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A COMPREHENSIVE REVIEW ON MICROPROPAGATION OF *BACOPA MONNIERI* L.

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ABSTRACT

Bacopa monnieri L. Pennell, is an important medicinal plant and is commonly known as Neer-brahmi that belongs to the family Scrophulariaceae. *Bacopa* is growing through vegetative propagation in nature. *Bacopa* is a well-known drug in Ayurveda and contain a variety of chemical compounds that can be used as medicines. It is used for increases intelligence & improves memory. Brahmi is also used for antioxidant, anti-inflammatory and anti-hepatotoxic activites. Presently, *Bacopa* raw materials huge demand in the market, particularly in medicinal industries. But, naturally propagation of *Bacopa* not sufficient to provided commercial demand. So, to overcome this problem resolved through by using micropropagation techniques to sufficient for the production of important secondary metabolites. Microprogation is useful for rapid propagation and conservation of medicinally important plants with increased production of bioactive compounds. Now this review article, we study and observed for the combination of on MS medium with auxin and cytokine (BAP, IAA, IBA, NAA, Kinetin, 2,4-D) for callus, shoot and root formation for cultivation of better propagation of *Bacopa monnieri*.

INTRODUCTION

Nature has furnished a comprehensive repository of medicines to address all of humanity's ailments. Higher plants function as solar-powered biochemical factories, using sunlight, air, water, and minerals to synthesize primary and secondary chemicals. Currently, extensive knowledge exists regarding the therapeutic applications of several plants. In recent years, maintaining an adequate supply of therapeutic plants has become difficult due to severe exploitation, inadequate environmental conservation, rising labour costs, economic and technical challenges related to their cultivation. Similar problem are there in selected medicinal plant of *B.monnieri* , for that reason we selected to investigation of *Bacopa* for this review article.

Scientific Classification:

Kingdom	Plantae
Division	Angiosperms
Class	Dicotyledones
Sub class	Gamopetalae
Series	Bicarpellate
Order	Personales
Family	Scrophulariaceae
Genus	*Bacopa*
Species	*monnieri*

Fig 1. *Bacopa monnieri* L.

Vernacular Names: Sanskrit: Brahmi, Nira-brahmi; Hindi: Brahmi; Marathi: Jalnaveri English: Brahmi-sak; Bengali: Jalanimba; Telugu: Sambrani chettu; Kannada: Nirubrahmi; Malayalam, Marathi and Tamil: Neer Brahmi

Botanical Description:

Bacopa monnieri is popularly called "Thyme Leaved Gratiola" or "Indian Pennywort." It is a widespread annual member of the Scrophulariaceae family that grows in marshy or moist environments. In conventional medicine, the entire plant is utilized as a nerve tonic, treating epilepsy and insanity and improving memory (Satyavati *et al.*, 1976). It can be grown in moist conditions around a pond or in a bog garden in Nepal, China, Sri Lanka, Taiwan, Florida, Vietnam, and several other southern states of the United States of America-a little succulent herb with glabrous roots that creeps and roots at nodes.

A little, prostrate, smooth, fleshy herb that roots at its nodes. Stem soft and obtusely angular, with ascending branches and numerous prostrate branches about 10–30 cm long. The leaves are sessile, oppositely arranged in a decussate pattern, succulent, and vary in shape from oblong-concave to obovate or oblanceolate. They are short, petiolate, and fleshy, measuring 0.6–2.5 cm by 3.3 mm. The blooms are solitary and axillary, featuring blue or white peduncles with purple veins, campanulate in shape, and pentamerous and ovoid capsules (Mathur and Kumar, 1998). Flora and fruit emerge during the summer season. The entire plant constitutes the medicinally beneficial component.

Medicinal Importance of *Bacopa monnieri*:

Brahmi is one of the important medicinal plants in the world, because of there is present huge amount saponins and it is called bacosides. Bacosides are a complex mix of chemicals with similar structures, like either jujubogenin or pseudojujubogenin glycosides. (Singh et al., 2006; Ali et al., 1999; Mathur et al., 2010; De et al., 2009; Jager et al., 2007; Rastogi et al., 1964; Zhang et al., 2011; Sivaramakrishna et al., 2005; Udgire and Pathade, 2012; Patil et al., 2009; Behera., 2016). *Bacopa* has many chemical compounds that can be used as medicines. These include bacosides, stigmasterol, sapogenins, and flavonoids (Rastogi et al., 1964). Bacoside is the major compound found in *Bacopa* and which help to improve memory enhancement of human being (Singh and Dhawan, 1997; Ashok et al., 2021). Chatterji et al.,(1965) reported that Bacoside A and B are the two saponins found in this plant. Bacoside A is made up of triglycosidic saponins like jujubogenin, bacopaside II, bacoside A3, and bacopasaponin C. Many research to work done on the related topic of quantitative and qualitative estimation of phytochemical studies of *Bacopa* plants (Deepak et al., 2005; Murthy et al., 2006; Mathur et al., 2002; Rahman et al., 2002). As antioxidants, Bacosides A help rats' defence systems work better (Anbarasi et al., 2006). In Ayurvedic medicine, Brahmi is often used to treat a wide range of conditions, including nervousness, cognitive impairment, and memory loss (Singh and Dhawan, 1997), as well as epilepsy, insanity, and developmental delays (Mathur et al., 2002). It is also used to heal sensory systems and ease mental stress and neurosis (Sivarajan and Balachandran, 1994). It is known as a nootropic plant because it has many health benefits, such as calming effects (Malhotra and Das, 1959), heart-strengthening effects (Mathur et al., 2002), cognitive enhancement effects (Nathan et al., 2001; Roodenrys et al., 2002), bronchodilator effects (Channa et al., 2003), antidepressant effects (Sairam et al., 2002), calcium antagonist effects (Dar and Channa, 1999), smooth muscle relaxant effects (Dar and Channa, 1997), neuropharmacological effects (Borrelli,2005), cell stabilising effects (Samiulla et al., 2001), and ulcer-fighting effects (Sairam, 2001).

Micropropagation of *Bacopa*:

In vitro culture techniques are applied to propagate elite plants by culturing a tiny part of them called explants. This technique often generates mother plants to type through appropriate *in-vitro* cultivation procedures. The term micropropagation derives its name from the miniature shoots/plantlets initially produced from tiny parts of the plant. This method offers a quick and dependable way to generate many genetically identical plantlets with beneficial agronomic characteristics. One of the critical advancements in plant tissue culture for industrial propagation is micropropagation. Additionally, this method can introduce genes significant to agronomy and improve genetics. The rate at which true-to-type plants multiply and the efficiency of transplanting can be advantageous for both conservation and the proliferation of elite plants for commercial exploitation (Jain et al., 2013; Himinish et al., 2017).Due to *Bacopa* extensive medical applications, various research groups have investigated ways to establish sustainable and reliable micropropagation and conservation processes through diverse tissue culture techniques. Techniques for *in vitro* enhancement of metabolite synthesis have been extensively utilized. An overview of several understated findings from these investigations is presented below:

Selection of Explants:

Micropropagation through shoot tip culture involves growing the shoot tip in suitable media to stimulate the proliferation of both apical and axillary meristems by ensuring optimal culture conditions. *Bacopa monnieri* is a diminutive creeping medicinal plant ideal for *in vitro* investigations owing to its rapid sensitivity to plant growth regulators, and numerous studies on the tissue culture characteristics of *B. monnieri* have been documented. In most instances, nodal segments from healthy inter-nodal shoot tips and leaves of *Bacopa monnieri* were used (Tiwari et al., 1998; Tiwari et al., 2001). Use of explants as node, internode and leaf (Shrivastava and Rajani 1999) and leaf and stem used as an explants of *B. monnieri* (Binita et al., 2005). Utilized axillary nodes, young leaves, internodes and shoot tips are also used as explants to micropropagation *B. monnieri* (Ramesh et al., 2006; Tiwari et al., 2006; Ceasar et al., 2010). Similarly, internode and leaf explants for direct shoot bud regeneration (Kumari et al., 2010; Sundriyal et al., 2013). Defoliated the removed shoots and sectioned them into 5-7 cm segments, each containing a minimum of 2-3 nodes (Joshi et al., 2010; Rout et al.,2011). Utilization of whole leaves as explants (Pandiyan et al., 2012). Umesh et al., (2014) suggested that *in vitro* propagation of *B. monnieri* can utilize leaf and nodal segments from the 2nd to the 6th node. Similarly, Richa et al., (2013) also reported that the use nodal, inter-nodal, leaves and shoot tip of *Bacopa monnieri* as explant material.

Sterilization Treatment:

Surface sterilization is the essential procedure before the inoculation of explants. Various methods have been employed for the treatment of explants. Mathur and Kumar (1998) used a method wherein leaf and stem explants were stirred for 10 minutes in a solution of Tween 20 and Savlon in water, subsequently rinsed in running water for 30 minutes, and then subjected to a 0.1 percent $HgCl_2$ treatment for 3-4 minutes, followed by multiple washes with sterile water to eliminate any residual $HgCl_2$ (Shrivastava and Rajani, 1999). The application of 0.1 percent mercuric chloride ($HgCl_2$) for 2 minutes has been detailed for the surface sterilization of *Bacopa monnieri*. They additionally indicated that excessive application of $HgCl_2$ results in browning and subsequent blackening of tissues. Consequently, 0.1 percent mercuric chloride was restricted to the plants 4 to 5 times. Multiple surfactants have been reported for sterilizing *Bacopa* (Tiwari et al., 2001). Nodal segments were submerged in running tap water for approximately one hour, after which the container was replaced, and the explants were cleansed with a few drops of Teepol and Tween 20 for about three minutes with constant agitation, followed by thorough rinsing with distilled water. The explants were subsequently treated in laminar flow with a 0.1 percent $HgCl_2$ solution, followed by washing with sterile double-distilled water (Binita et al.,2005). It was recommended that for the micropropagation of *B. monnieri*, the explants be thoroughly washed under running tap water for 30 minutes and subsequently treated with a 0.2 percent (v/v) aqueous surfactant Teepol for 15 minutes, and then rinsed repeatedly with distilled water. The explants were treated for 20 minutes with 0.1 percent (w/v) Carbendenzim. Subsequently, in a laminar airflow hood, explants were subjected to surface sterilization for 1 minute using 50 percent (v/v) ethanol, followed by a 3-minute treatment with 0.01 percent (w/v) $HgCl_2$. The explants were thoroughly rinsed with sterile distilled water four to five times (Ramesh et al. 2006). Explants of *Bacopa* were rinsed under running tap water for 20 minutes, treated with 3 percent (v/v) labolin for 3 minutes, and then briefly washed with sterile distilled water. Subsequently, explants were subjected to surface sterilization using 70 percent

(v/v) ethanol for 30 seconds, followed by a 3-minute treatment with 0.1 percent $HgCl_2$ (w/v), and then rinsed four times with sterile distilled water. Escandon et al., *(*2006) recommended that, explants be washed with 70 percent ethanol for 30 seconds to treat nodal segments of *B. monnieri*, followed by a 25-minute treatment containing 25 percent sodium hypochlorite and 0.01 percent Tween 80. The segments were washed thrice with sterile distilled water. Showkat et al., (2010) developed an alternative approach for the sterilizing treatment of *B. monnieri*. The nodal explants were subjected to a 30-minute wash under running tap water, followed by a 15-minute immersion in a solution comprising 0.2 percent bavistin and 0.01 percent neomycin. Afterward, they were rinsed multiple times with distilled water for 5 minutes. In the laminar airflow hood, explants were subjected to surface sterilization using 70 percent (v/v) ethanol for 1 minute, followed by treatment with 0.01 percent (w/v) $HgCl_2$ for 3 minutes. The explants were rinsed thoroughly with sterile distilled water three to five times. Kumari et al., (2010) also recommended the application of Tween 20, succeeded by 0.08 percent $HgCl_2$ for 10-12 minutes, to surface sterilize the nodes of *B. monnieri*. The explants were that rinsed three times with sterile distilled water. Sharma et al., (2010) suggested that the applying a 1-2 percent Cetavelon solution to remove dust particles and a 0.1 percent (w/v) $HgCl_2$ solution for surface sterilizing. Vijayakumar et al., (2010) reported that the contamination-free explants of *Bacopa monnieri* were successfully established by washing the explants under tap water, followed by treatment in a solution containing 0.2-0.5 percent bavistin and 0.03 percent streptomycin for 10 minutes. Explants were subsequently submerged in an aqueous solution of Savlon (1.5 percent v/v Chlorhexidine gluconate solution and 3 percent w/v Centrimide) for 10 minutes. The explants were properly cleansed twice with sterile double distilled water. After this treatment, the explants underwent surface sterilization using 0.01 percent $HgCl_2$ for 1 minute (Joshi et al., 2010;Tanveer et al., 2010; Narayan et al., 2011; Gurnani et al., 2012). Recommended washing explants with teepol, followed by treatment with 0.1 percent $HgCl_2$ (w/v), and thereafter rinsing thoroughly with sterile water (Joshi et al., 2013). Leaves and stem explants of *B. monnieri* were rinsed with running tap water, subsequently treated with a solution of 2-3 drops of Tween 20 in 100 ml of water, and finally subjected to a 0.01 percent $HgCl_2$ treatment. The explants were that rinsed three times with sterile distilled water. Kumari et al., (2014) suggested the application of bavistin and streptomycin, wherein nodal segments and shoot tips were rinsed in running tap water and subsequently immersed in a 0.2-0.5 percent bavistin and 0.04 percent streptomycin aqueous solution for 8-10 minutes. The explants were subsequently washed with distilled water. This procedure was reiterated thrice. The explants were treated with a 0.01 percent aqueous solution of $HgCl_2$ for 1 minute, followed by washing with sterile distilled water to eradicate residual chemicals.

Media Preparation:

The composition of the medium is contingent upon the explant being cultivated and the physiological process being encouraged, such as growth vs development. Media formulation is an empirical process, as various explants from the same cultivar and identical explants from different cultivars may exhibit distinct media requirements (Hammerschlag, 1982).

Basal Medium:

The Murashige and Skoog medium (1962), modified with various combinations of growth regulators, has been utilized to cultivate herbaceous species. Singh et al., (1999) reported that employing shoot tips (1-2 cm) as explants resulted in the production of several shoots from field-cultivated *Bacopa monnieri* in Murashige and Skoog's media enriched with 0.5 mg/liter BAP within six days of culture. Mohapatra and Rath (2005) suggested that the nodal explants inoculated (5-6 nodes; 7-8 cm length) on MS medium and B5 medium, but MS medium has better results as compared to B5 medium. Three Sources of Carbon: Most of the media used for *in vitro* shoot regeneration and rooting invariably contain 2 to 3 percent (w/v) sucrose as a carbon source for energy and osmolarity requirements. However, tests have also been done on alternative carbon sources. Sindhu et al., (2010) proposed that the using 3 percent table sugar as the carbon source in the medium for *B. monnieri* micropropagation; this approach lowers the cost and has no discernible influence on the morphogenetic response of the shoots.

Solidifying Agents:

Plant tissue culture has long used agar as a hardening agent. It is the most expensive element because it frequently contains contaminants. According to Prakash (1993), 70 percent of media expenditures are attributable to the gelling agent, agar, which is typically added to increase media viscosity. Suppose less expensive, reusable alternatives to tissue culture grade pure agar were available. In that case, the high production costs associated with micropropagation techniques may be significantly decreased without sacrificing the quality of regenerated plants. Physical and chemical investigations found significant variations in how cultures responded to different brands or varieties of agar (Scholten and Pierik, 1998). *Isabgol* serves as an effective solidifying agent. Sindhu et al., (2010) reported that *isabgol* (1.2 percent) serves as a gelling agent without significantly affecting shoot morphogenetic response, merely reducing costs. Yusuf et al., (2011) indicated that i*sabgol*, utilized as a gelling agent, facilitated consistent culture multiplication compared to microbiology-grade agar, with cultures preserved in a medium containing 3 percent (w/v) *isabgol* for 190 days, in contrast to an average of 120 days in 1 percent (w/v) agar media.

Phytohormones:

Phytohormones are plant growth regulators and are essential in tissue culture research. They regulate growth, development, and responses to stimuli. Among the several classical plant hormones, auxins (NAA, IAA, IBA, and 2,4-D), cytokinins (BAP, KIN, Zeatin, 2iP, and TDZ), gibberellins, and ethylene are the most extensively utilized. Simultaneously, numerous structurally analogous substances with phytohormonal regulating functions are continuously synthesized.

Shoot Induction:

Shrivastava et al., (1999) reported that the induction of adventitious shoot buds from leaf and stem explants of *B. monnieri* on Murashige and Skoog's media enriched with benzyladenine or kinetin. The optimal response was achieved using leaf explants from shoot cultures cultivated in media enriched with 2 µM benzyladenine and solidifying with 0.2 percent generated. Tiwari et al., (2000) proposed an efficient and quick technique for the *in vitro* growth of *Bacopa* utilizing liquid shake cultures. Nodal explants were cultivated in liquid MS media, supplemented with or without 6-benzyladenine. Compared to single axillary shoot proliferation on a growth regulator-free agar medium, the corresponding liquid media facilitated the induction of 4 or 5 shoots per nodal explant after four weeks of culture. An enhancement in morphogenetic response (quantity of shoots, average shoot length, and number of roots per node explant) was noted with 6-benzyladenine (0.01-0.1 l) in both types of culture media. Tiwari et al., (2001) suggested using various cytokinins to induce numerous shoots in *B. monnieri* of the four cytokinins tested-6-benzyladenine, thidiazuron, kinetin, and 2-isopentenyladenine-thidiazuron (6.8 µM) and 6-benzyladenine (8.9 µM) exhibited superior responses. Optimal induction of adventitious shoot buds occurred with 6.8 µM thidiazuron, resulting in an average production of 93 shoot buds in leaf explants after seven weeks of incubation. Ramesh et al., (2006) reported that MS media augmented with various combinations of auxins and cytokinins effectively induced shoot formation, excluding those containing either auxin or cytokinin. 0.2 mg/l IAA and 1.5 mg/l BAP yielded a high percentage of multiple shoot development (96.3). The combination of 60 mg/l adenine sulphate, 0.2 mg/l IAA, and 1.5 mg/l BAP was the most effective in generating the maximum shoot. Sharma et al., (2007) reported an alternative protocol for the *in vitro* clonal propagation of *B. monnieri*, wherein single node explants were cultured on Murashige and Skoog's medium augmented with BA (0.2 mg/l), leading to vigorous shoot proliferation (20-22 shoots per explant in 8 weeks) without the formation of callus. Patil et al., (2009) suggested employing MS medium augmented with 0.5 µM BAP and 0.5 µM NAA to facilitate caulogenesis from nodal segments of *Bacopa monnieri*. Ceasar et al., (2010) developed a two-stage culture protocol for effectively regenerating shoots from leaf and internode explants of *B. monnieri*. Adventitious shoot buds were generated on the shoot induction medium comprising MS basal salt augmented with

1.5 mg/l thidiazuron and 0.5 mg/l naphthalene acetic acid. Parale et al., (2010) reported that shoots were raised in liquid Murashige and Skoog's (MS) medium, which was fortified with 5 µM 6-benzyladenine BA. Yusuf et al., (2011) investigated the impact of antioxidants and gelling agents on the regeneration of *Bacopa monnieri*. It was claimed that shoot regeneration was accomplished from several explants using 4.4 µM benzylaminopurine (BA). It was reported that callus biomass was cultivated on agar-solidified MS medium with 1 µM 2,4-dichlorophenoxyacetic acid (2,4-D) alongside 5 µM 1-naphthaleneacetic acid (NAA). At the same time, shoots were developed in liquid Murashige and Skoog's (MS) medium enriched with 5 µM 6-benzyladenine (BA). Nodal explants produced 75-80 shoots, leaf explants generated 55-65 shoots, and internodal explants yielded 50-55 shoots within 40 days of culture. Adding citric acid (25 mg/l) and ascorbic acid (50 mg/l) increased the number of shoots and the duration of culture conservation. Pandiyan et al., (2012) suggested that nodal explants of *Bacopa monnieri* exhibited superior growth compared to shoot tip explants, yielding the highest number of shoots on a medium supplemented with BAP, KIN, and NAA (0.5-2.0 mg/l). Sundriyal et al., (2013) demonstrated that axillary bud break occurred in all aseptic cultures on MS medium supplemented with 0.5-4.0 mg/l BAP within 2 weeks of inoculation. The quantity of proliferating shoots varied between 2 and 4. A diminished bud break response (25 percent) was noted when axillary buds were cultivated on hormone-free media, resulting in subsequent shoot mortality. MS medium enriched with 2.0 mg/l BAP exhibited an optimal bud break reaction of 90-95 percent. Kumari et al., (2014) also advocated using BAP and Kinetin, individually or in conjunction with IBA. At a concentration of 0.5 mg/l, BAP yielded 15.0 shoots in shoot-tip explants and 18.0 shoots in nodal explants, whereas KIN produced 12.0 shoots in shoot-tip explants and 16.0 shoots in nodal explants at similar concentrations.

Root Induction:

Singh et al., (1999) stated that root induction in *B. monnieri* (L.) was observed in Murashige and Skoog medium supplemented with 0.5 mg/l BAP within six days of culture. Tiwari *et al.*, (2000) investigated rooting in *Bacopa* using various media, specifically MS media with and without hormones. They determined that the highest rooting rate (9 %) occurred on full-strength MS medium supplemented with 2.46 µM IBA. Rooting has also been reported to occur on the shoot proliferation medium. The root induction was accomplished using the same medium employed for shoot proliferation, specifically MS media augmented with 1.1 µM BA and 0.2 µM IAA (Binita *et al*. 2005). Several researchers have examined the impact of utilizing a half-strength medium. Joshi et al., (2010) mentioned that the regenerated shoots of *B. monnieri* were successfully rooted in a half-strength MS medium containing 1 percent sucrose and supplemented with 2 µM IBA. Root development was observed to commence within two weeks from the nodes of the shoots immersed in the media. By the conclusion of four weeks, the roots had sufficiently elongated. Showkat et al., (2010) studied that optimal results were attained, when developed shoots were placed into rooting media devoid of hormones, namely in MS medium containing 20 g/l sucrose and 7 g/l agar. Patil et al., (2009) supported by using hormone-free media for rooting in *Bacopa monnieri*. The surgically separated individual shoots were planted in liquid 1/4 MS basal media augmented with different concentrations of NAA and IBA. NAA at 3.0 mg/l and IBA at 5.0 mg/l produced high-quality roots. The absence of agar does not adversely affect root induction; instead, it lowers costs and enhances greenhouse survival rates, as removing agar from regenerated plantlets harms root hairs and is laborious (Sindhu *et al*. 2010). The regenerated shoots of *B. monnieri* were rooted on MS medium supplemented with 0.5 mg/l NAA and 1mg/l IBA yielded favourable outcomes after ten days (Pandiyan et al., 2012). Ahire et al., (2012) tried rooting in solid and liquid MS medium devoid of plant growth regulators and determined that liquid MS medium is more conducive for roots. They also conducted rooting in liquid MS medium augmented with varying concentrations of IAA and NAA (0.1-0.5 mg/l) and achieved complete roots of shoots within two weeks of culture. Kaur et al., (2013) investigated that the influence of several hormones (IBA, NAA, IAA) on rooting and determined that IBA at a concentration of 1.0 mg/l resulted in optimal rooting.

Callus Induction:

Plant callus is a proliferating aggregation of disorganized plant parenchyma cells. In living plants, callus cells are the cells that encapsulate a plant wound. In biological research and biotechnology, callus development is created from plant tissue samples (explants) following surface sterilization and subsequent plating onto tissue culture media *in vitro*. The administration of auxin and cytokinin stimulates callus formation in various plant species (Skoog and Miller 1957). They indicated that a moderate ratio of auxin to cytokinin facilitates callus induction. Still, a high auxin-to-cytokinin ratio or a high cytokinin-to-auxin ratio stimulates root and shoot regeneration. The several hormones, including brassinosteroids and abscisic acid, also promote callus development and may, in certain species, replace auxin with cytokinin in this process (Goren et al., 1979; Hu et al., 2000). Tiwari et al., (1998) suggested that calli derived from nodal explants of *Bacopa*, cultured on MS medium supplemented with 0.5 mg/l 2,4-D, yielded somatic embryos upon culturing on MS media containing either 0.1 or 0.5 mg/l BA or 0.2 mg/l 2,4-D in conjunction with 0.1 or 0.5 mg/l Kinetin. The somatic embryos germinated on either the same medium or MS base medium, and the resulting plantlets were successfully transplanted into the soil. Leaf petiole explants were used for callus induction. The most significant growth was noted in MS medium augmented with 0.25 mg/l 2, 4-D, and 0.5 mg/l Kn, and in MS medium enhanced with 0.25 mg/l 2, 4-D, and 0.1 mg/l BAP (Mehta et al., 2012). Singh et al., (2012) induced callus formation from the leaves of *B. monnieri*. The callus was begun on medium with two distinct hormonal compositions: MS + (0.5 mg/l) BAP + (1.0, 2.0 mg/l) NAA and MS + (2.0 mg/l) 2, 4-D + (0.5, 1.0 mg/l) BAP. The callus was effectively sustained on MS medium enriched with 1.0 mg/l 2,4-D and 0.5 mg/l BAP. Talukdar (2014) observed that callus induction from Brahmi's nodal and leaf explants. For callus induction, he utilized MS media individually augmented with several auxins, including IAA, NAA, IBA, and 2,4-D (0.2-0.5 mg/l). The optimal outcomes were achieved with the treatment MS + 2 mg/l 2, 4-D. Callus biomass on agar solidified MS medium containing 1µM 2,4-dichloro phenoxy acetic (2,4-D) in conjunction with 5 µM 1-naphthalene acetic acid (Parale et al., 2010; Yusuf et al., 2011).

Acclimatization and Transfer of Plantlets to Soil:

A significant constraint of micropropagation technique on a commercial scale is the elevated mortality rate observed in *in vitro* cultivated plants during their transition from the laboratory to the field. When transitioned to natural circumstances, Tissue-cultured plants encounter many factors, such as modified temperature, light intensity, and water stress, necessitating acclimation for plantlets' effective establishment and survival (Chandra et al., 2010). Attempts have been undertaken to harden and acclimatize *Bacopa* plantlets, a crucial feature of *in vitro* cultivated plants. Several accounts indicate varying soil media were utilized and standardized with a 50-100 percent success rate, contingent upon the soil composition. Binita et al., (2005) suggested that for acclimatization, *in vitro* regenerated plantlets of *B. monnieri* were placed in small plastic pots filled with a mixture of sand, soil, and farmyard manure in a 1:1:1 ratio. Initially, elevated humidity was sustained with five water sprays daily at 5-6 hour intervals and A survival rate of 98 percent for plants was recorded. Patil et al., (2009) tested various soil mixtures and recommended that *in vitro* regenerated brahmi plantlets be transferred to polybags containing a sterile soil and sand mixture (3:1) for hardening. When irrigated every 24 hours with a half-strength MS salt solution, the survival frequency was 75 ± 5 percent. Similarly, Narayan et al., (2011) suggested that the most significant shoot length and root length were recorded in plants cultivated in soil combined with VAM (10:2) and soil with vermicompost (4:1), respectively. A survival rate of 80-90 percent was noted in the transplantation. The plant thrived in the cultivated field soil after 45 days. A survival rate of 90 percent for the plant was observed in clay and red soils. Pandiyan et al., (2012) observed that when rooted plantlets were relocated to poly caps and PVC pots filled with sterile soil and perlite in a 1:1 ratio, the plantlets demonstrated adequate acclimatization and

achieved a 92 percent survival rate upon transfer to the glasshouse.

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