medium containing MS media supplemented with 0.75 mg/l (6-BA). Later the callus was subcultured on M.S. media supplemented with 1.0 mg/l BAP + 0.25 mg/l Kinetin, for shoot multiplication. Every four weeks, a subculture was performed. Rooting in MS medium with 1.0 mg/l IAA + 0.2 mg/l NAA yielded the best response (85%) from several roots and an average shoot length of 4.30 cm was obtained. The percentage germination rate was 94 percent with an average number of 4.5 roots per shoot over a period of 4 weeks. As a result, both the current and earlier research demonstrated that growth hormones of auxin with BAP and NAA as well as BAP, Kinetin, and NAA in combinations had a significant impact on callus induction in the nutritional media. Among the various variables affecting the formation and regeneration of calluses are the plants' genotypes, the environment during incubation, and the primary elements that determine the composition of nutritional media chosen in which the cultures grow in vitro.

When the nodal explants were implanted on MS media for the purpose of multiplication, it was discovered that the shoot length was shorter when compared with the shoot tip as an explant. Additionally, growth kinetics was slower with the nodal explants. The percentage germination rate was 14% lower as compared with shoot tip as explant. Nodal explants were injected into MS media that contained various growth hormone (6-BA) concentrations.

Subculturing: Nevertheless, it was seen that the most favorable callus growth occurred after a period of 21 days in culture. This growth was observed specifically in the tip of the shoot, which served as the explants. The medium used for this observation consisted of MS media supplemented with a concentration of 0.50 mg/l of 6-Benzylaminopurine (6-BA). Later the callus was subcultured on M.S. media supplemented with 1.0 mg/l BAP + 0.2 mg/l NAA for shoot multiplication. Every four weeks, a subculture was performed. The best response (78%) was recorded from multiple roots, and the average shoot length was 3.90 centimeters with an average of 4.0 roots per shoot throughout the course of a period of 4 weeks. The MS medium was supplemented with 1.0 mg/l IAA and 0.2 mg/l NAA for rooting. The presence of auxins in root growth demonstrates that there is sufficient cytokinin present in shoots; hence, little or no cytokinins are required in the rooting medium. This is due to the fact that auxins play a role in root development.

Transplantation to greenhouse:
Rooted plantlets of B. monnieri (L.) were transferred from culture bottles to polythene bags carrying a variety of sterile soils containing vermicompost (4:1) and soil with VAM (10:2) in accordance with the results. The plantlets were grown in a controlled environment. It was noted that the survival rate was 80% to 90% of the graft. Thus, it is evident that the physicochemical characteristics of the soil had a significant impact on the growth and biochemical processes of the plants, in addition to the nature and texture of the soil, on plant growth.

The plants underwent a three-week period of confinement within the mist chamber, whereby they were irrigated with a solution consisting of a half-strength MS medium. Subsequently, in order to facilitate acclimatization, the polybags were transferred to a two-tiered agro net open shade house with high-density occupancy. After duration of one month in the shade house, the plants were transplanted into larger polybags or pots, maintaining the same soil composition. Subsequently, the plants were irrigated using tap water. The plants were subjected to a shade house environment for duration of two months, during which an estimated survival rate of 78-84% was observed among the plantlets. The procedure outlined in this study suggests that Bacopa monnieri holds potential for the preservation and cultivation of superior plant specimens, with the aim of facilitating their economic utilization. Hence, it is apparent that the growth of plants is influenced by both the soil's composition and texture, as well as its physicochemical features, which play a substantial role in shaping the plants' growth and biochemical mechanisms.

Table: 1 Effect of various combinations of growth regulators on shoot growth

Explant	M.S media + PGA	Number of shoots/explants after 28 days of culture	Average no. of shoots/explants after 28 days of culture	The length of shoots/explants after 28 days of culture (cm)	The average length of shoots/explants after 28 days of culture (cm)
Leaf	M.S +1.0 BAP + 0.25 Km	8	7.4	9.0	8.4
		8		8.4	
		7		7.6	
		7		8.8	
		7		8.2	
	M.S +1.0 BAP + 0.2 NAA	6	6.4	8.9	8.2
		8		9.1	
		6		7.8	
		6		8.0	

		6		7.2	
	M.S +1.0 BAP + 0.5 Kn	9	7.2	8.9	8.1
		8		8.4	
		6		7.2	
		7		8.8	
		6		7.2	
	M.S +1.5 BAP	7	6.0	7.7	6.8
		5		6.9	
		7		5.9	
		5		6.6	
		6		6.9	
	M.S +1.0 BAP + 0.5 NAA	7	6.4	6.9	7.0
		7		7.1	
		6		6.4	
		5		7.6	
		7		7.0	
Nodal Piece	M.S +1.0 BAP + 0.25 Kn	7.1	6.9	7.4	7.8
		6.7		8.2	
		6.4		7.8	
		7.4		8.1	
		6.9		7.5	
	M.S +1.0 BAP + 0.2 NAA	6.1	6.1	6.9	7.0
		7		7.1	
		5.5		6.4	
		5.9		7.6	
		6		7.0	
	M.S +1.0 BAP + 0.5 Kn	6	6.8	7.1	7.4
		8		7.1	
		8		7.3	
		6		7.9	
		6		7.6	
	M.S +1.5	6	5.8	6.1	6.4

	BAP				
		7		7.9	
		6		6.0	
		5		5.8	
		5		6.2	
	M.S +1.0 BAP + 0.5 NAA	7	6.0	6.1	6.6
		5		7.9	
		7		7.0	
		5		5.8	
		6		6.2	

Table: 2 Effect of various combinations of growth regulators on root growth

Explant	**M.S media +Rooting PGA (mg L^{-1})**	**Number of roots/shoot after 28 days of culture**	**Average no. of roots/shoot after 28 days of culture**	**The length of roots/shoot after 28 days of culture (cm)**	**The average length of roots/shoot after 28 days of culture (cm)**
Leaf	M.S +1.0 IAA + 0.25 IBA	5	4.4	5.2	4.4
		4		3.8	
		4		4.2	
		4		3.8	
		5		5	
	M.S +1.0 IAA + 0.5 NAA	3	3.4	3.1	3.0
		4		3.1	
		4		3	
		3		3.2	
		3		2.6	
	M.S +1.0 IAA + 0.2 NAA	5	4.6	3.7	3.8
		4		3.5	
		5		3.8	
		5		4.2	
		4		3.8	
	M.S +15 IAA	4.7	4.2	4	3.8

		3.8		5	
		3.7		4	
		3.8		3	
		5.0		3	
	M.S +1.0 IAA + 0.5 (2,4 D)	4	3.8	5.2	4.4
		5		3.8	
		4		4.2	
		3		3.8	
		3		5	
Nodal Piece	M.S +1.0 IAA + 0.25 IBA	4	3.8	5.2	4.4
		5		3.8	
		4		4.2	
		3		3.8	
		3		5	
	M.S +1.0 IAA + 0.5 NAA	3	3.4	3	3.1
		4		3.1	
		4		3.4	
		3		2.9	
		3		3.1	
	M.S +1.0 IAA + 0.2 NAA	5	4.6	3.6	3.7
		4		3.7	
		5		3.6	
		5		3.8	
		4		3.8	
	M.S +15 IAA	5	4.6	3.6	3.4
		4		3.2	
		5		3.6	
		5		3.2	
		4		3.4	
	M.S +1.0 IAA + 0.5 (2,4 D)	4	3.8	4.6	4.0
		5		3.8	

		4		4	
		3		3.8	
		3		3.8	

The photosynthetic rate of plants (Bacopa monnieri) rose under field conditions in different modes of propagation and was recorded after sixty days of growth. The photosynthetic rate of plants obtained through in vitro regeneration from Nodal explant was 18.27µmol/m2/s, and from shoot tip as explants 18.62µmol/m2/s (Table 3).

b) Estimation of chlorophyll content: The total chlorophyll content of plants (Bacopa monnieri) rose under field conditions in different modes of propagation and was measured after seventy days of growth. Plants rose through Nodal explant was 1.48 mg/g fresh wt and 1.98 mg/g fresh wt for Shoot tip as explants (Table 3).

c) Net assimilation rate: The net assimilation rate of plants (Bacopa monnieri) rose under field conditions in different modes of propagation and was measured after seventy days of growth. Plants rose through Nodal as explant was 54.32 g m-2 w-1, 58.96g m-2 w-1 for Shoot tip as explants (Table 3).

d) Nitrogen content: The nitrogen content of plants (Bacopa monnieri) rose under field conditions in different modes of propagation and was measured after seventy days of growth. Plants rose through Nodal as explant was 4.56 mg/g dry wt 1, 5.32 mg/g dry wtfor Shoot tip as explants (Table 3).

e) Protein content: The protein content of Bacopa monnieri plants cultivated under several ways of propagation in field circumstances was assessed after a growth period of seventy days. The plants exhibited an increase in growth when subjected to Nodal as an explant, with a recorded weight of 3.59mg/g. Similarly, the Shoot tip explants demonstrated a weight of 4.01mg/ (Table 3). The shoot tip explants exhibited a higher maximum photosynthetic rate compared to the plant raised using nodal explants. A comparable pattern was noted in Bacopa monnieri with regard to chlorophyll content, nitrogen content, protein content, and net absorption rate.

Table: 3 Evaluation of in vitro raised plants for growth parameters grown under field condition

Parameters	**Shoot tip as explants**	**Average Shoot tip as explants**	**Nodal as explants**	**Average Nodal as explants**
Photosynthetic Rate (µmol/m^2/s)	18.8	**18.62**	18.4	**18.27**
	18.8		18.3	
	18.1		17.98	
	18.6		18.4	
	18.8		18.3	
Total Chlorophyll Content (mg/g fresh wt.)	2.02	**1.98**	1.55	**1.48**
	1.94		1.41	
	1.98		1.46	
	2.00		1.52	
	1.96		1.46	
Net Assimilation Rate (g m^{-2} w^{-1})	58.96	**58.96**	54.02	**54.32**
	58.9		55.02	
	58.02		55.04	

	60.00		53.02	
	58.92		54.5	
Nitrogen content (mg/g dry wt.)	5.44	**5.32**	4.56	**4.56**
	5.12		4.88	
	5.08		4.48	
	5.42		4.22	
	5.6		4.64	
Protein content (mg/g)	3.67	**4.01**	4.01	**3.59**
	4.1		4.2	
	3.4		4.12	
	3.4		3.98	
	3.4		3.93	

Discussion

Techniques for cultivating plant tissues for both herbaceous and ornamental plants are well established. Tissue culture represents the most expedient approach for achieving large-scale plant replication, as in vitro propagation serves as a highly effective means for preserving plant genetics. The production of seedlings directly from explants is a crucial aspect of the clonal micropropagation of superior germplasm (Ignacimuthu, 1997). Plant biotechnology encompasses a wide range of methodologies aimed at producing plant tissues and explants to facilitate experimental investigations, particularly in the field of developmental biology in grain legumes, with the ultimate goal of crop modification (Ramawat, 2003). In this study, the current research utilized nodal and shoot tips as explants obtained from high-yielding plants grown in the field for the purpose of establishing a stock culture. These explants were injected into MS media that contained various growth hormones of auxin with BAP and NAA as well as BAP, Kinetin, and NAA in combinations. Similarly, shoot tip and nodal explants were employed by Pandiyan and Selvaraj, (2012) for their micropropagation research on B. monnieri (L.) In the context of initiating plant culture, a significant number of prior research studies on diverse medicinal plant species have indicated the use of cytokinin, either in isolation or in conjunction with other specific quantities. Singh et al. (1999) cultivated several shoots of Paederia foetida and Centella asiatica plants in a growth medium known as MS, which was enriched with 1.0 mg/l of BAP. Similarly, Sehrawat et al. (2001) grew Rauwolfia serpentina in MS media supplemented with benzyl adenine and NAA.

The other growth regulators also caused a moderate amount of shoot development. For Wedeliachinensis, Kumari et al. (2023) reported similar types of work. However, after 21 days of culture, the optimal conditions for callus obtained from the tip of the shoot as explant were discovered on the medium containing MS media supplemented with 0.75 mg/l (6-BA). It was discovered that MS media containing various combinations and doses of growth regulators encouraged numerous shoots from both nodal and shoot tip explants. After 18 to 22 days following inoculation, several shoots begin to emerge from nodes. Combinations of the growth regulators BAP (2 mg/l) and IBA (0.5 mg/l) produced the greatest number of shoots (5.21.55) and longer shoot lengths (5.21.12 cm) from nodal segments. As a result, it has been reported that plant growth regulators have a significant impact on the induction of shoots, however, the exact impact varies depending on the plant chosen.

The MS medium was augmented with a combination of 1.0 IAA and 0.2 NAA in order to promote root development. The experimental results indicated that this supplementation yielded the most favorable outcome, with 85% of the samples exhibiting multiple roots. Additionally, the average length of the shoots was measured to be 4.30 cm. The germination rate was observed to be 94%, with an average of 4.5 roots per shoot recorded throughout a 4-week duration. Ahmed et al. (2007) revealed that the highest percentage of root induction (97.66%) was observed on MS media supplemented with 0.1 mg/1 of indole-3-acetic acid (IAA). The root induction of Stevia rebaudiana Bertoni, a sweetener plant, exhibited a quick decline in response to

higher concentrations of auxin, except for the concentrations of 0.1, 0.2, and 0.5 mg/l IAA and 0.1 mg/l IBA. Rooting was not observed on a medium lacking auxin.

According to Monirul Islam et al. (2005), the key stages in crop plants that can be managed using biotechnological methods and utilized to harness soma clonal diversity include the development of callus and its subsequent regeneration. Subsequently, the callus was subjected to subculturing on Murashige and Skoog (M.S.) medium, which was supplemented with 1.0 benzyl aminopurine (BAP) and 0.25 kinetin. This particular shoot multiplication medium resulted in the production of a substantial number of shoots. The highest callus formation was observed in Bacopa nodal explants cultivated on MS media supplemented with 0.5 mg/l 2,4-D, as reported by Tiwari et al. (1998).

Accordingly, the current and earlier findings showed that growth hormones, specifically auxin or cytokinin in the nutritional media depending on the source of explants, had a significant impact on callus induction. The genotype of the plants employed, the incubation environment, and the nutritional media's composition are the primary determinants of the in vitro-produced cultures among the various parameters influencing callus induction and regeneration.

The effective transplantation of a plant into field soil without the utilization of growth hormones or synthetic media indicates the achievement of successful in vitro micropropagation. Bacopa monnieri L. rooted plantlets were transferred from a culture bottle and transplanted into polythene bags containing eleven distinct sterile soil mixtures. The shoot and root growth of plants exhibited modest to minimal enhancement when subjected to the remaining soil combinations. Consequently, it has been posited that the presence of organic molecules, which

facilitate plant growth, has a regulatory role in the rate of plant growth within soil environments. Previous studies have conducted transplantation experiments under in vitro conditions using various plant species, including Terminalia arjuna Roxb. (Thomas et al., 2003), and Withaniasomnifera Dunal. (Sivanesan and Murugesan, 2005), Morusindica L. (Ahmed et al., 2007), Hyptis suaveolens Poit. (Jain and Chaturvedi, 2005), and Musa acuminata Colla (Anilkumar and Sajeevan, 2005).

Conclusion

Brahmi contain a significant amount of bioactive secondary metabolites, such as bacosides, which possess therapeutic potential for addressing many health conditions. The established literature extensively supports the antioxidant, anti-inflammatory, and anti-hepatotoxic qualities of Brahmi. The raw components of this particular substance are highly sought after, particularly for the purpose of extracting bioactive chemicals. The conventional method of information distribution was insufficient to meet the essential requirements of the business demand. In order to tackle this issue, researchers have devised biotechnological approaches, such as plant tissue culture methodologies. These techniques facilitate the synthesis of important secondary metabolites by employing several culture methods, including callus and cell suspension cultures, as well as organ cultures. This facilitates the rapid dissemination and conservation of pharmaceutically important plants, hence enhancing the synthesis of bioactive chemicals. A comparative analysis was conducted to assess the field performances of plants derived from distinct explants. Plants propagated using shoot tip as an explant are being compared to plants propagated using nodal tissue. A higher number of plants were grown from a shoot tip explant as compared to plants raised from a nodal explant. The findings of this study demonstrate that the utilization of shoot tip explant grown plants is a viable and secure practice for Bacopa monnieri cultivators. This approach holds significant potential for farmers, as it has the capacity to substantially decrease costs. This methodology has the ability to safeguard medicinal plant resources that are at risk of endangerment and facilitate their commercial replication. Plant tissue culture is a widely employed technique in the realm of physiological and biochemical research on plants, enabling the investigation of several aspects such as the cell cycle, cellular metabolism, nutrition, morphogenetics, and developmental processes. Given its ability to generate numerous plantlets, this approach exhibits a remarkable capacity for mass production.

Acknowledgment: The first author is thankful to the Haryana State Council for Science, Innovation, and Technology (HSCSIT) for providing financial support in the form of a fellowship.

References

[1] Ahmed MB, Salahin M, Karim R, Razvy MA, Hannan MM, Sultana R, Hossain M and Islam R (2007) An efficient method for in vitro clonal propagation of a newly introduced sweetener plant (Stevia rebaudianaBertoni.) in Bangladesh. Amer-Eur. J. Sci. Res. 2, 121- 125.

[2] Alkahtani, J., Elshikh, M. S., Dwiningsih, Y., Rathi, M. A., Sathya, R., & Vijayaraghavan, P. (2022). In- vitro antidepressant property of methanol

extract of Bacopa monnieri. Journal of King Saud University- Science, 34(8), 102299.

[3] Anand, U., Tudu, C. K., Nandy, S., Sunita, K., Tripathi, V., Loake, G. J., ... &Proćków, J. (2022). Ethnodermatological use of medicinal plants in India: From ayurvedic formulations to clinical perspectives–A review. Journal of ethnopharmacology, 284, 114744.

[4] Anilkumar M and Sajeevan RS (2005) Micropropagation of Musa acuminatacolla. Plant Cell Biotechnol. Mol. Biol. 6, 159-162.

[5] Anilkumar M, Mathew SK, Mathew P, John S, Deepa KP and Kiran VS (2005) In vitro shoot multiplication in Ocimumbasilicum L. Plant Cell Biotechnol. Mol. Biol. 6, 73-76.

[6] B Aggarwal, B., Prasad, S., Reuter, S., Kannappan, R., R Yadav, V., Park, B., ... & Sung, B. (2011). Identification of novel anti-inflammatory agents from Ayurvedic medicine for prevention of chronic diseases:"reverse pharmacology" and "bedside to bench" approach. Current drug targets, 12(11), 1595- 1653.

[7] Balaramaswamy Yadav P and Padmaja V (2005) Plantlet regeneration through multiple shoot induction in Cajanuscajan (L.). Plant Cell Biotechnol. Mol. Biol. 6, 65-68.

[8] Bharti, N., Yadav, D., Barnawal, D., Maji, D., &Kalra, A. (2013). Exiguobacteriumoxidotolerans, a halotolerant plant growth promoting rhizobacteria, improves yield and content of secondary metabolites in Bacopa monnieri (L.) Pennell under primary and secondary salt stress. World Journal of Microbiology and Biotechnology, 29, 379-387.

[9] Bonjoch, N. P., & Tamayo, P. R. (2001). Protein content quantification by Bradford method. In Handbook of plant ecophysiology techniques (pp. 283-295). Dordrecht: Springer Netherlands.

[10] Deepika, Priyanka, Minakshi Pal and Ikbal. (2015). In vitro studies of the Ocimum sanctum: Tulsi, Medicinal herb, American journal of pharmatech research 5(6): 33-50. ISSN: 2249-3387.

[11] Dixit, H., & Thakur, A. (2017). In vitro propagation of a medicinally important plant Bacopa monnieri from nodal explants. Indian Journal of Research in Pharmacy and Biotechnology, 5(1), 1-4.

[12] Fatima, U., Roy, S., Ahmad, S., Al-Keridis, L. A., Alshammari, N., Adnan, M., ... & Hassan, M. I. (2022). Investigating neuroprotective roles of Bacopa monnieri extracts: Mechanistic insights and therapeutic implications. Biomedicine & Pharmacotherapy, 153, 113469..

[13] Hu, C. Y., & Wang, P. J. (1983). Meristem, shoot tip and bud cultures. Handbook of plant cell culture (USA).

[14] Ignacimuthu S (1997) Plant Biotechnology, Oxford and IBH publishing Co. Pvt. Ltd, p. 180

[15] Jain A and Chaturvedi A (2005) In vitro proliferation of Hyptis suavelolens point: An ethno-medicinal herb. Plant Cell Biotechnol. Mol. Biol, 6, 151-154.

[16] Kumari, A., Kumar, A., Mandal, S., Roy, P., & Sircar, D. (2023). A metabolic reprogramming in Bacopa monnieri plants induced by methyl-jasmonate and enhanced biosynthesis of triterpene saponins. Industrial Crops and Products, 204, 117241.

[17] Kumari, R., Ashraf, S., Bagri, G. K., Khatik, S. K., Bagri, D. K., &Bagdi, D. L. (2018). Extraction and estimation of chlorophyll content of seed treated lentil crop using DMSO and acetone. Journal of Pharmacognosy and Phytochemistry, 7(3), 249-250.

[18] Lala, S. (2020). Enhancement of secondary metabolites in Bacopa monnieri (L.) Pennell plants treated with copper- based nanoparticles in vivo. IET nanobiotechnology, 14(1), 78-85.

[19] Mokat, D. N., & Jadhav, B. B. (2003). Species diversity and germplasm

collection of medicinal plants from Western Ghats of Maharashtra. Indian Journal of Plant Genetic Resources, 16(2), 126-132.

[20] Pandiyan, P., & Selvaraj, T. (2012). In vitro multiplication of Bacopa monnieri (L.) Pennell from shoot tip and nodal explants. J Agric Technol, 8(3), 1099-1108.

[21] Pothiaraj, G., Manoranjani, M., Pitchaikani, S., Seker, G. K., Saravanan, K. M., Rajan, M., &Shakila, H. (2022). Investigation of therapeutic and immunomodulatory activity of Bacopa saponin from Bacopa monnieri. South African Journal of Botany, 151, 639-650.

[22] Rahe, M. A., Mollika, S. R., Khan, M. S., Banu, T. A., Al Amin, G. M., Habib, M. A., ... &Sharmin, R.
A. (2020). In vitro micropropagation of Bacopa monnieri (L.) Penn.-an important medicinal plant. Plant Tissue Culture and Biotechnology, 30(1), 57-63.

[23] Ramawat KG (2003) Plant Biotechnology, S. Chand and Co. pp:1-37.

[24] Ray T, Saha P and Roy SC (2005) In vitro plant regeneration from young capitulum explants of Gerbera jamessonii. Plant Cell Biotechnol. Mol. Biol. 6, 35-40.

[25] Ray T, Saha P and Roy SC (2005) In vitro plant regeneration from young capitulum explants of Gerbera jamessonii. Plant Cell Biotechnol. Mol. Biol. 6, 35-40.

[26] Rebelo, D., Mariz-Ponte, N., Loureiro, J., Castro, S., Debiasi, C., Domingues, J., ... & Gonçalves, J. C. (2022). A protocol for micropropagation of the medicinal species Tuberarialignosa provides ploidy true- to-type plants with high antioxidant capacity. Plant

[27] Richa, J., Bheem, P., & Manju, J. (2013). In-vitro regeneration of Bacopa monnieri (L.): a highly valuable medicinal plant. International Journal of Current Microbiology and Applied Sciences, 2(12), 198-205.

[28] Saha, P. S., Sarkar, S., Jeyasri, R., Muthuramalingam, P., Ramesh, M., & Jha, S. (2020). In vitro propagation, phytochemical and neuropharmacological profiles of Bacopa monnieri (L.) Wettst.: a review. Plants, 9(4), 411.

[29] Saha, S., Adhikari, S., Mukhopadhyay, S., & Ghosh, P. (2023). Comparative studies on regeneration of different tissues of Bacopa monnieri (L.) Wettst in vitro and analysis of genetic homogeneity by random
DNA markers (RDMs). Acta EcologicaSinica, 43(3), 513-524.

[30] Sambyal M, Dogra A, Koul S and Ahuja A (2006) Rapid in vitro propagation of Potentilla fulgens wall - A Himalayan alpine herb of medicinal value. J. Plant Biochem. Biotechnol. 15,143-145.

[31] Sambyal M, Dogra A, Koul S and Ahuja A (2006) Rapid in vitro propagation of Potentilla fulgens wall - A Himalayan alpine herb of medicinal value. J. Plant Biochem. Biotechnol. 15,143-145.

[32] Sanyal, R., Nandi, S., Pandey, S., Chatterjee, U., Mishra, T., Datta, S., & Dey, A. (2022). Biotechnology for propagation and secondary metabolite production in Bacopa monnieri. Applied Microbiology and Biotechnology, 106(5-6), 1837-1854.

[33] Sehrawat AR, Sanjogta U and Anita P (2001) In vitro culture and multiplication of Rauwolfia serpentine
- a threatened medicinal plant. Crop Res. 22, 68-71.

[34] Sehrawat, A. R., Uppal, S., &Punia, A. (2001). In vitro culture and multiplication of Rauwolfia serpentina-a threatened medicinal plant. CROP RESEARCH-HISAR-, 22(1), 68-71.

[35] Shahid, M., Subhan, F., Ahmad, N., & Ullah, I. (2017). A bacosides containing Bacopa monnieri extract alleviates allodynia and hyperalgesia in the chronic constriction injury model of neuropathic pain in rats. BMC complementary and alternative medicine, 17, 1-12.

[36] Sharma, S., Kamal, B., Rathi, N.,

Chauhan, S., Jadon, V., Vats, N., ... & Arya, S. (2010). In vitro rapid and mass multiplication of highly valuable medicinal plant Bacopa monnieri (L.) Wettst. African Journal of Biotechnology, 9(49), 8318-8322.

[37] Singh, S., Ray, B. K., Mathew, S., Buragohain, P., Gogoi, J., Gogoi, S., ... & Deka, P. C. (1999). Micropropagation of a few important medicinal plants. Annals of Biology, 15, 1-8.

[38] Sudheer, W. N., Thiruvengadam, M., &Nagella, P. (2023). A comprehensive review on tissue culture studies and secondary metabolite production in Bacopa monnieri L. Pennell: A nootropic plant. Critical Reviews in Biotechnology, 1-15.

[39] Sulaiman, M. K., & Lakshmanan, J. (2022). Systemic and Anticancer Potential of Adaptogenic Constituents Isolated from Traditional Herbs-A Mini-Review. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 22(16), 2811-2821.

[40] Thomas TV, Rema Shree AB, Nabeesa E, Neelakandan N and Nandakumar S (2003) In vitro propagation of Terminalia arjuna Roxb. a multipurpose tree. Plant Cell Biotehnol. Mol. Biol. 4, 95-98.

[41] Tiwari V, Singh BD and Tiwari KN (1998) Shoot regeneration and somatic embryogenesis from different explants of Brahmi [Bacopa monniera (L.) Wettst.]. Plant Cell Rep. 17, 538-543.

[42] Tiwari V, Tiwari KN and Singh BD (2001). Comparative studies of cytokinins on in vitro propagation of Bacopa monniera. Plant Cell Tiss. Org. Cult. 66, 9-16..

[43] Torrey, J. G. (1976). Root hormones and plant growth. Annual review of plant physiology, 27(1), 435- 459.

[44] Watson, D. J. (1958). The dependence of net assimilation rate on leaf-area index. Annals of Botany, 22(1), 37-54.

[45] Werner, W., &Tölg, G. (1975). Optimisation of the Kjeldahl method for the determination of nitrogen in high-purity metals. Fresenius' ZeitschriftfüranalytischeChemie, 276, 103-110.

Fig; 1A Cultured raised plant from nodal as explant

Fig; 1B Cultured raised plant from shoot tip as explants

Fig 2A Hardening of plants raised through micro propagation from nodal explant

Fig 2B Hardening of plants raised through micro propagation from shoot tip as explant