EPH - International Journal Of Applied Science

ISSN (Online): 2208-2158
Volume 10 Issue 01-March, 2024

DOI:https://doi.org/10.53555/eijas.v10i1.173

PLANT TISSUE CULTURE OF AN ENDANGERED MEDICINAL HERB BACOPA MONNIERI

Vaisnavi pandey[1], Aishwarya Samantaray[2], Sradhanjali Moharana[3], Sushree Sangeeta Sahoo[4], Rajalaxmi Baliarsingh[5], Sanjay ku. Pattnaik[6], Somali Das[7*]

[1,2,3,4]Students of Botany of NIIS Institute of Information Science and Management
[5,6]Faculty in Botany, NIIS Institute of Information Science and Management
*[7]*Faculty in Biotechnology, NIIS Institute of Information Science and Management*

***Corresponding author :- Email:-somali536@gmail.com**

Abstract :-

Bacopa monnieri, a valuable medicinal herb, is endangered and is often propagated vegetatively. It is well-known for its versatility in applications. Bacopa monnieri ,a plant with strong roots in the Indian ancient Ayurvedic system, has long been known as an effective treatment. Commonly known as "Brahmi," this medicinal plant contains important phytopharmaceuticals substances.

Given its importance in treating a variety of disorders, producing Bacopa monnieri via in vitro cultivation is crucial. Aseptic cultures were produced and begun on Murashige and Skoog (MS) media enriched with 6-benzyl adenine (0.5µM). For shoot multiplication, a combination of BA, Kinetin (KIN), and Thidizuron (TDZ) was used. BA surpassed KIN and TDZ in terms of multiplication of shoots and elongation.MS medium with 2.5µM of BA resulted in the maximum shoot multiplication. Furthermore, the greatest shoot regeneration frequency was found on MS medium enriched with 5.0µM of BA and 1.0µM of α-naphthalene acetic acid (NAA).

This plant is suggested in the Indian Materia Medica (Bhavaprakasha Nighantu AD 1500) for treating a wide range of mental illnesses, including stress, poor cognition, lack of focus, sleeplessness, insanity, depression, psychosis, epilepsy, and Alzheimer's disease [1,2]. Commercially accessible extracts of B. monnieri have been shown to improve memory focus, cognitive development, and general brain function in young as well as older people. Furthermore, the plant has a long history of usage as a heart tonic and digestive aid in India and Pakistan, with reports of improved respiratory performance during bronchoconstriction. Clinical studies demonstrate the favorable benefits of bacopa-based compounds in the recovery of brain functions in children with diagnoses of attention deficit hyperactivity disorder (ADHD), as well as the augmentation of cognitive capacities in patients recovering from stroke.

It is also used in cosmetic industries as it contains essential oils, sterols, flavonoids, glycosides, as well as triterpenoid saponins. Two commercially available hair care formulations—Brahmi oil and soft extract—make use of Brahmi's positive characteristics. It is also classified as classified as a Rasayana in Ayurveda, is known for its ability to postpone indications of aging in the body, such as hair graying. Furthermore, Brahmi helps to alleviate mental weariness, which contributes to the preservation of a favorable body environment for healthy hair [3].

Keywords:- *Medicinal plant, in-vitro cultivation, Endangered plant*

Introduction :-

Small rectangular leaf and white to purple flowers define Bacopa monnieri, a curative creeper perennials belonging to the Scrophulariaceae family that is widely used in ayurvedic medicine. It is also known as the herb of grace, thyme-leaved gratiola, water hyssop, and brahmi [4]. The word "Brahmi" in Hindu mythology comes from "Brahma," which denotes the all-powerful creator[5]. Furthermore, historically, the term "Brahmi" has been used to refer to either Bacopa monnieri, the Asian Centella asiatica (Gotu kola), as well as a mixture of the two plants[4].This species of plant is a creeper, subsucculent herb that is commonly found in swampy environments. The leaves of this plant are sessile, barely obovate-oblong, or spathulate, along with stems up to 30 cm tall (Saxena and Brahmam, 1995). The blooms are single as well as axillary, having bracteoles which are straight as well as smaller compared to the calyx. The calyx lobes differ significantly, with the two outside lobes having ovate-oblong and reaching 4.3-6.0 mm in length, and the inner one being lanceolate. The corolla's hue ranges from purplish white to blue or pink, and it is widely campanulate, ranging 7-11 mm long and 7.5 mm in wide. The calyx has an ovoid and sharp capsule. This species is commonly found in moist areas near water sources. is a member of the Scrophulariaceae family and is also known by the name "Brahmi". The Indian Materia Medica, which is notably referenced in the around 1500 AD Bhavaprakasha Nighantu, recommends Bacopa monnieri L. as a treatment for a variety of mental illnesses. This includes include Alzheimer's disease, anxiety, sadness, psychosis, sleeplessness, insanity, anxiety, and cognitive problems . This age-old medicinal herb is well- known for its fabled ability to improve memory(6). It is also used in treatment of a variety of mental illnesses. This includes include Alzheimer's disease, anxiety, sadness, psychosis, sleeplessness, insanity, anxiety, and cognitive problems. Smooth, creeping stems with many branches define the perennial, non-aromatic plant Bacopa monnieri. The plant normally grows to a height of 60-90 cm, having branch spanning 5-35cm long. Its roots are thin and wiry, becoming light yellowish as they expand. The seeds are abundant, irregular in shape, or oblong in form. The stem is fragile, varying in colour from grey to purplish green, around 1mm thick, and made up of nodes as well as internodes. The flavour is rather bitter. The leaves are curled, straight, reversed or crisscrossed, greenish in colour, sessile, and measure 8-15mm in length as well as 4mm wide. The bottom surface has elongated spots and small flecks. The little blooms are axillary and have five petals that might be white, purple, pink, or pale violet (7-11). Bacopa monnieri contains alkaloids like brahmine and herpestine. The plant contains notable phytochemicals such as saponins terpenoids (12), monnierin, hersaponin, tannins, flavonoids (13), glycosides (14), and specialized compounds such as Bacoside A and B. Saponins are further classified as pseudojujubogenin and jujubogenin glycosides, which are an essential component of the plant (15).Bacosides A and B have been recognized for their memory-enhancing qualities, with Bacoside A having the potential to produce nitric oxide. This release helps to dilate veins as well as aorta, resulting in better circulation of blood throughout the body. This property distinguishes it to be a remarkable nootropic medication (16). Additionally, Brahmi components are known for their anti-carcinogenic qualities (17).The saponin composition of Bacopa monnieri contains Bacoside A. Bacoside B,A1, A3, bacogenin A1,A2,A3,A4, bacopa saponin-C, bacopasides I ,II,III,IV,V,VI,VII bacobitacins A-D, monnieraside I,III, monnieri, plantioside B, jujubogenin, pseudojujubogenin, Nicotine, betulinic acid, wogonin, oroxidin, luteolin, luteolin 7-glucoside, luteolin-7-glucuronide, apigenin7-glucuronide, 3-O-β-D-glucopyranosyl-3 β-D-glucopyranosyl, jujubogenin, References 38 and 39 list the following compounds: 3-formyl-4-hydroxy-2H-pyran, bacosine, bacostcrol, bacosterol-3-O-β-D-glucopyranoside, stigmasterol, stigmastanol, β-sitosterol, D- mannitol, as well as unidentified glycoside (18,19).

Review in literature: -

Traditional medicines play an important role in meeting healthcare demands within rural areas across many nations where basic health-care systems may be lacking[20-23]. Bacopa monnieri has been utilized by various cultures around the world, demonstrating its diverse ethnobotanical value. Around the Nara Desert of Sindh, Pakistan, entire natives utilize this for a blood purifier, powdering the entire plant [24]. Furthermore, in Rajasthani traditional medicine, Bacopa monnieri is used to treat a variety of health conditions, including stomach difficulties, fractures of the bones, respiratory conditions, bladder duct inflammatory conditions, rheumatic lung disease, swelling in the legs, improvement in memory, faintness in voice, and blisters. Joyawake tea, a combination of Bacopa monnieri along with the tea plant Camellia sinensis, is a nervine tonic [25]. In Orissa, Bacopa monnieri leaves are traditionally used to treat coughs, colds, and nasal congestion. The root extract is used for a solution for the eye for cataract treatment, and the leaves are used to cure constipation and asthma. Furthermore, the plant is used to treat migraines, in both oil as well as powder form, and is known for its antiseptic

characteristics [26]. Brahmi has been utilized to treat diarrhea across the Southern Western Ghats area of Tamil Nadu's Virudhunagar region [27]. It is also renowned for its ability to improve memory. Bacopa monnieri is used by the people of Dakshin Dinajpur, West Bengal, for both memory improvement and the treatment of neurological illnesses. B. monnieri leaves are used by Malayan tribes in Southern Kerala to treat urinary problems and energize the pubic area [28-29]. Furthermore, some tribal groups in Bangladesh employ the leafy parts of this plant internally to purify their blood [30]. In Ayurveda, The Brahmi is very important. First records of its characteristics and applications can be found in the Charaka Samhita, Atharva- Ved, and Susurtu Samhita [31]. It is classified as a "Medhya Rasayan" in Ayurveda, signifying that it aids memory, addresses intellectual weaknesses, and improves mental capacity. The word "Medhya" refers to the intellect. Practitioners of Ayurveda in India have been using these characteristics for almost 3000 years [32,33]. This method is critical in Ayurveda for treating psychological disorders related with aging [34,35]. The Bacopa plant is extremely important in the Ayurvedic system, particularly in formulations like Brahmighritam, Brahmirasayanam, and more. Brahmigritham as well as Brahmigritham have long been used to treat epilepsy in Ayurveda [36]. Brahmi has become one of the most often used medicines, with well- established neuropsychological benefits. In Ayurveda, it is commonly used in the preparation of polyherbal remedies which include Saraswatarishta (SW), Brahmi Ghrita (BG), Saraswat Choorna, as well as others [37,38]. The plant has long been utilized throughout India and Pakistan to be a heart stimulant and digestive aid. Additionally, it has been shown to improve respiratory function in cases of bronchoconstriction. Scientific research has confirmed that bacopa-containing formulations have a positive impact on the restoration of cognitive functioning in children having Attention Deficit Hyperactivity Disorder (ADHD). Furthermore, these formulations help to improve cognitive performance in individuals recuperating from strokes and epilepsy [39-40]. Additionally, the plant is used in phytoremediation to remove heavy metals including cadmium and chromium. Because B. monnieri is the only known medicinal source of bacosides, pharmaceutical researchers and herbal traders actively gather it from its natural habitat. There has been a boom in research focused on large-scale plant multiplication and alternative biotechnological ways for the manufacture of bacopa saponins, the plant's active components, due to its enormous and varied therapeutic relevance. Many articles about the species' in vitro propagation have surfaced. Explants of B. monnieri have the morphogenic potential that makes it an invaluable model plant for in vitro studies. This allows researchers to avoid the effects of external phytohormones and investigate the impact of transgenes on organogenesis as well as the functional aspects of bacoside synthesis in vitro. In vitro methods, including tissue and organ culture, provide fresh prospects to breeders of plants in regard to clonal propagation, genetic modification, and the development of inbred lines, which is especially useful for uncommon species. In vitro technologies enable an incredible million-fold increase in clonal multiplication per year over conventional methodologies. The high rate for true-to-type plants multiplication and effective transplant of B. monnieri are beneficial in the conservation and replication of exceptional plants for commercial use. As a result, an effective in vitro regeneration strategy is required for large multiplication under a variety of circumstances and conservation efforts. Furthermore, depending on field agriculture for secondary metabolite production has intrinsic limitations, such as poor yields and variability caused by regional, periodical, along with environmental variables [42].

Materials and Methods Shoot culture :-
Researchers picked B. monnieri plants at the institute's botanical garden. They started by cleaning the plant material (which includes stems) using liquid detergent, then sterilizing the surface with ethanol for 30 seconds. The item was then submerged in a 0.1% HgCl2 aqueous solution for 10 minutes before being washed four times using sterilized distilled water. They next cut the plant material to appropriate-sized explants (about 1 cm) and infected them on the surface of solid MS (the Murashige & Skoog 1962) media. Finally, the colonies were cultured in a liquid media of same composition [43].

Culture conditions :-
The cultures were kept in a culture environment at 26.0±0.5 °C with white, fluorescent lighting (Phillips cool TL 12 W) with an overall irradiation of 36 µmol m−2 s−1 for a photoperiod of sixteen hours and 55-60% relative humidity. The medium's pH was adjusted to 5.8 & sterilized by sterilization in an auto at 121 °C for fifteen minutes. Each treatment used a minimum of six culture flasks, each with 120 explants as duplicates. To calculate the growth index (GI), cultured were removed from the flasks and their fresh weight was measured. subsequently, they were allowed to air out in an oven at 60 °C until they reached a consistent weight, and their dry weight was measured. GI was computed as ½ [43].

Optimization of shoots :-
Using nodal and internodal explants, different doses of 6-benzylamniopurine (BAP, 0.5 to 10 mg/l) were coupled with 3-indole acetic acid (IAA, 0.01 mg/l) to evaluate shoots induction, multiplication, and length. . Following the selection of the best medium, the size and number of nodal explants were adjusted to find the ideal circumstances for shoot multiplication. The shoot cultures were grown in MS medium enriched with the BAP (2.5 mg/l) and IAA (0.01 mg/l), as well as 3% sucrose, with or without agar. The growth development was observed over a 60-day period using time-course research. Cultures containing optimal explants of nodal tissue (adjusting for quantity and size) were collected every ten days till 60 days.

Culture vessels: -
The experiment used four different types of vessels: magenta boxes (400 mL), conical flasks (100 and 250 mL), a Growtek bioreactor (1 L), as well as commercial glass jars (500 mL). The Growtek bioreactor (1 L, Tarson, India) was oxygenated at 10% v/v using Sharma et al.'s [44] design, which required inserting a needle through a septum in the side tube.

The experiment used single nodal pieces of 0.5 cm from tissue-cultured plants aged 6 weeks. In glass jars and pink containers, the explants were supported by cotton pads. A rotary shaker set to 80 rpm was used to agitate liquid cultures [45]. After 40 days, the cultures were harvested, and several parameters such as shoot count, new dryweight, growth index (GI), total phenolic content, and potential for antioxidants were evaluated.
In addition, antioxidant strength and overall phenolic content of in vivo material were measured to compare in vitro regenerated plants with in vivo B. monnieri.

Total phenolic content: -
The dried and powdered materials (100 mg) were extracted for four hours at ambient temperature using a test tube rotator and 5 mL of 60% methanol. After extraction, the sample suspensions were centrifuged at 10,000 g for 15 minutes at 10°C, and the supernatants were passed through Whatman No.1 filter paper. The filtrate was then kept at -20°C until analysis. A spectrophotometer was used to measure total phenolic content (TPC), following the method described by Farkas and Kiraly in 1962 [46]. Calibration was performed using gallic acid as the reference standard. The assays were performed in triplicate, and the results were represented as mg gallic acid equivalent per gram (mg GAE/g) of extract.

DPPH radical scavenging activity: -
The DPPH radical scavenging activity was determined using the technique published by Sreeramulu et al. in 2009 [47]. This approach depends on the antioxidant's capacity for neutralizing the DPPH cation radical. The % inhibition of the sample extract was then computed.
Superoxide radical scavenging activity: -
The superoxide activity for radical scavenging was determined using the technique provided by Jain et al. [48]. The PMS/NADH system created superoxide anions, which were subsequently used to decrease NBT, yielding a chromogenic product at 560 nm. Percent inhibition was than computed.

Statistical Analysis: -
The data were collected in triplicate (n = 3). The analysis consisted of a determination of variances accompanied by a Dunnett numerous comparing test (comparing all versus control) with Prism statistical software. A regression analysis was performed to link the antioxidant potential values acquired using various approaches, and the correlation coefficients were then derived.

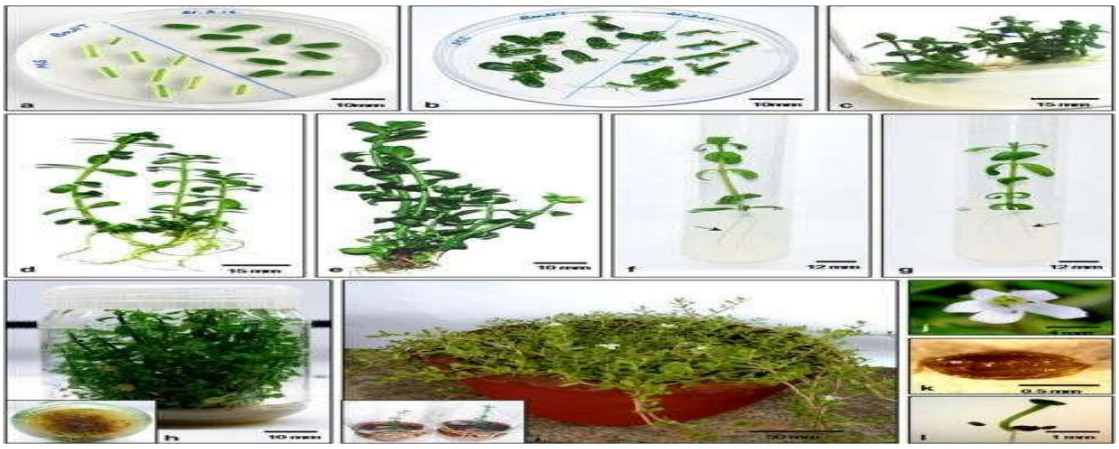

FIG :-1 Shows Tissue culture of Brahmi plant by Shoot Explant Result and Discussion

Effect of BAP :-
Almost all examined medium showed shoot induction from both stem explants. Despite shoot development, the first culture growth was slow. It shows the results of using different doses of BAP in MS media to regenerate shoots from stem explant. MS media enriched with BAP (2.5 mg l−1), IAA (0.01 mg l−1), and 3% sucrose resulted in the most shoots per explant, increased explant response, and longer shoot length. As a result, these medium and shoot explants were chosen for the ensuing experiment. Callusing was seen at a BAP concentration of 10 mg l-1 in media

Explant number and size :-
The experiment used explants of different sizes and numbers in 40 ml of MS media with the BAP (2.5 mg l−1) and IAA (0.01 mg l−1). The results show that a 0.5 cm explant size and 20 explants per 40 ml (equal to 1 explant per 2 ml) gave the best circumstances for obtaining a strong explant response, as well as a larger number of shoots per explant regenerated and longer shoot length. In contrast, a greater amount of explants did not yield favourable growth compared to the use of 10 explants per 40 ml medium. Furthermore, expanding the amount of explants did not roughly enhance the number of shoots, and using large-sized explants also resulted in a higher consumption of primary material for culture.

Time course of growth :-

Explants grew rapidly in MS media with BAP (2.5 mg l−1), IAA (0.01 mg l−1), and 3% sucrose over the first three weeks (Fig. 1). Throughout this period, there was a significant rise of both shoot number and dry mass production, but the growth rate steadily slowed over the next 20 days. The cultures' growth rate began to slow on the 40th day.

Total phenolic content and antioxidant capacity :-
The total phenolic quantity and antioxidant capability of in vitro regenerated plants were shown to be higher than in vivo plants. In vitro regenerated plants produced in a 250 ml conical flask had a total phenolic content of 55 mg GAE/g, but in vivo plants had only 5.3 mg GAE/g, showing a 10-fold greater activity in the in vitro system. The in vitro system not only provides an ideal platform for biomass generation, but it also shows promise for increased phenolic content and antioxidant activity. As a result, this technology might be used to produce huge quantities of biomass from this medicinal plant, removing the requirement for heterogeneous material collection in the field.
The study demonstrated a strong correlation between SOD and DPPH (R=0.9352) indicating a meaningful link between the two tests. Furthermore, a significant connection was found between the TPC and DPPH assays, with a correlation value of R=0.8763. In contrast, the correlation between TPC and SOD was rather modest (R=0.8374). Prior research by Wangensteem et al. [49] and Zheng and Wang [50] found a high positive association between free radical-scavenging activity and total phenolic component concentrations in plant extracts.

Explant-Source/Type	Culture Medium, PGRs and Additives	Remarks, Experimental Outcome and Maximum Productivity, Acclimatization etc.	References
Terminal shoots bearing 4–5 nodes from field-grown plants	MS + 2.0 mg L−1 BA (SIM); MS + 0.1 mg L −1 BA + 0.2 mg L−1 IAA (SELM). MS + 1.0 mg L−1 NAA (RIM). MS + 0.5 mg L−1 BA (SEIM; callus explants).	79 shoots/leaf explant, 20 shoots/node and 26 shoots/internode formed on SIM within 4-w. 100% of the shoots rooted on RIM. SE developed after 4-w of culture on SEIM. Histological analysis of the calli revealed typical heart-shaped and cotyledonary stage somatic embryos. Plantlets acclimatized in sterilized soilrite with 95% survival rate.	51
Shoot apex and nodes (1–1.2 cm) of young greenhouse plant	MS + 5.0 mg L−1 BA + 0.2% (w/v) NaCl/10% Mannitol.	20 shoots/culture without root formed after 15-d under salt stress. Shoots with 4–5 roots/explant formed after 15-d under drought stress.	52
Shoot tips (0.7–1.2 cm) of field grown plants	MS + 1.0 mg L−1 BA	86% of encapsulated nodal explants germinated into plantlets after 6-8 w. Acclimatization in a potting mix of sand: soil (1:1) and finally transferred to net house.	56
Shoot tips (≤5 mm) from in vitro shoot cultures	2.5% Na- alginate in MS + 3% sucrose→100 mM CaCl2 .2H2O (encapsulation).	93% of encapsulated shoot tips showed regrowth with 10 shoots/explant after 6 months of storage at 4 ◦C. 100% of shoots showed rooting. Acclimatization in pot mixture of peat-moss and sand (1:1) with 93% survival rate in greenhouse.	57
Shoot tips of ex vitro plants	PGR free MS medium	Explants excised from different positions showed variable frequency of direct shoot organogenesis in unsupplemented MS medium with optimum of 8 shoot buds/leaf and 15/internode explant within 4-w. 100% of micro shoots (5–6 cm long) rooted within 2-w of culture. Acclimatized plants flowered within 3 m	58

Table 1 shows some other result of tissue culture by Shoot Explant

Nodal Explant :-
Explant preparation :-
Nodal explants of Bacopa monnieri were collected at the Botany Department's Experimental Farm at Andhra University in Visakhapatnam, Andhra Pradesh. They were then surface sterilized with 0.1% HgCl2 for 3 minutes before being rinsed three to four times with sterile distilled water. These surface-sterilized explants were subsequently administered with various growth mediums to determine their in vitro response [55].

Micropropagation :-
The basal MS medium was supplemented with various concentrations and combinations of the following substances: 6-benzylaminopurine (BAP; 0.5, 1.0, 1.5, 2.0, and 2.5mg/l), Kinetin, which (KIN; 0.5, 1.0, 1.5, 2.0, and 2.5mg/l), and Indole-3-acetic acid (0.5mg/l) in addition to BAP or KIN concentrations (0.5, 1.0, 1.5, 2.0, and 2.5mg/l). These combinations were examined for bud break and multiple shoot induction, and they were sub cultured on the same medium every 15 days. The total amount of buds on the shoot and their length (cm) were counted after the 3rd subculture. To assess their rooting ability, shoots produced in vitro were removed and transferred to MS medium enriched with various doses of Naphthaleneacetic acid (NAA; 0.1, 0.2, 0.3, and 0.4mg/L). After two weeks of culture, the effectiveness of rhizogenesis was measured, including the frequency of rooting (%), the number of roots per shoot, and the root length (cm) [55].

In vitro conditions :-
The mediums were all supplemented with 3% (w/v) sucrose and 0.8% agar. Prior to autoclaving, the media's pH was adjusted to 5.8 using 1N NaOH or 1N HCl. Cultures were cultured at 22±2ºC in the culture room, with a 16-hour photoperiod and illumination of 20m mol m-2 s-1 photosynthetic photons flux density, provided by cool-white fluorescent light.

Acclimatization :-
The plants with roots were removed from the culture media, washed under water from the faucet to remove any agar residue, and transferred to sterilized vermiculite-filled plastic pots. They were hardened for four weeks before being transplanted into clay pots containing a 1:1 combination of sand and dirt. These pots were kept in greenhouse conditions for up keep.

Statistical Analysis :-
The studies were repeated three times and data were reported as Mean ± Standard error using Minitab statistical software.

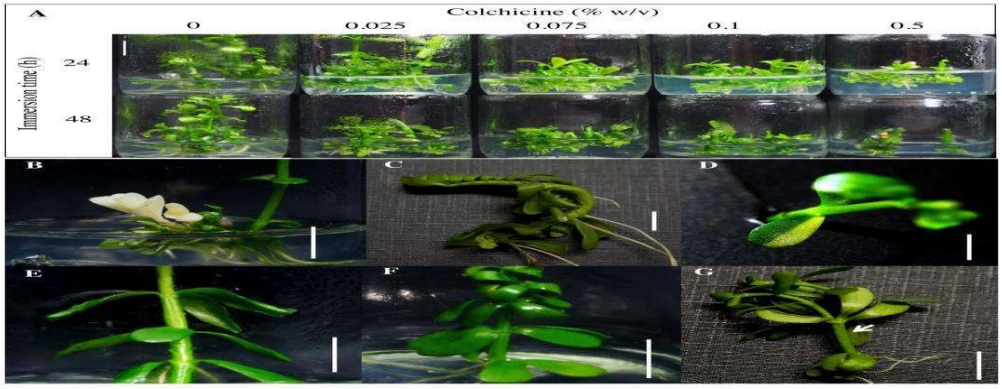

Fig 2 :- shows Tissue culture of Brahmi plant by Nodal Explant

Result and Discussion :-
The study attempted to elicit adventitious multiple shoots in B. monnieri nodal explants by direct organogenesis employing cytokinins (BAP and KIN) alone or in combination with IAA. Within forty-five days of culture, all hormone treatments showed swelling and bud break, accompanied by multiple shoot development and synchronous shoot extension from nodal explants. Notably, the study accomplished shoot multiplication and subsequent elongation in the same media, simplifying the procedure by eliminating the requirement for microshoot elongation and shortening the length of micropropagation. However, it is worth mentioning that in numerous species of plants, regeneration may need the use of various propagation and shoot elongation medium, thereby complicating and raising the expense of micropropagation techniques. The results showed that nodal explants cultivated on MS baseline media without growth inhibitors did not promote shoot proliferation. BAP alone or paired with IAA outperformed KIN alone or with IAA in terms of percent shot induction, shoot number per explant, and shoot duration. This finding is consistent with prior research by Balaraju et al. [59], Mehta et al. [60], and Molsaghi et al. [61] in medicinal plants such as Vitex agnus-castus, B. monnieri, and Aloe vera. Among the BAP doses studied, 1.5mg/l had the best in vitro response, resulting in 78.2 shoots per explant, 70% regenerated shoots, and a shoot length of 5.3cm. MS medium supplemented with varying doses of KIN, on the other hand, demonstrated an encouraging response, with 1.5mg/l resulting in 65% regenerated shoots, 58 shoots per node, and a shoot length of 5cm after forty-five days of culture. However, concentrations more than 1.5mg/l of BAP or KIN caused stunted shoots. In contrast, the use of BAP in combination with IAA or KIN blended with IAA resulted in high-frequency regeneration of shoots and respectable shoot length, possibly due to cytokinin's synergistic activity with auxin, which facilitated fast cell division and the growth of plenty of relatively small and undifferentiated cells [62,63]. Among the different combination media evaluated, MS+BAP(1.5mg/l)+IAA(0.5mg/l) outperformed the other media in terms of percentage shoot induction (95%), 168 shoot number per node , and shoot length (5.9cm).

Explant-Source/Type	Culture Medium, PGRs and Additives	Remarks, Experimental Outcome and Maximum Productivity, Acclimatization etc.	References
Nodal explants (1.0 cm) of field grown plants	MS + 6.8 µM TDZ (SIM)/+2.2 µM BA (SMM)/4.9 µM IBA (RIM).	92 shoot buds/leaf explant, 42 adventitious shoot buds/node and 28 adventitious shoot buds/internode after 7-w of culture on SIM. 129 shoot buds/leaf explant on SMM after 3 subcultures (each with 4 weeks duration). 100% of shoots rooted on RIM within 2-w. Acclimatization in sterilized soilrite with 100% survival rate.	53
Nodal segments (5–6 nodes; 7–8 cm) of ex vitro plants	MS + 2.0 mg L−1 BA (SIM). MS + IBA (0.5–2.0 mg L−1) (RIM)	Nodal explants formed 9.4 shoots/explant after 7-d. on SIM while leaf explants formed 4.3 shoots/explants after 15-d.	54
Nodal explants internodes and leaf of 4-week-old in vitro grown shoots	MS + 300 mg L−1 BVN (SIM)/+0.2 mg L −1 IAA + 0.1 mg L−1 BA (SELM)/	100% of explants formed shoots with 98 shoots/internode explant, 81 shoots/leaf explant and 21 shoots/nodal explant on SIM within 4-w. Optimum shoot growth in SELM. Acclimatization in sterilized soilrite with 85% survival rate in field.	67
Nodal segments of ex vitro plants	MS + 0.1 mg L−1 BA (SIM, SMM). MS + 0.15 mg L−1 IBA (RIM).	100% of cultures showed axillary bud break; 41 shoots/explant after 4-w. 100% shoots formed roots with 24 roots/shoot within 3–4 w. Plantlets acclimatized in a mixture of sand, farmyard manure and soil (1:1:1) irrigated with 1 2 MS medium and finally shifted to shade house.	64
Nodal segments (1.0–1.5 cm) of ex vitro plants	MS + 3.0 mg L−1 BA (SIM)/+ 1.0 mg L−1 + GA3 (SMM, SELM). 1 2 MS + 0.2 mg L−1 IBA (RIM).	Shoot bud induction after 4-w. 114 shoots/explant with shoot length 6.4 cm after 3-w of sub-culture. Shoots rooted with 10 roots/shoot on RIM after 2-w. Acclimatization in plastic pots containing garden soil with 100% survival rate.	65

Table 2 shows some other result of tissue culture by Nodal Explant Leaf Explant :-

Plant Material :-

Small shoots (1.5 - 2 cm) bearing leaves (0.7 - 0.9 cm length, 0.3 - 0.5 cm width) were gathered from healthy vegetatively propagated net house grown plants of five different accessions of Bacopa monnieri in the Herbal Garden of SMVDU, Katra, Jammu and Kashmir (Latitude = 28°66′ North, Longitude = 77°21' East, and Altitude = 754m). The leaves were properly washed under tap water to remove solid debris and a 2% (w/v) Tween 20 (Himedia, India) solution before being treated with 1% (v/v) Sodium hypochlorite for 3 minutes and kept under running tap water for a period of sixty minutes. The explants were aseptically surface sterilized for 2 minutes with a 0.2% (w/v) HgCl2 solution, then washed 3-4 times with sterile distilled water to eliminate any remaining sterilants [66].

Aseptic Culture Conditions :-

The experiment used small shoots (1.5 - 2 cm) with leaves (0.7 - 0.9 cm length, 0.3 - 0.5 cm width) gathered from healthy vegetatively propagated net house grown plants of five distinct accessions of Bacopa monnieri at the SMVDU Herbal Garden in Katra, Jammu and Kashmir (Latitude = 28°66′ North, Longitude = 77°21' East, Altitude = 754m). The leaves were thoroughly washed with tap water, treated with a 2% (w/v) Tween 20 solution, then 1% (v/v) Sodium hypochlorite, and sterilized with a 0.2% (w/v) HgCl2 solution. The leaves were used as explants to grow cultures on Murashige & Skoog (MS) and Gamborg's (B5) medium, which were autoclaved at 121ºC under 15-20 pressure for 15 minutes. Throughout the experiment, the media's pH was kept at 5.8 and gelled with 0.7% (w/v) agar. Explants were injected into culture tubes (15 ml) and put in a culture environment at a temperature of 25 ± 2ºC. Cool white fluorescent CFL lights (3000 lux) supplied a 16-hour photoperiod. Five replicates were used for each treatment and accession of Bacopa monnieri, and they were observed for eight weeks, with a four-week subculture interval. Data were gathered weekly, and the experiment was repeated twice [66].

FIG 3 :- Shows Tissue culture of Brahmi plant by Leaf explant

Results and Discussion :-

After 20 days of inoculation, de novo shoot initiation was observed from leaf explants on both MS and B5 medium, followed by rooting in the form of a single tap root, shoot multiplication beginning in the sixth week, and leaf regeneration beginning in the seventh week. These investigations took use of the fact that both MS and B5 medium were adequate for in vitro shoot regeneration of Bacopa monnieri from a single leaf explant, without the need for an extra plant growth regulator. Although the regeneration pattern was consistent throughout the five accessions investigated, the regeneration mechanism differed. Accession BM003 produced the most leaves, roots, and shoots in MS medium, whereas Accession BM004 produced the fewest of these characteristics in B5 media.The findings obtained reinforced the fact that among all the accessions investigated, BM003 demonstrated stronger regeneration capacity than the other accessions and that MS media were superior to B5 media for in vitro regeneration in Bacopa monnieri. Earlier investigations documented in vitro regeneration and shoot multiplication of monnieri utilizing leaf explants on MS medium, although at the expense of plant growth regulators (PGRs) [68,69]. The procedure utilized in our work might be used to mass micropropagation in order to lower the cost of PGRs and the number of different explants necessary to save a single plant. The findings demonstrated that large-scale propagation and conservation of B. monnieri using tissue culture were possible, cost-effective, and simple in this endangered and medicinally essential plant.

Explant-Source/Type	Culture Medium, PGRs and Additives	Remarks, Experimental Outcome and Maximum Productivity, Acclimatization etc.	References
Leaf explants of ex vitro plants	MS + 2.0 mg L−1 BA + 0.5 mg L−1 NAA + 3% sucrose (CIM, SIM). 1 2 MS + 2.0 mg L −1 IAA + 2% sucrose (RIM).	61% callus induced formed shoots with 16 shoots/callus after 5-w. 6 roots/shoot formed on RIM after 3-w. Acclimatization in sterilized sand: soil: dry powdered cow dung (1:1:1) with mild irrigation at 2-day interval and supplied with 1 4 strength MS inorganic solutiontwice a week and transferred to filed with 86% survival rate.	70
Leaf and stem of ex vitro plants	MS + 2.5 mg L−1 BA + 0.01 mg L−1 IAA (SIM). Liquid MS + 2.5 mg L−1 BA + 0.01 mg L−1 IAA (SIM; node) in bioreactor.	20 nodal explant/40 mL medium was optimal for high explant response. Maximum growth index (10.0) was recorded in bioreactor producing ~2000 shoots/L with 16.5 g/L DW. The total phenolic content and antioxidant capacity of in vitro grown plants was higher to that recorded for in vivo plants.	71
Leaf and internodes of ex vitro plants	MS + 2.0 mg L−1 BA (SIM). MS + 0.5 mg L −1 GA3 (SMM). 1 2 MS + 2.0 mg L−1 IBA (RIM).	Shoot organogenesis with 104 shoots/leaf explant and 89shoots/internode explant on SIM. 100% of shoots rooted with 57 roots/shoot and on RIM. Acclimatization in sterile vermiculate: sand: soil (1:2:2) with 90% survival rate in greenhouse.	72
Leaf of ex vitro plant	MS + 2 mg L−1 Kn (SIM; leaf). MS (SELM, RIM). MS + 1 mg L−1 2,4-D (CIM).	126 shoots/leaf explant formed after 45-d. Micro shoots elongated and rooted on RIM in 15-d. Acclimatization in soil rite (mixture of coco brick, cocopeat perlite and vermiculite). Detection of Bacopaside I and II in micro shoots by HPLC.	73
Leaf, internode shoot buds of ex vitro plants	MS + 1.5 mg L−1 TDZ + 0.5 mg L−1 NAA (SIM). MS + 0.5 mg L−1 BA (SMM). 1 2 MS + 1.0 mg L−1 IBA + 0.5 mg L−1 Phloroglucinol (RIM).	56 shoots/leaf explant and 49 shoots/internode explant after 3-w. 135 shoots/leaf explant and 112 shoots/internode explant on SMM after 4-w. 16 roots/shoot on RIM after 4-w. Acclimatization in sterilized vermicompost supplied with diluted MS basal salts with a 100% survival rate. RAPD profile confirmed clonal fidelity.	74

Table 3 shows some other results of tissue culture by Leaf explant

CONCLUSION :-

In this paper we have shown ways of Tissue Culture on Bacopa monnieri plant. We have done its tissue culture by using its different organs as explant like Nodal explant, Shoot explant as well as Leaf explant.

In Shoot explant B.monnieri plants were cleaned by using liquid detergent,then the surface was sterilized for 30 secs with ethanol. Then the item was submerged in a 0.1% HgCl2 aqueous solution for 10 minutes before being washed four times using sterilized distilled water. Climatic conditions were kept in mind during the process. Almost all examined medium showed shoot induction from both stem explants. Despite shoot development, the first culture growth was slow. the study's findings suggest that growing B. monnieri in large vessel sizes under in vitro conditions might be an effective strategy for producing biomass with potential antioxidant activity.

In Nodal explant ,these were surface sterilised with 0.1% HgCl2 for 3 mintues before being rinsed three to four times with sterile distilled water. These surface sterilised explant were subsequently adminstered with various growth mediums to determine their in vitro response. The standardized technique created in this study demonstrates the possibility of large-scale growth within the medicinal herb Bacopa monnieri via nodal explants. Furthermore, the study stresses the effect of different cytokinin concentrations, such as BAP and KIN, either alone or in combination using IAA , by fast multiplication of adventitious several shoots.

In Leaf explant leaves were thoroughly washed with tap water, treated with a 2% (w/v) Tween 20 solution, then 1% (v/v) Sodium hypochlorite, and sterilized with a 0.2% (w/v) HgCl2 solution. The given procedure results in the observation that Bacopa monnieri leaflets are able to be effectively regenerated in vitro utilizing only one leaf on MS/B5 media, without the requirement for any additional plant growth regulator. MS medium outperformed the two tested media types in terms of highest shoot as well as leaf multiplication. BM003 produced the highest yield of the five investigated accessions, which was followed by BM001, BM002, BM005, and BM004.

This study provides important insights, demonstrating that cost-effective tissue culture options can be used to propagate and protect equally vulnerable and elite plant species. Ongoing efforts are aimed at evaluating the phytochemical abilities of various regenerants and their bacoside content.

Acknowledgement
All the authors have contributed equally having equal positions.

REFERENCE: -

1. Russo, A.; Borrelli, F. Bacopa monniera, a reputed nootropic plant: An overview. Phytomedicine 2005, 12, 305–317. [CrossRef] [PubMed]
2. Majumdar, S.; Basu, A.; Paul, P.; Halder, M.; Jha, S. Bacosides and Neuroprotection. In Natural Products; Ramawat, K., Mérillon, J.M., Eds.; Springer: Berlin/Heidelberg, Germany, 2013; pp. 3639– 3660
3. Sairam, K.; Dorababu, M.; Goel, R.K.; Bhattacharya, S.K. Antidepressant activity of standardized extract of Bacopa monniera in experimental models of depression in rats. Phytomedicine 2002, 9, 207–211. [CrossRef] [PubMed]
4. Aguiar S, Borowski T. Neuropharmacological review of the nootropic herb Bacopa monnieri. Rejuvenation Res. 2013 Aug;16(4):313-26. [PMC free article] [PubMed] 5. Shinomol GK, Muralidhara, Bharath MM. Exploring the Role of "Brahmi" (Bacopa monnieri and Centella asiatica) in Brain Function and Therapy. Recent Pat Endocr Metab Immune Drug Discov. 2011 Jan;5(1):33-49. [PubMed]
6. Saxena HO and Brahmam M. (1995). The flora of Orissa. Regional Research Laboratory. Orissa Forest Development Corporation Limited. 2: 1229
7. CL M. Pharmacological studies of Herpestis monniera, Linn., (Brahmi). The Indian journal of medical research. 1959 May 1; 47(3): 294-305.
8. Oudhia P. Glory Lily or Kalihari (G. superba L.). Society for Parthenium Management (SOPAM). 28-A. College Rd., Geeta Nagar, India. 2004
9. Wallis TE. Textbook of pharmacognosy. 1946.
10. Basak A, Hossain ML, Parvin MN. Evaluation of phytochemical and pharmacological activities of Bacopa monnieri (L.). Int J Sci Rep. 2016 Oct;2(10):242.
11. Tripathi YB, Chaurasia S, Tripathi E, Upadhyay A, Dubey GP. Bacopa monniera Linn. as an antioxidant: mechanism of action. Indian Journal of Experimental Biology. 1996 Jun 1; 34(6): 523-6
12. Zhou Y, Shen YH, Zhang C, Su J, Liu RH, Zhang WD. Triterpene saponins from Bacopa monnieri and their antidepressant effects in two mice models. Journal of natural products. 2007 April 27; 70(4):652-5.
13. Singh SK. Phytochemical analysis of leaf callus of Bacopa monnieri L. Int J Sci Res Pub. 2012; 2:1-3.
14. Sivaramakrishna C, Rao CV, Trimurtulu G, Vanisree M, Subbaraju GV. Triterpenoid glycosides from Bacopa monnieri. Phytochemistry. 2005 Dec 1; 66(23): 2719-28.
15. Basu N. Chemical examination of Bacopa monniera, Wettst: Part III Bacoside B. Indian J. Chemistry. 1967; 5: 84-6.
16. Elangovan V, Govindasamy S, Ramamoorthy N, Balasubramanian K. In vitro studies on the anticancer activity of Bacopa monnieri. Fitoterapia (Milano). 1995; 66(3): 211-5.
17. Singh HK, Rastogi RP, Srimal RC, Dhawan BN. Effect of bacosides A and B on avoidance responses in rats. Phytotherapy Research. 1988 Jun;2(2):70-5.
18. Shanmugasundaram ER, Akbar GM, Shanmugasundaram KR. Brahmighritham, an Ayurvedic herbal formula for the control of epilepsy. Journal of ethnopharmacology. 1991 July1; 33(3): 269-76.
19. Chatterjee N. Chemical examination of Bacopa monniera Wettst. Part II: The Constitution of Bacoside A. Indian J. Chemistry. 1965; 3: 24-9.
20. Sheldon JW, Balick MJ, Laird SA, Milne GM. Medicinal plants: can utilization and conservation coexist? Advances in economic botany. 1997 Jan 1; 12:i-104.
21. Teh OH. The role of traditional medical practitioners in the context of the African traditional concept of health & healing. International mental health workshop. Retrieved May 1998; 25: 2004
22. Shrestha PM, Dhillion SS. Medicinal plant diversity and use in the highlands of Dolakha district, Nepal. Journal of ethnopharmacology. 2003 May 1; 86(1): 81-96.
23. Tabuti JR, Dhillion SS, Lye KA. Traditional medicine in Bulamogi county, Uganda: its practitioners, users and viability. Journal of Ethnopharmacology. 2003 Mar 1; 85(1):119-29.
24. Qureshi R, Bhatti GR. Ethnobotany of plants used by the Thari people of Nara Desert, Pakistan. Fitoterapia. 2008 Sep 1; 79(6): 468-73.
25. Verma M. Ethno medicinal and antimicrobial screening of bacopa monnieri (l.) pennell. J. phytol. 2014; 1(6).
26. Panda A, Misra MK. Ethnomedicinal survey of some wetland plants of South Orissa and their conservation. 2011;10(2):296-303
27. Suresh M, Irulandi K, Siva V, Mehalingam P. An Ethnobotanical Study on Medicinal Plants in Southern Western Ghats of Virudhunagar district, Tamil Nadu, India.2016; 6(4):2321-29
28. Das H, Chakraborty U. Ethnobotanical Study of Medicinal Plants in the Dakshin Dinajpur District. 2019; 8(3): 18-24

29. Kumar V. Ethnomedicines of Malayan tribes of southern region of Kerala, India. Recent Advances in Ethnobotany, Sanjeev kumar, Deep Publication, New Delhi. 2015.
30. Akter S, Das PR, Islam MT, Kabir MH, Haque MM, Khatun Z, Nurunnabi M, Khatun Z, Lee YK, Jahan R, Rahmatullah M. A selection of medicinal plants used as blood purifiers by folk medicinal practitioners of Bangladesh. American-Eurasian J Sustain Agri. 2012 Jul 1; 6(3): 188-94.
31. Rai K, Gupta N, Dharamdasani L, Nair P, Bodhankar P. Bacopa monnieri: A wonder drug changing fortune of people. International Journal of Applied Sciences and Biotechnology. 2017 Jun 29; 5(2):127-32.
32. Russo A, Borrelli F. Bacopa monniera, a reputed nootropic plant: an overview. Phytomedicine. 2005 Apr 20; 12(4): 305-17.
33. Shinomol~ GK, Bharath MM. Exploring the role of Brahmi (Bacopa monnieri and Centella asiatica) in brain function and therapy. Recent patents on endocrine, metabolic & immune drug discovery. 2011 Jan 1; 5(1):33-49.
34. Ernst E. Herbal remedies for anxiety–a systematic review of controlled clinical trials. Phytomedicine. 2006 Feb 13; 13(3):205-8.
35. Russo A, Borrelli F. Bacopa monniera, a reputed nootropic plant: an overview. Phytomedicine. 2005 Apr 20; 12(4):305-17.
36. Tyagi A, Delanty N. Herbal remedies, dietary supplements, and seizures. Epilepsia. 2003 Feb;44(2):228-35
37. Singh HK, Dhawan BN. Neuropsychopharmacological effects of the Ayurvedic nootropic Bacopa monniera Linn. (Brahmi). Indian Journal of Pharmacology. 1997 Sep 1;29(5):359.
38. Giramkar SA, Kulkarni OP, Jagtap SD, Kuvalekar AA, Mukherjee S, Jagtap RR, Wagh AR, Bandawane DD, Nipate SS. Anticonvulsant potential of commonly practiced formulations of Brahmi (Bacopa monnieri Linn.) in Wistar rats. journal of pharmacy research. 2013 Sep 1; 7(9):787-91
39. Jyoti, A.; Sharma, D. Neuroprotective role of Bacopa monniera extract against aluminium- induced oxidativestress in the hippocampus of rat brain. NeuroToxicology 2006, 27, 457. [CrossRef]
40. Jyoti, A.; Sethi, P.; Sharma, D. Bacopa monniera prevents from aluminium neurotoxicity in the cerebral cortexof rat brain. J. Ethnopharmacol. 2007, 111, 56–62.
41. Kamkaew, N.; Scholfield, C.N.; Ingkaninan, K.; Taepavarapruk, N.; Chootip, K. Bacopa monnieri increasescerebral blood flow in rat independent of blood pressure. Phytother. Res. 2013, 27, 135–138. [CrossRef]
42. Jain M, Rajput R, Mishra A. Enhancement of secondary metabolite biosynthesis in Bacopa monnieri: An in vitrostudy. Res. J Rec. Sci. 2013;2(1):13-16.
43. Jain N, Sharma V, Ramawat KG. Shoot culture of Bacopa monnieri: standardization of explant, vessels and bioreactor for growth and antioxidant capacity. Physiol Mol Biol Plants. 2012 Apr;18(2):185-90. doi: 10.1007/s12298-012-0103-0. Epub 2012 Mar 14. PMID: 23573056; PMCID: PMC3550505.
44. Sharma V, Goyal S, Ramawat KG. Increased puerarin biosynthesis during in vitro shoot formation in Pueraria tuberosa grown in Growtek bioreactor with aeration. Physiol Mol Biol Plants. 2011;17:87–92. doi: 10.1007/s12298-011-0049-7. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
45. Ruuhola TM, Julkunen-Tiitto MRK. Salicylates of intact Salix myrsinifolia plantlets do not undergo rapid metabolic turnover. Plant physiol. 2000;122:895–906. doi: 10.1104/pp.122.3.895. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
46. Farkas GL, Kiraly Z. Role of phenolic compound in the physiology of plant diseases and disease resistance. Phytopath Z. 1962;44:105–150. doi: 10.1111/j.1439-0434.1962.tb02005.x. [CrossRef] [Google Scholar]
47. Sreeramulu D, Reddy CVK, Raghunath M. Antioxidant activity of commonly consumed cereals, millets, pulses and legumes in India. Indian J Biochem Biophy. 2009;46:112–115. [PubMed] [Google Scholar]
48. Jain PK, Ravichandran V, Agrawal RK. Antioxidant and free radical scavenging properties of traditionally used three Indian medicinal plants. Current Trends Biotechnol Pharm. 2008;2:538–547. [Google Scholar]
49. Wangensteem H, Samuelsen AB, Malterud KE. Antioxidant activity in extracts from coriander. Food Chem. 2004;88:293–297. doi: 10.1016/j.foodchem.2004.01.047. [CrossRef] [Google Scholar]
50. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem. 2001;49:5165–70. doi: 10.1021/jf010697n. [PubMed] [CrossRef] [Google Scholar]
51. Tiwari, V.; Singh, B.D.; Tiwari, K.N. Shoot regeneration and somatic embryogenesis from different explants of Brahmi [Bacopa monniera (L.) Wettst.]. Plant Cell Rep. 1998, 17, 538–543. [CrossRef]
52. Debnath, M. Responses of Bacopa monnieri to salinity and drought stress in vitro. J. Med. Plants Res.2008, 2, 347–351.
53. Tiwari, V.; Tiwari, K.N.; Singh, B.D. Comparative studies of cytokinins on in vitro propagation of Bacopa monniera. Plant Cell Tiss. Organ Cult. 2001, 66, 9–16. [CrossRef]
54. Mohapatra, H.P.; Rath, S.P. In vitro studies of Bacopa monnieri—An important medicinal plant with reference to its biochemical variations. Indian J. Exp. Biol. 2005, 43, 373–376
55. Sape, S. T., Kandukuri, A. V., & Owk, A. K. (2020). DIRECT AXILLARY SHOOT REGENERATION WITH NODAL EXPLANTS OF Bacopa monnieri (L.) Pennell – A MULTI MEDICINAL HERB. Journal of Applied Biological Sciences, 14(2), 190–197.
56. Sharma, M.; Raina, H.; Verma, V.; Mallubhotla, S.; Ahuja, A. Synthetic seeds a viable approach for conservation and propagation of phytoremediant herb: Bacopa monnieri (L.) Wettst. J. Env. Res. Dev. 2012, 7, 399–404.
57. Hegazi, G.A.E.M. In vitro preservation of Bacopa monnieri (L.) Pennell as a rare medicinal plant in Egypt. J. Basic Appl. Sci. Res. 2016, 6, 35–43
58. Sarkar, S.; Jha, S. Morpho-histological characterization and direct shoot organogenesis in two types of explants from

Bacopa monnieri on unsupplemented basal medium. Plant Cell Tiss. Organ Cult. 2017, 130, 435–441. [CrossRef]19 / 20
59. Balaraju, K., Agastian, P., Preetamraj, J.P, Arokiyaraj, S., Ignacimuthu, S. (2008): Micropropagation of Vitex agnus-castus, (Verbenaceae) a valuable medicinal plant. In Vitro Cellular & Developmental Biology – Plant 44: 436-441.
60. Mehta, J., Ansari, R., Syedy, M., Khan, S., Sharma, S., Gupta, N., Rathore, R., Vaishnav, K. (2012): An effective method for high frequency Multiple shoots regeneration and callus induction of Bacopa monnieri (L.) Pennel.: An important medicinal plant. Asian Journal of Plant Science and Research 2(5): 620-626
61. Molsaghi, M., Moieni, A., Kahrizi, D. (2014): Efficient protocol for rapid Aloe vera micropropagation. Pharmaceutical Biology 52(6): 735–739.
62. Casado, J. P., Navarro, M. C., Utrilla, M. P., Martinez, A., Jimenez, J. (2002): Micropropagation of Santolina canescens Lagasca and invitro volatiles production by shoot explants. Plant Cell, Tissue Organ Culture 69: 147-153
63. Hassani, B., Saboora, A., Radjabian, T., Fallah Husseini, H. (2008): Somatic embryogenesis of Ferula assa-foetida. Journal of Science and Technology 33(4): 15-23.
64. Sharma, S.; Kamal, B.; Rathi, N.; Chauhan, S.; Jadon, V.; Vats, N.; Gehlot, A.; Arya, S. In vitro rapid andmass multiplication of highly valuable medicinal plant Bacopa monnieri (L.) Wettst. Afr. J. Biotechnol.2010, 9, 8318–8322.
65. Behera, S.; Nayak, N.; Shasmita, D.P.; Naik, S.K. An efficient micropropagation protocol of Bacopa monnieri(L.) Pennell through two-stage culture of nodal segments and ex vitro acclimatization. J. Appl. Biol. Biotech.2015, 3, 16–21.
66. Koul, Anuja & Sharma, Arti & Gupta, Sofia & Mallubhotla, Sharada. (2014). Cost Effective Protocol for Micropropagation of Bacopa Monnieri Using Leaf Explants. International Journal of Science and Research. 3. 201-212.
67. Tiwari, V.; Tiwari, K.N.; Singh, B.D. Comparative studies of cytokinins on in vitro propagation of Bacopa monniera. Plant Cell Tiss. Organ Cult. 2001, 66, 9–16. [CrossRef]
68. A.Tanveer, M. Khan and F. Shah, "In Vitromicropropagation of Brahmi- Bacopa monnieri (L.) Pennel- A step for conservation," Nanobiotechnica Universale (1), pp. 139-150, 2010
69. G.A. Joshi, R.A. Pathak, M.A. Sharma and S. Singh, "High frequency of shoot regeneration on leaf explants of Bacopa monnieri," Environmental and Experimental Biology (8), pp. 81- 84, 2010.
70. Rout, J.R.; Sahoo, S.L.; Ray, S.S.; Sethi, B.K.; Das, R. Standardization of an efficient protocol for in vitro clonal propagation of Bacopa monnieri L.—An important medicinal plant. J. Agric. Tech. 2011, 7, 289–299.
71. Jain, N.; Sharma, V.; Ramawat, K.G. Shoot culture of Bacopa monnieri: Standardization of explant, vessels and bioreactor for growth and antioxidant capacity. Physiol. Mol. Biol. Plants 2012, 18, 185–190. [CrossRef] [PubMed]
72. Rao, S.; Rajkumar, P.; Kaviraj, C.; Parveen, P.A. Efficient plant regeneration from leaf explants of Bacopa monniera (L.) Wettst.: A threatened medicinal herb. Ann. Phytomed 2012, 1, 110–117.
73. Umesh, T.G.; Sharma, A.; Rao, N. Regeneration potential and major metabolite analysis in nootropic plantBacopa monnieri (L.) Pennell. Asian J. Pharm. Clin. Res. 2014, 7, 134–136.
74. Ceasar, S.A.; Maxwell, S.L.; Prasad, K.B.; Karthigan, M.; Ignacimuthu, S. Highly efficient shoot regeneration of Bacopa monnieri (L.) using a two-stage culture procedure and assessment of genetic integrity of micropropagated plants by RAPD. Acta Physiol. Plant 2010, 32, 443– 452. [CrossRef]