Advances in Bioresearch
Adv. Biores., Vol 11 (5) September 2020: 97-103

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.11.5.97103

Advances in Bioresearch

ORIGINAL ARTICLE

Tissue culture propagation of a medicinal plant *Bacopa monnieri* (L.) Pennell

[1]Diwakar Aggarwal[*], [2]Sushil Kumar Upadhyay[#], [3]Raj Singh, [4]Nirmala Sehrawat, [5]Mukesh Yadav, [6]Manoj Singh, [7]Vikas Kumar
[1-7]Department of Biotechnology, Maharishi Markandeshwar (Deemed to be University)
Mullana-(Ambala) 133207, Haryana, India.
[*#]Corresponding Email**:** diwakaraggarwal@yahoo.co.in; sushil.upadhyay@mmumulla.org

ABSTRACT
Present investigation displays a fast and reproducible in vitro propagation method for Bacopa monnieri, which can be utilized for large scale propagation of plants. Aseptic cultures were built up and initiated on Murashige and Skoog (MS) medium fortified with 6-benzyle adenine (BA, 0.5µM). For shoot multiplication, BA, Kinetin (KIN) and thidizuron (TDZ) were utilized. The BA was seen as better for shoot multiplication and elongation as contrasted with KIN and TDZ. Maximum shoot multiplication was seen on MS medium containing 2.5µM of BA. Further maximum shoot regeneration frequency was observed on MS medium containing 5.0µM of BA in blend with 1.0µM of α-naphthalene acetic acid (NAA). Indole 3-butyric acid (IBA) was seen as better auxin for root induction and maximum rooting frequency was seen on MS medium supplemented with 5.0µM of IBA. Micropropagated plants showed healthy growth and survival during polyhouse and nursery conditions.
Keywords; *Brahmi, Micropropagation, Medicinal Plants, Cytokinins, PGRs, Shoot regeneration*

Received 01.05.2020 Revised 19.06.2020 Accepted 05.08.2020

How to cite this article:
D Aggarwal, S K Upadhyay, R Singh, N Sehrawat, M Yadav, M Singh, V Kumar. Tissue culture propagation of a medicinal plant *Bacopa monnieri* (L.) Pennell. Adv. Biores., Vol 11 (5) September 2020: 97-103

INTRODUCTION

In spite of advances in development of synthetic drugs and antibiotics, medicinal plant preparation enjoyed the maximum representation in the indigenous system of medicine [1]. Medicinal plants are found to be major sources of drugs in modern as well as traditional system of medicine [2]. Plant based drugs are considered safe and more effective in comparison to synthetic drugs, which have higher risk of side effects [3]. This has led to an increased interest in screening of plant species and reviewing of traditional remedies for development of new and effective drugs. India harbors rich diversity of medicinal plants and occupies top most position in use of herbal drugs [4]. The worldwide market of therapeutic plants and home grown prescriptions is assessed to be US$ 80 billion every year [5]. Around 32, 600 Tons of therapeutic plants are sent out from India consistently adds to the foreign trade in the Indian economy [6].

Increased demand of medicinal plants led to expanded rate of plant extraction from natural habitat. In this way, populaces of high worth medicinal plant species are diminishing at a disturbing rate and status of numerous such plants is currently now threatened to critically endangered [7]. Thus arise the need of an elective strategy for proliferation of plants; an effective and most relevant method is development of *in vitro* frameworks for the production of therapeutic plants and their concentrates. The *in vitro* propagated therapeutic plants presents a reliable and uniform plant material for biochemical portrayal and identification of active constituents [8]. In a report by the National Medicinal Plant Board (NMPB), Government of India and Technology Information Forecasting and Assessment Council (TIFAC) has prescribed prompt consideration regarding preservation of some significant endangered plants, among which *Bacopa monnieri* is listed as profoundly jeopardized plants in India [9]. Recently, *B. monnieri* was put on second place in priority list of most significant Indian medicinal plants assessed based on therapeutic significance, business worth and potential for further innovative work [10].

The *B. monnieri* commonly referred to in India as Brahmi or Neera-Brahmi is an individual from family Scrophulariaceae. It is a significant ayurvedic herb utilized in India for over 3000 years. It is a small prostate herb that develops wild in subtropical places close to water bodies all through India [11]. This plant has been widely worked upon for its chemical constituents [12]. It is an old and eminent medicinal plant with incredible reputation as a memory vitalizer. In the Ayurveda system of medication, 'Brahmi' is named 'medhya rasayana' *i.e.*, a medication that should balance the impacts of mental pressure and improve knowledge and memory work. 'Brahmi' is seen as successful in instances of uneasiness and mental issues [13]. The ongoing revelation of the memory–improving property of the bacosides has upgraded the interest of this plant and brought about its broad use in a few business creations [14]. Nearly the whole business requirement is met from the wild natural populaces bringing about its listing as threatened plant by the International Union for Conservation of Nature and National Resources [4,15]. With an expanding overall interest for plant based drugs and definitions, there has been an increment in the interest for crude material [16]. Thus, there is a need to create approaches for guaranteeing the accessibility of crude material of a reliable quality from customary and feasible sources which may likewise decrease pressure on regular populaces [17]. Therefore, the present investigation was taken up for the development of an efficient and reproducible *in vitro* propagation method for commercial scale production of *B. monnieri* plants.

MATERIAL AND METHODS

Plant material, chemicals, glassware: The plants of *B. monnieri* were obtained from Tau Devi Lal Herbal Nature Park at town Chuharpur, District Yamunanager (Haryana), India and kept up at Maharishi Markandeshwar (Deemed to be University) nursery under optimum nursery conditions. All chemicals utilized were from HiMedia Laboratory unless specified. The experiments were carried out in 300ml glass bottles (Kasablanka, Mumbai) containing 30ml of medium. Medium pH was settled at 5.8 before autoclaving at 21^{o}C for 20min. Cultures were raised using nodal segments as explants utilizing methodology after Aggarwal *et al.* [18]. The shoot cultures were kept on Murashige and Skoog medium (MS medium) containing 58mM sucrose and gelled with 0.7% (*w/v*) agar supplemented with 0.5µM BA (benzyladenine) [19].

Effect of cytokinins on shoot proliferation and elongation: The impact of various cytokinins in particular, BA (6-benzyl adenine), KIN (Kinitin, 6-furfurylaminopurine) and TDZ (Thidiazuron (1-phenyl-3 (1,2,3-thiadiazol-5-yl) urea) on shoot multiplication and development was tried. The shoot clumps (2.0-2.5cm/3-4 shoots, three shoot clusters/culture vessel) were cultured on MS medium differently supplemented with various concentrations (0.0-5.0µM) of these cytokinins. The data for shoot multiplication and elongation was recorded after three weeks of culture.

Effect of Plant growth regulators (PGRs) on shoot regeneration: The fully extended leafs (about 1.0 to 1.5cm size) from three weeks old micro-shoots were used for shoot organogenesis. Leaf segments were cut transversely were inoculated on MS medium variously supplemented with different concentrations of BA (0.0–12.5 µM) and 0.0–25.0µM NAA (α-naphthalene acetic acid) with adaxial surface facing the medium.

Rooting of micro-shoots and acclimatization of plantlets: Micro-shoots were excised from clumps just below the node, leaves were removed from lower nodes and were cultured on MS medium supplemented with different concentrations (0-5 µM)of NAA, indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) for the induction of roots. Acclimatization of plantlets was carried out in polyhouse with controlled temperature (25-28^{o}C) and humidity (90-95%). Plantlets were planted in a mixture of soil and vermicompost in equal ratio in polybags and kept in polyhouse. During the initial periods 90% relative humidity was maintained and slowly it was reduced to 40 % over a period of one month.

Statistical analysis: Unless otherwise stated, all experiments were conducted using four replicates with three explants in each culture vessel and repeated four times. The data were recorded after 4 week of subculture. Data were analyzed by analysis of variance and the means were compared with Duncan's multiple range test.

RESULTS

Effect of cytokinins on shoot proliferation and shoot elongation: Aseptic cultures were built up utilizing mercuric chloride as surface sterilizing agent, it was found be good for establishment of aseptic cultures (Figure 1A). The impact of various concentrations of BA, KIN and TDZ was inspected on shoot multiplication and elongation of micro-shoots. Maximum shoot multiplication frequency per culture vessel (51) were seen on MS medium supplemented with 2.5µM BA (Figure 1B; Table 1), while maximum number of elongated shoots per culture vessel (16) alongside maximum shoot length (5.3cm) was seen on

MS medium supplemented with 0.5µM BA (Figure 1C; Table 1). Out of the three tried cytokinins *i.e.* BA, KIN and TDZ; BA was seen as best for both shoot multiplication and shoot elongation pursued by KIN and TDZ (Table 1).

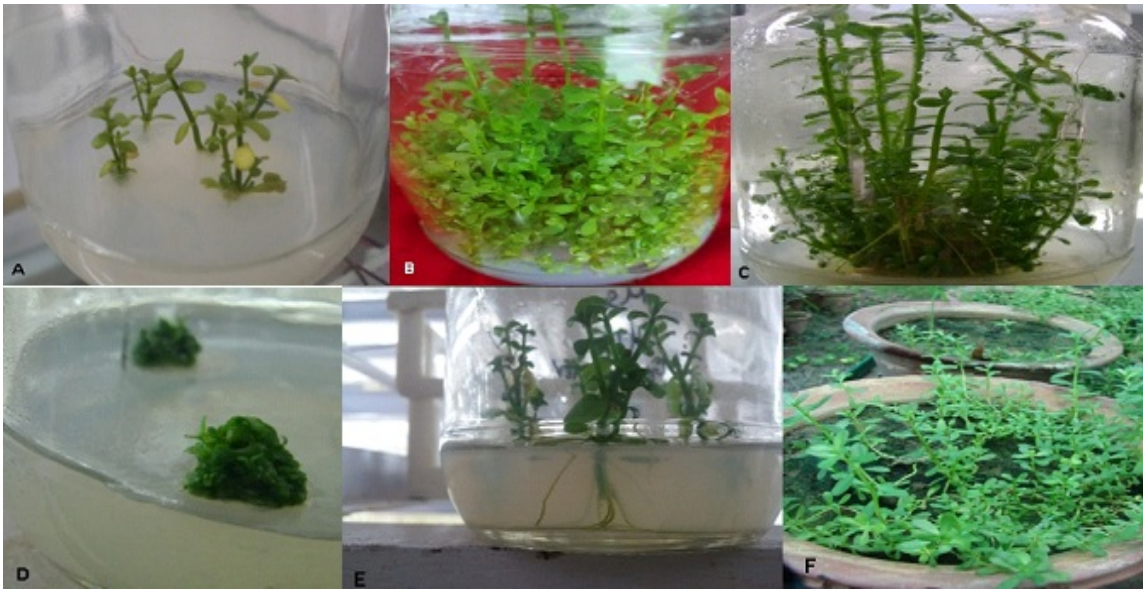

Figure 1: Micropropagation of *B. monnieri*. Contamination free aseptic cultures of *B. monnieri* (A), Shoot multiplication on MS medium supplemented with 2.5µM BA (B), Shoot elongation on MS medium supplemented with 0.5µM BA (C), Shoot regeneration on MS medium supplemented with 5.0µM BA and 1.0µM NAA (D), Rooting of microshoots on MS medium supplemented with 5µM IBA (E), Acclimatized plantlets (F).

Table 1: The effect of different cytokinins on proliferation and elongation of microshoots of *B. monnieri* propagated on MS medium.

Plant growth regulators (µM)			Morphogenic Responses			
BAP	KIN	TDZ	% Explants responded	Average no. of shoots proliferated	Average no. of shoots elongated	Average shoot length (cm)
0.0	0.0	0.0	20m	15l	05i	3.1i
0.1	0.0	0.0	24i	28g	13b	4.3be
0.5	0.0	0.0	37h	37d	16a	5.3a
1.5	0.0	0.0	45e	47b	11c	4.6b
2.5	0.0	0.0	65a	51a	10d	4.3bcd
5.0	0.0	0.0	58b	42c	08f	3.9def
0.0	0.1	0.0	20m	13n	08f	3.6fgh
0.0	0.5	0.0	29k	18k	10d	4.0def
0.0	1.5	0.0	38g	29f	09e	4.2bcd
0.0	2.5	0.0	56c	34e	06h	3.7efg
0.0	5.0	0.0	46d	28g	05i	3.5efg
0.0	0.0	0.1	15n	14m	04j	4.1cde
0.0	0.0	0.5	24l	18k	07g	4.3bcd
0.0	0.0	1.5	32j	21i	06h	3.7efg
0.0	0.0	2.5	42f	23h	04j	3.4ghi
0.0	0.0	5.0	35i	19j	03k	3.2hi

Means with the same letter within a column are not significantly different according to Duncan's multiple range test (P< 0.05).

Effect of PGRs on shoot regeneration: The impact of BA alongside 2,4–D (2,4-Dichlorophenoxyacetic acid) or NAA was inspected on shoot regeneration from sections of leaves taken from micro-shoots on MS medium supplemented with various concentrations of BA alongside 2,4–D or NAA. Shoot regeneration was seen in all the combinations (Table 2). Higher percent of explants demonstrated shoot regeneration on medium containing NAA when contrasted with those containing 2,4–D. Maximum shoot regeneration

frequency was seen in MS medium enhanced with 5.0µM BA in mix with 1.0µM NAA with 13.5 shoots per explants (Figure 1D; Table 2).

Table 2: The effect of plant growth regulators on shoot regeneration from leaf segments taken from micro-shoots of *B. monnieri* on MS medium.

Plant growth regulator (µM)		% explants with shoot regeneration	Average no. of shoots /explant
0.0	0.0	9.5l	1.2l
BA 1.0	2,4-D 1.0	12.5k	2.1k
	2,4-D 2.5	20.1i	6.7g
BA 5.0	2,4-D 1.0	35.6e	9.6e
	2,4-D 2.5	33.1f	8.6f
BA 12.5	2,4-D 1.0	22.3h	5.7ij
	2,4-D 2.5	19.2j	5.2j
BA 1.0	NAA 1.0	44.5c	11.2c
	NAA 2.5	40.9d	10.4d
BA 5.0	NAA 1.0	54.6a	13.5a
	NAA 2.5	50.1b	11.9b
BA 12.5	NAA 1.0	35.6e	6.1h
	NAA 2.5	28.3g	5.8hi

Cultures were sub-cultured on same medium at 4-week interval. Data were scored after 8 weeks of inoculation. Means with the same letter within a column are not significantly different according to Duncan's multiple range test (P< 0.05).

Rooting of micro-shoots and acclimatization of plantlets: The effects of various auxins *i.e.* NAA, IBA and IAA, was examined on rooting efficiency of micro-shoots. The IBA was found to be most effective amongst all auxins (Table 3). Maximum rooting frequency of micro-shoots (79.8%) was observed on MS medium supplemented with 5.0 µM IBA (Figure 1E; Table 3). The IBA was found to be best for induction followed by IAA and NAA. Rooted microshoots were successfully acclimatized under polyhouse conditions (80%, Figure 1F). Later acclimatized plants were successfully established in their natural conditions.

Table 3: The effect of different auxins on rooting of *B. monnieri* micropropagated shoots on MS medium.

Auxin (µM)	Percentage of shoots showing rooting	Average no. of roots per shoot	Average root length (cm)
0.0	0.0i	0.0f	0.0e
1.0 NAA	45.8h	2.6c	0.95d
2.5 NAA	56.2f	2.8c	0.84d
5.0 NAA	63.4c	2.9bc	1.1cd
1.0 IBA	62.3d	3.1b	1.8b
2.5 IBA	70.1b	3.7a	1.9a
5.0 IBA	79.8a	4.1a	2.5a
1.0 IAA	55.6g	1.9d	1.5bc
2.5 IAA	57.1e	1.8de	1.1d
5.0 IAA	62.8d	1.5e	1.05d

Means with the same letter within a column are not significantly different according to Duncan's multiple range test (P< 0.05).

DISCUSSION

Plant tissue culture is considered as elective strategy to conventional vegetative propagation techniques utilized for plant propagation as it offers fast and large scale generation of valued planting material for business supplies from constrained explants material [20]. Different tissue culture systems, for example, axillary and adventitious shoot multiplication, shoot organogenesis and somatic embryogenesis, and are utilized for the large scale production of significant plant species [21]. One of the problems faced in continues and uniform supply of quality planting material has been the absence of a reproducible *in vitro* propagation protocol for medicinal important plants [22]. Few reports could be traced on *in vitro* propagation of *Bacopa*, that too are genotype dependent and there is lack of reproducibility for reported protocols [23-28]. In this manner the present investigation was engaged towards the improvement of a proficient and reproducible micropropagation method for the commercial scale production of *B. monnieri*. Surface sterilization is one of the most significant steps towards establishment of aseptic cultures. A few surface disinfecting agents are suggested for removal of surface microorganisms from explants [29]. In

the present examination mercuric chloride ($HgCl_2$) at the concentration of 0.1% (*w/v*) was used as surface sterilizing agent and was seen to be satisfactory (Fig. 1A). Mercuric chloride was the choice of sterilizing agent for foundation of aseptic cultures in various examinations [18,29,30].

Effective micropropagation of any plant under *in vitro* conditions relies on utilization of right kind of plant growth regulator and in right concentration as PGRs are known to promote cell division along with shoot multiplication and axillary bud improvement [15]. Therefore, in present examination also PGRs in various concentrations were tried for maximum production of plantlets of *B. monnieri*. Out of the three tried cytokinins *i.e.* BA, KIN and TDZ for acquiring *in vitro* shoot multiplication and elongation, Maximum frequency for shoot initiation was seen on MS medium enhanced with 2.5µM of BA (Table 1), besides maximum shoot multiplication and elongation was also seen on MS medium supplemented with BA (Table 1) trailed by kinetin and thidizuron. BA is adenine type cytokinin and answered to be utilized as often as possible for growth, development and increase in many plant species including *Bacopa* [15,18,31].

Productive shoot regeneration protocol is essential for any genetic transformation method to be fruitful and furthermore depicted as valuable strategy for mass propagation of value plant material [32]. In spite of the fact that there are as of now a few reports on the development of shoot regeneration protocols for *B. monnieri* [23,30,33]. However, the frequency of regeneration varied from species to species and clone to clone inside similar species [34,35]. Along these lines emerge the need to create clone-specific protocols. To accomplish successful shoot regeneration from leaf explants, impact of PGRs was assessed. Among the various mixes of PGRs tried, shoot regeneration was seen on MS media containing blends with higher amount of BA and lower amount of auxins (2,4-D or NAA) (Table 2). Maximum shoot regeneration frequency (54.6%) was seen on MS media supplemented with 5.0µM of BA in blend with 1.0µM of NAA. The necessity of BA and NAA for the induction of shoot separation in *B. monnieri* has been accounted for by many earlier researchers [17,33]. Appropriate auxin and cytokinins ratio is required for the optimum shoot regeneration in many plants. Further appropriate auxin and cytokinins ratio is reported to regulated shoot regeneration through regulation of intercellular auxin distribution [36]. Further NAA is answered to play significant job in cytokinin metabolism and stability which may have helped in acquiring higher shoot organogenesis recurrence in leaf explants [37].

Rooting of micro-shoots is the initial step during hardening of plantlets and pre imperative for any micropropagation protocol to be effective at business level. It helps in establishment of plantlets in soil under normal conditions. Auxins are generally utilized for induction of roots in micro-shoots. Out of the different auxins tried (IBA, NAA and IAA), IBA at concentration of 5.0µM showed maximum rooting frequency (Table 3). Utilization of IBA for root induction of micro-shoots for different plant species has been very well surveyed and answered to be more helpful than different auxins like NAA and IAA [37,38]. Yet, the concentration of IBA must be streamlined for various plant species [39]. The rooted plantlets showed healthy growth and survival upon transfer to pots containing soil and vermicompost in equal ratio under poly house conditions.

CONCLUSION

Conclusively, present study presents the fast, reliable and most importantly reproducible micropropagation protocol for the selected plants of *B. monnieri* which can be utilized for commercial level production of quality planting material to insure to reproducibility of developed plantlets with efficient medicinal property.

ACKNOWLEDGMENTS

The authors are thankful to Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala (HR), India and TIFAC-CORE Thapar Institute of Engineering and Technology, Patiala for providing necessary facilities required to carry out the work.

REFERENCES

1. Rates SMK. (2001). Plants as source of drugs. Toxicon; 39:603–13.
2. Newman DJ, Cragg GM, Sander KM. (2003). Natural products as sources of new drugs over the period 1981-2002. J Nat Prod; 66:1022–37.
3. Butler MS. (2004). The role of natural product chemistry in drug discovery. J Nat Prod; 67:2141–53.
4. Pandey NK, Tewari KC, Tewari RN, Joshi GC, PandeyVN, Pandey G. (1993). Medicinal plants of Kumaon Himalaya, strategies' for conservation. In: Dhar U, editor. Himalayan biodiversity conservation strategies'. Himavikas Publ, Nainital; 3:293–302.
5. Purohit SS, Vyas SP. (2004). Marketing of medicinal and aromatic plants in Rajasthan. In: National consultative workshop on medicinal and aromatic plants. GBPUAT, Pantnagar.

6. Rawat GS, Garg GP. (2005). Medicinal plants: Trade and commerce opportunities with India. The Indian For; 131:275–87.
7. Sang W, Ma K, Axmacher JC. (2011). Securing a future for China's wild plant resources. Biosci; 61:720–5.
8. Wakhlu, AK, Bajwa PS. (1986). Regeneration of uniform plants from somatic embryos of *Papaver somniferum* (opium poppy). Phytomorphol; 36:101–5.
9. Banerjee M, Shrivastava S. (2008). An improved protocol for in vitro multiplication of *Bacopa monnieri* (L.). World J Microbiol Biotechnol; 24:1355–9.
10. Anonymous. (1997). Wealth of India, raw materials. Council Scient Indust Res, New Delhi.
11. Chopra RN, Nayar L, Chopra IC. (1956). Glossary of Indian medicinal plants. Vol. 32. Council Scient Indust Res, New Delhi.
12. Darokar MP, Suman PSK, Shasany AK, Kumar S. (2001). Low levels of genetic diversity detected by RAPD analysis in geographically distinct accessions of *Bacopa monnieri*. Genet Resour Crop Ev; 48:555–8.
13. Singh HK, Dhawan BN. (1982). Effect of *Bacopa monniera* extract on avoidance responses in rat. J Ethnopharmacol; 5:205–14.
14. Bansal M, Kumar A, Reddy MS. (2014). Diversity amongst wild accessions of *Bacopa monnieri* (L.) Wettst. and their morphogenetic potential. Acta Physiol Plant; 36:1177–86.
15. Tiwari V, Tewari KN, Singh BD. (2001). Comparative studies of cytokinins on *in vitro* propagation of *Bacopa monniera*. Plant Cell Tiss Org Cult; 66:9–16.
16. Jawla S, Gupta AK, Singla R, Gupta V. (2009). General awareness and relative popularity of allopathic, ayurvedic and homeopathic systems. J Chem Pharm Res; 1(1):105–12.
17. Shrivastava N, Rajani M. (1999). Multiple shoot regeneration and tissue culture studies on *Bacopa monnieri* (L.) Pennell. Plant Cell Rep; 18:919–23.
18. Aggarwal, D, Kumar A, Sharma J, Reddy MS. (2012). Factors effecting micro propagation and acclimatization of an elite clone of *Eucalyptus tereticornis*. In Vitro Cell Dev Biol Plant; 48:521–9.
19. Murashige, T, Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant; 15:473–97.
20. Aggarwal D, Kumar A, Kumar A. (2019). Plant tissue culture for commercial propagation of economically important plants. In: Yadav M, Kumar V, Sehrawat N, editors. Industrial biotechnology: Plant systems, resources and products. Walter de Gruyter, Germany.
21. Vengadesan, G, Pijut PM. (2009). *In vitro* propagation of northern red oak (*Quercus rubra* L.). In Vitro Cell Dev Biol Plant; 45:474–82.
22. Rout GR, Samantaray S, Das P. (2000). *In vitro* manipulation and propagation of medicinal plants. Biotechnol Adv; 18(2):91–120
23. Tiwari, KN, Tiwari V, Singh J, Singh BD, Srivastava P. (2012). Synergistic effect of trimethoprim and bavistin for micropropagation of *Bacopa monnieri*. Biol Plant; 56:177–80.
24. Kaur J, Nautiyal K. (2013). *In vitro* propagation of *Bacopa monnieri* (L.) Wettst A medicinally priced herb. Int J Curr Microbiol Appl Sci; 2:131–8.
25. Dharishini MP, Balasubramanian K, Radha A. (2014). *In vitro* micropropagation of *Bacopa monnieri* and detection of bacosides from secondary callus. J Acad Ind Res; 3:233–6.
26. Behera S, Nayak N, Shasmita H, Barik DP, Naik SK. (2015). An efficient micropropagation protocol of *Bacopa monnieri* (L.) Pennell through two-stage culture of nodal segments and *ex vitro* acclimatization. J Appl Biol Biotech; 3:16–21.
27. Kumari U, Vishwakarma RK, Gupta N, Ruby SMV, Khan BM. (2015). Efficient shoots regeneration and genetic transformation of *Bacopa monniera*. Physiol Mol Biol Plants; 21:261–7.
28. Mohammad F, Abdulrahman AA, El-Sheikha MA, Eslam MA, Qahtan AA. (2018). Thidiazuron induced in vitro morphogenesis for sustainable supply of genetically true quality plantlets of Brahmi. Ind Crops Prod; 118:173–9.
29. Aasim M, Karatas M, Khawar KM, Dogan M. (2013). Optimization of sterilization and micropropagation of water lettuce (*Pistia stratiotes* L.). J Appl Biol Sci; 7(3):71–7.
30. Tiwari, V, Tiwari KN, Singh BD. (2006). Shoot bud regeneration from different explants of *Bacopa monniera*(L.) Wettst. by trimethoprim and bavistin. Plant Cell Rep; 25:629–35.
31. Kajla, S, Kala S, Kumar A, Mir GH, Singh MK. (2018). Effect of growth regulators on *in vitro* shoot multiplication and plant regeneration of *Rosa hybrida* L. from nodal explants. Int J CurrMicrobiol Appl Sci; 7:3804–11.
32. Yasodha R, Sumathi R, Gurumurthi K. (2004). Micropropagation for quality production in plantation forestry. Indian J Biotechnol; 3:159–70.
33. Tiwari V, Singh BD, Tiwari KN. (1998). Shoot regeneration and somatic embryogenesis from different explants of Brahmi [*Bacopa monniera* (L.) Wettst.]. Plant Cell Rep; 17:538–43.
34. Mullins KV, Llewellyn DJ, Hartney VJ, Strauss S, Dennis ES. (1997). Regeneration and transformation of *Eucalyptus camaldulensis*. Plant Cell Rep; 16:787–91.
35. Aggarwal D, Kumar A, Reddy MS. (2010). Shoot organogenesis from elite plants of *Eucalyptus tereticornis*. Plant Cell Tiss Org Cult; 102:45–52.
36. Bishopp A, Benkova E, Helariutta Y. (2011). Sending mixed messages: Auxin-cytokinin crosstalk in roots. Curr Opin Plant Biol; 14:10–16.
37. Palni LMS, Burch L, Horgan R. (1988). The effect of auxin concentration on cytokinin stability and metabolism. Planta; 174(2):231–4.

38. Kato M. (1985). Regeneration of plantlets from tea stem callus. Jpn J Breed; 35:317–22.
39. Jha T, Sen SK. (1992). Micropropagation of an elite Darjeeling tea clone. Plant Cell Rep; 11:101–4.