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AN EFFICIENT IAA AND BAP-ASSISTED *IN VITRO* MICROPROPAGATION THROUGH SHOOT TIP CULTURE OF *BACOPA MONNIERI* (L.) WETTST. – A MEDICINALLY IMPORTANT PLANT

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ABSTRACT

A rapid, simple and efficient protocol for *in vitro* multiple shoot induction and plantlet regeneration was achieved from shoot tip explants of *Bacopa monnieri* (L.) Wettst. For this, the effect of three different cytokines on *in vitro* micropropagation from shoot tip explants was studied in *B. monnieri*. The cytokinins used were 6-benzylaminopurine (BAP), Kinetin (KN) and Thidiazuron (TDZ). They were used either individually or in combination with Indole acetic acid (IAA). Although organogenesis could be obtained on medium supplemented with BAP/KN/TDZ, the regenerated shoots failed to elongate on the same medium and showed an albino phenotype. BAP stimulated shoot regeneration and this effect was significantly enhanced when combined with IAA. BAP (3.0 mg/L) in combination with IAA (0.5 mg/L) proved to be optimal for induction maximum number of shoots from shoot tip explants within three weeks of culture. The highest number of shoots (24.0 ± 0.04) was achieved on MS medium augmented with IAA (0.5 mg/L) + (3.0 mg/L) BAP. The medium supplemented with IAA (0.5 mg/L) + (3.0 mg/L) BAP better than all other media concentrations in shoot tip explants. Individual shoots were aseptically excised and sub cultured in the same media for shoot elongation. The elongated shoots were transferred to Indole Butyric Acid (IBA) (0.5-1.0 mg/L) and Indole Acetic Acid (IAA) (0.5-1.0 mg/L) for root induction. Rooting was observed within two weeks of culture. MS medium supplemented with (1.0 mg/L) IBA proved better with seventy percent rooting after 25 days of implantation. Most of the roots were long and healthy. Rooted plantlets were successfully hardened under culture conditions and subsequently established in the field conditions. The recorded survival rate of the plants was 76.3%. Plants looked healthy with no visually detectable phenotypic variations. The plantlets (10-12 weeks old) were successfully acclimatized in soils with 85% survival frequency.

KEYWORDS: ***in vitro Micro propagation; shoot tip; Multiple Shoots; Rooting; Hardening***

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INTRODUCTION

Medicinal plants (MP) and aromatic plants (AP) are known to human beings since antiquity. Examples were cited on the use of these plants for religious ceremonies, rituals forcuring diseases and as beauty aids around in the ancient literature of different countries including India. The study of "Vedas" indicates that over 200 types of vegetable drugs were inuse during Vedic Period. "Charak Samhita" mentioned the Sanskrit names of 1270 MP, while "Shushruta Samhita" and Vagbhata's "AstangaHridaya" mentioned the names of 1100MP and 1150 AP, respectively. America, Arabia, China, Egypt, Europe, Greece, Mexico and Rome were recorded the uses of many medicinal plants. 1800 species of medicinal plants and 800 aromatic plants, along with 5000 herbal formulations are recorded in "Ayurveda". 750 species in Unani, 500 species in Siddha, 400 species in Tibetan Medicine, and 500 species in Chinese Medicine are on the records. Brahmi which played a very important role in Ayurvedic therapies[1] is considered to be the main rejuvenating herb. "Brahmi" has been used in the Ayurvedic system of medicine for centuries. *B. monnieri*, commonly known as ''Brahmi'', is a member of the Family Plantaginaceae, and is placed second in the priority list of Indian medicinal plants[2]. It is commonly found on the banks of rivers and lakes. It has been used for centuries in legends and traditional system of medicine as a memory enhancer [3,4], anti-inflammatory[5], analgesic, Antidiarrhoeal, Cytotoxic activity [6], antipyretic [7], sedative and antiepileptic agent [8]. In addition to its unique medicinal use, *B. monnieri* has also been linked to phytoremediation programmes for the removal of heavy metals such as cadmium and chromium [9]. Brahmi leaves are oblong, sessile and fleshy. In the Ayurvedic Bacopa has been recognized for its brain enhancement personality. *B. monnieriis* a small, creeping herb with numerous branches, small oblong leaves and light purple flowers. In India and the tropics, it grows naturally in wet soil, shallow water and marshes of *B.monnieri*[10].

MATERIALS AND METHODS

Seed germination

Seeds of *B. monnieri* were obtained from CIMAP at Hyderabad, Telangana India. The seeds were soaked in distilled water for 24 hours and surface were sterilized using 0.1% $HgCl_2$ for 3 min. These are followed by four to five rinses with sterile distilled water. Seeds were germinated aseptically on[11] medium containing 2% sucrose and solidified with 0.8% agar (Hi-media).

Rooting medium and greenhouse transfer

After approximately one month's growth on regeneration medium the elongated shoots 3-4 cm in length were transferred for rooting onto MS medium supplemented with (1.0 mg/L) IBA. The effect of plant growth regulators on rooting was investigated 3-4 weeks after culture on rooting medium. Rooted plants (4-5 weeks old) were weaned away from the tubes, the roots cleaned for agar by washing with sterile distilled water and the plantlets were transferred to pot containing soil and compost (1:1) followed by transfer to greenhouse. A 65% relative humidity was maintained in the green house and the pots were covered with polythene bags until the plantlets were acclimated under greenhouse conditions. All media pH were adjusted to 5.7 with 1M KOH prior to addition of 0.8% agar, and the media were autoclaved at a pressure of 1.05 kg cm^{-2} for 15 min at 121°C. All cultures were incubated at 25 ± 2°C under white fluorescent light 40-60 µmol $m^{-2}s^{-1}$ with a 16 h photoperiod. All data were statistically analyzed by ANOVA followed by Duncan's multiple range tests for mean comparison. Data pertaining to shoot regeneration was obtained from 10 explants in each of two replicates for each treatment and the experiment was repeated twice.

STATISTICAL ANALYSIS

The statistic analysis called sample standard deviation, is a measure of the spread (variability) of the scores in the sample on a given variable and is represented by: s = sqrt [Σ (x_i – x_bar)^2 / (n – 1)] The term 'Σ (x_i – x_bar)^2' represents the sum of the squared deviations of the scores from the sample mean.

RESULTS AND DISCUSSION

In the present study three kinds of cytokinins (TDZ, Kn and BAP 0.5-6.0 mg/L) were used either alone or in combination with IAA to investigate *in vitro* micro propagation in *B. monnieri.*

Effect of TDZ

To find out the influence of TDZ on direct regeneration the shoot tip explants were excised from the surface sterilized *in vitro* grown 8-day old seedlings and cultured on MS medium fortified with TDZ (0.5-6.0 mg/L) for multiple induction of all different concentrations of TDZ tested .Highest percentage of response was observed at 3.0mg/L with (18.0±0.04 shoots/explants) (Figure 1a). The percentage of response was increased up to 3.0mg/L TDZ and later gradually decreased at high concentrations.

Effect of KN

Morphogenesis response of shoot tip explant cultures on various concentrations of cytokinin such as KN (0.5-6.0mg/L) alone. At (3.0mg/L) resulted in maximum number of shoots (14.0±0.04) was observed with 100 percentage response. A considerable decrease in shoot length was observed from 3.0mg/L to 6.0mg/L the multiple number of shoots was also increased (Table1).

Effect of BAP

BAP showed efficient shoot bud induction and regeneration from shoot tip explants. Medium containing BAP induced formation of shoot buds predominantly at the cut surface of the cultured explants within 1-2 weeks of culture. Shoot buds were proliferated and developed into shoot primordia. Among the tested concentrations of BAP (0.5 - 6.0 mg/L), lower (1.0 mg/L) and higher (6.0 mg/L) concentrations showed least regenerative response with fewer shoots produced per explant compared to (3.0 mg/L) and (4.0 mg/L) of BAP (Table1) BAP at (3.0 mg/L) proved to be the optimal concentration producing a maximum number of shoots from shoot tip (20.0 ± 0.05) explants with 100 percent responded. These regenerated shoots did not elongate on the same medium and showed an albino phenotype

(Figure 1b). BAP at (4.0 mg/L) was less significant in terms of the number of shoots/explant produced as compared to (3.0 mg/L) BAP. At (5.0 mg/L) BAP, the numbers of shoots produced from shoot tip (9.0 ± 0.04) with average length of (2. ± 0.04) shoots the number of shoots more than with (1.0 mg/L) BAP (Table1). In the control treatment, no shoot development was observed.

Table 1
Influence of TDZ, KN and BAP on shoot bud induction from Shoot tip explants cultures of B. Monnieri.

Hormone concentration (mg/L)	%of cultures responding	Average No. of shoots / Explants ± (SE)*	Average length of shoots (cm) ± (SE)*
TDZ			
0.5	75	6.0 ± 0.03	1.0 ± 0.03
1.0	82	9.0 ± 0.02	2.0 ± 0.04
2.0	90	12.0 ± 0.05	2.2 ± 0.04
3.0	100	18.0 ± 0.04	3.0 ± 0.03
4.0	73	13.0 ± 0.03	2.6 ± 0.02
5.0	62	08.0 ± 0.03	2.1 ± 0.03
6.0	50	03.0 ± 0.02	1.3 ± 0.02
Kn			
0.5	78	4.0 ± 0.03	0.5 ± 0.02
1.0	86	7.0 ± 0.04	1.8 ± 0.02
2.0	92	10.0 ± 0.03	2.0 ± 0.04
3.0	100	14.0 ± 0.04	2.8 ± 0.05
4.0	70	09.0 ± 0.05	2.4 ± 0.03
5.0	60	05.0 ± 0.03	2.1 ± 0.05
6.0	50	02.0 ± 0.04	1.6 ± 0.04
BAP			
0.5	80	8.0 ± 0.04	1.3 ± 0.04
1.0	87	10.0 ± 0.02	2.3 ± 0.04
2.0	95	16.0 ± 0.02	2.5 ± 0.02
3.0	100	20.0 ± 0.05	3.0 ± 0.04
4.0	75	16.0 ± 0.02	2.8 ± 0.02
5.0	65	09.0 ± 0.04	2.6 ± 0.04
6.0	58	04.0 ± 0.04	2.0 ± 0.02

**** Mean ± Standard Error***

Table 2
Effect of IAA in combination with TDZ/Kn/BAP on induction of shoots proliferation from shoot tip cultures of B. Monnieri.

Hormone concentration (mg/L)	% of frequency of plantlet production	Average No. of shoots / Explants ± (SE)*	Average length of shoots (cms) ± (SE)*
IAA + TDZ			
0.5 + 0.5	80	8.0 ± 0.34	1.3 ± 0.22
0.5 + 1.0	85	10.0 ± 0.36	2.2 ± 0.25
0.5 + 2.0	90	12.0 ± 0.57	2.3 ± 0.34
0.5 + 3.0	100	20.0 ± 0.32	4.2 ± 0.24
0.5 + 4.0	70	15.0 ± 0.24	3.2 ± 0.44
0.5 + 5.0	65	10.0 ± 0.62	2.0 ± 0.24
0.5 + 6.0	58	4.0 ± 0.75	1.0 ± 0.45
IAA + Kn			
0.5 + 0.5	80	6.0 ± 0.03	0.8 ± 0.05
0.5 + 1.0	86	8.0 ± 0.02	1.9 ± 0.02
0.5 + 2.0	90	12.0 ± 0.02	2.4 ± 0.05
0.5 + 3.0	100	15.0 ± 0.05	2.9 ± 0.04
0.5 + 4.0	75	10.0 ± 0.02	2.6 ± 0.42
0.5 + 5.0	63	07.0 ± 0.02	2.0 ± 0.04
0.5 + 6.0	57	04.0 ± 0.04	1.2 ± 0.04
IAA + BAP			
0.5 + 0.5	85	10.0 ± 0.02	1.5 ± 0.04
0.5 + 1.0	88	12.0 ± 0.05	2.5 ± 0.
0.5 + 2.0	97	15.0 ± 0.04	2.6 ± 0.03
0.5 + 3.0	100	24.0 ± 0.04	4.0 ± 0.02
0.5 + 4.0	78	14.0 ± 0.03	2.4 ± 0.04
0.5 + 5.0	68	07.0 ± 0.03	2.0 ± 0.04
0.5 + 6.0	60	03.0 ± 0.04	1.8 ± 0.04

**** Mean ± Standard Error***

Effect of TDZ and IAA combination

Shoot tip explants showed a positive morphogenic response on medium containing BAP (0.5 to 6.0 mg/L) in combination with IAA (0.5mg/L). Interestingly, TDZ at lower concentrations (0.5 and 1.0 mg/L) induce less *in vitro* regeneration however, when combined with IAA (0.5 mg/L) shoot formation was initiated from shoot tip explants (Table 2). This clearly implies the additive effect of IAA with TDZ on shoot tip regeneration in *B. monnieri*. Further, a higher BAP concentration (3.0 mg/L) when combined with IAA (0.5 mg/L) produced more shoots/explant from shoot tip explants than when lower levels of TDZ (1.0 and 2.0 mg/L) in combination with IAA were used. The shoots that regenerated from shoot tip explants in the presence of TDZ and IAA were of albino type.

Table 3

Rooting ability of regenerated shoots from shoot tip explants culture of B. monnieri cultured on MS medium supplemented with IAA and IBA.

Growth Hormones (mg/L)		Percentage of response	Average no of roots (S.E)*
IAA	IBA		
00	00	23	1.0 ± 0.12
0.5	-	60	2.3 ± 0.37
1.0	-	70	3.2 ± 0.38
2.0	-	73	5.6 ± 0.38
-	0.5	54	4.3 ± 0.36
-	1.0	73	8.3 ± 0.87
-	2.0	70	6.3 ± 0.36

**** Mean ± Standard Error,p <0.01***

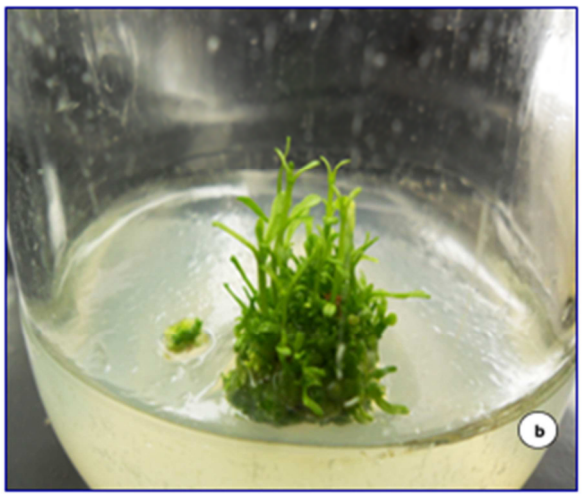
Effect of KN and IAA combination

Morphogenetic response of shoot tip culture on various concentration of cytokinin such as KN in combination with (0.5 mg/L) IAA is presented in (Table 2) on MS + (0.5 mg/L) IAA + (3.0 mg/L) KN resulted in maximum number of shoots (15.0 ± 0.05) shoots/explants with 100 percent resulted as the concentration of KN was increased, considerably the number of shoot induction was found to be reduced. At (0.5 mg/L) KN supplemented with (0.5 mg/L) IAA resulted in lowest number of shoots (6.0 ± 0.03 shoots/explant) induced with (0.8 ± 0.05) average length of roots. The percentage of response was increased up to (3.0 mg/L) KN and later gradually decreased at high concentrations. At the concentration of KN increased from (1.0mg/L to 3.0mg/L the shoot number and response increased. At (4.0, 5.0 and 6.0 mg/L KN induced (10.0 + 0.04, 7.0 + 0.05 and 4.0 + 0.04 shoots/explants) with 75, 63 and 57 % of cultures were responded. Percentage of response was gradually increased up to (3.0 mg/L) KN and after wards decreased the percentage of responding cultures. Low number of shoots per explants at (6.0 mg/L).

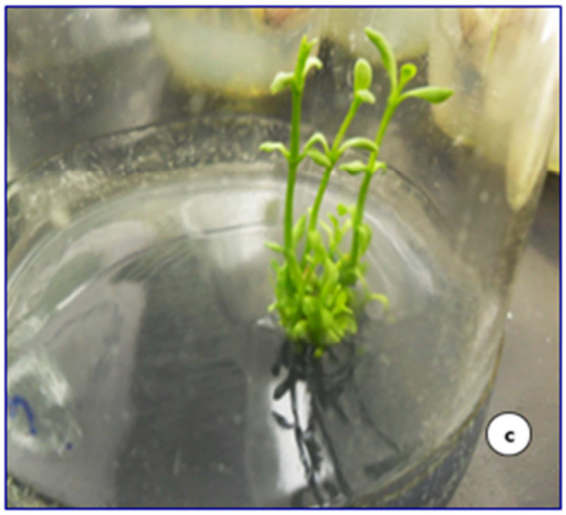
Effect of IAA and BAP combination

In the present study the effect of BAP and IAA combination on *in vitro* shoot proliferation and elongation was analyzed in shoot tip explants of *B. monnieri*. For this, BAP (0.5- 6.0 mg/L) and IAA (0.5 mg/ L) concentrations were tested in combination (Table 2).

a) Formation of multiple shoots on MS+TDZ (3.0 mg/L) after two weeks



b) Proliferation of multiple shoots on MS+BAP (3.0 mg/L) after six weeks



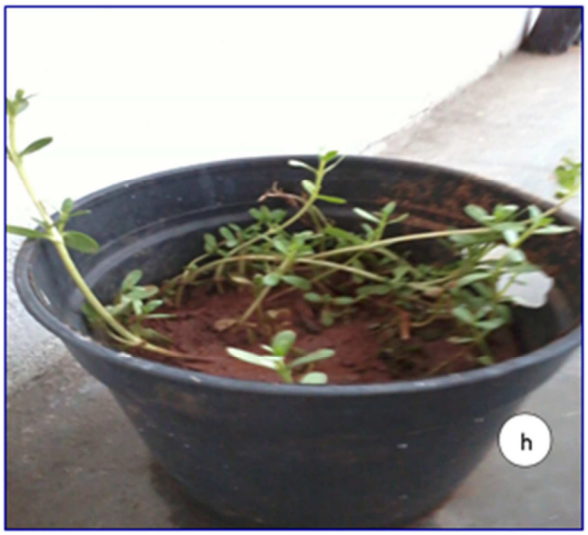
c) Initiation of shoots on MS+KN (3.0 mg/L)

d) induced multiple shoots in culture bottle on MS+IAA (0.5 mg/L) BAP (3.0 mg/L) after six weeks

e) ***Proliferation of multiple shoots in many culture bottles on MS+IAA (0.5 mg/L) BAP (3.0 mg/L) after six weeks***

f) ***Measurements of lengths of In vitro shoots from in vitro shoots formation on MS+IAA (0.5 mg/L) BAP (3.0 mg/L);***

g) ***Rooting of shoots MS+IBA (1.0 mg/L) after six weeks***



h) *Hardening of plantlets.*

Figure 1
(a) to (h):- In vitro Micropropagation of B. monnieri from shoot tip culture

Whereas with the addition of IAA + BAP induced maximum number of shoots/ explants was developed on MS medium (0.5 mg/L) IAA + BAP (2.0 mg/L) resulted (15.0 ± 0.04 Shoots/ explant) with (2.6 ± 0.03) length of shoots. Highest number of (24.0 ± 0.04 Shoots/ explant) (Figure 1d & e) with 100 Percentage response was observed at (0.5 mg/L) IAA+ (3.0 mg/L) BAP. The percentage of response increased up to (3.0 mg/L) BAP and later gradually decreased at high concentrations. Low frequency of shoots (3.0 + 0.04) was induced per explant at (6.0 mg/L) BAP with less percentage (60) were responded and the induction of ability was decreased as the concentration of BAP increased. An average of (24.0 ± 0.04 Shoots/ explant) shoots/explant with average shoot length of (4.0 ± 0.02 cm/explant) was recorded in this optimal medium (Table 2, Figure1f).

In vitro rooting

Fully elongated healthy shoots were transferred on to MS root induction medium (RIM) [11] fortified with different concentration of IAA (0.5 – 2.0 mg/L) and IBA (0.5 - 2.0 mg/L). Profuse rhizogenesis was observed on (1.0 mg/L IAA, compared to (0.5 - 2.0 mg/L) IAA/ IBA on MS medium containing (1.0 mg/L) IBA whereas 73% of plants produced roots with (8.3 ± 0.04 roots/ explant) (Table 3) (Figure 1g & h). Rooted plantlets were removed from the culture medium and the roots were washed under running tap water to remove agar. Then the plantlets were transferred to polypots containing pre-soaked vermiculite and maintained inside a growth chamber set at 28^{o}C and 70 - 80 % relative humidity. After three weeks they were transplanted to poly bags containing mixture of Soil + Sand + Manure in 1:1:1 ratio and kept under shade house for a period of three weeks. The potted plantlets were irrigated with Hogland's solution every 3 days for a period of 3 weeks. The result of present investigation show that the shoot tip explants from mature plants of *B. monnieri* could be induced to produce multiple shoots *In vitro.* Maximum number of shoots was induced on MS medium fortified with various concentrations of BAP, Kn and TDZ (0.5 - 6.0 mg/L) alone either IAA (0.5 mg/L) in combination with TDZ, Kn and BAP (05-6.0 mg/L) in this study IAA (0.5 mg/L) in combination with BAP (3.0 mg/L) is the most efficient phytohormones in promoting adventitious shoot formation in many plants[12]. BAP was superior to BAP in inducing high frequency shoot regeneration in many numbers of plants[13-17]. Combination of auxin and cytokinin favored shoot bud differentiation in many plants[18;19.] BAP was superior to KN in inducing high frequency shoot regeneration in many numbers of plants [13,16,17.] Combination of auxin and cytokinin favored shoot bud differentiation in many plants [18, 19]. These results are also in agreement with those in *Tectona grandis*[20] *Abizzialeb beck*[21] multiple shoot induction was also observed in *Ziziphus manritiana*[22], and *Vanilla plantifolia*[23] shoot tips cultured on MS + cytokinin alone as it was observed in the present studies. Clonal propagation of Mulberry plants through *in vitro* techniques[24] and *Solanum torvum*[24] have studied the effect of different cytokininsviz. BAP, KN, 2-ip and Zeatin on multiple shoot induction from shoot tip culture in mulberry. According to their observation, BAP and KN were superior to 2-ip and Zeatin. The superiority of BAP over other cytokinins for multiple shoot formation has been reported as it was observed in the present investigations in Mulberry 24.

CONCLUSION

Here we have successfully developed an efficient plant regeneration protocol for *B. monnieri* using shoot tip culture and *in vitro* rooting. The regenerated plants will be useful for constant supply of uniform raw materials for commercial secondary metabolite extraction. This will reduce the pressure on natural population of this valuable medicinal plant species and thus be indirectly useful for conservation of this plant species.

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AUTHOR CONTRIBUTION STATEMENT

Authors hereby declare that the corresponding author had designed and performed the experiments, analysed the data and manuscript writing. Co-authors have reviewed the experimental work and manuscript

CONFLICT OF INTEREST

Conflict of interest declared none

REFERENCES

1. Kapil SS, Sharma V. *In-Vitro* Propagation of *Bacopa monneri*: An Important Medicinal Plant. Int j curr biotechnol. 2014 Jan;2(1):7-10.
2. Anonymous. Indian Medicinal Plants: A Sector Study, Occasional Export-Import Bank of India. Quest Publication, Bombay, India).1997 Jan paper No 54. Available from: http://www.academia .edu/7484635/Floriculture_A_Sector_Study_EXP ORTIMPORT_BANK_OF_INDIA
3. Bhattacharya SK, Ghosal S. Anxiolytic activity of a standardized extract of *Bacopa monniera*: an experimental study. Phytomed. 1998 Apr 1;5(2):77-82.
4. Shankar G, Singh HK. Anxiolytic profile of standardized Brahmi extract. Indian j pharmacol. 2000;32(152):5.
5. Williams R, Münch G, Gyengesi E, Bennett L. *Bacopa monnieri* (L.) exerts anti-inflammatory effects on cells of the innate immune system *in vitro*. Food & Function. 2014;5(3):517-20.
6. Afjalus SM, Chakma N, Rahman M, Salahuddin M, Kumar SS. Assessment of Analgesic, Antidiarrhoeal and Cytotoxic activity of ethanolic extract of the whole plant of *Bacopa monnieri* Linn. Int res j pharm. 2012;3(10):98-101.
7. Bammidi SR, Volluri SS, Chippada SC, Avanigadda S, Vangalapati M. A Review on pharmacological studies of *Bacopa monniera*. J. Chemical, Biol. Phys. Sci. 2011;1(2):250
8. Srivastava S, Mishra N, Misra U. A Review on *Bacopa monniera* -a Future Perspective. Int. J. Pharm. Sci. Drug Res. 2009;1(3):154-7.
9. Subashri B, Pillai YJK. *In Vitro* Regeneration of *Bacopa Monnieri* (L.) Pennel.-A Multipurpose Medicinal Plant. Int. J. Pharm. Pharm. Sci. 2014;6(4):559-63. Available from; http://www.ijppsjournal.com/Vol6Issue4/9255.p df
10. Mohapatra HP, Rath SP. *In vitro* studies of *Bacopa monnieri*- an important medicinal plant with reference to its biochemical variations. Indian J Exp Biol. 2005 Dec:43(4):373-6. Available from: https://www.ncbi.nlm.nih.gov/pubmed/15875724
11. Murashig T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 1962;15(3:)473-97.
12. Pierik RLM. "*In vitro* culture of higher plants". Martinus night off, Dordrecht. Springer 1962 pp. 183-230.
13. Devendra BN, Srinivas N, Sandeep Reddy A. High frequency somatic embryogenesis and plant regeneration in nodal explants culture of *Eclipta alba* L. Hassk. Ann boil res. 2011;2(3):143-9.
14. Johnson M, Manickam VS. In vitro micropropagation of *Baliospermum montanum* (Willd) Muell – Arg- a Medcinal Plant. Indian J. Exp. Biol. 2003;41(11):1349-51. Available from: http://nopr.niscair.res.in/bitstream/123456789/17202/1/IJEB%2041(11)%201349-1351.pdf
15. Johnson M, Sonalidas, Yasmin N, Rajasekarapandiam M. Micropropagation studies on *Vitex negundo* L. A medicinally important Plant. Ethnobot Leaflet. 2008;12:1-5.
16. Sudha CG, Krishnan PN, Pushpangadan, Seeni S. In vitro propagation of *Decalepis arayalpathra*, a critically endangered ethnomedicinal plant. *In Vitro* Cell Dev. Biology-Plant. 2005;41(5):648-54.
17. SanjayaRathore, TS, RavishankarRai, V. Micropropagation of *Pseudoxytenanthera stocksii* Munro. *In vitro* Cell. Dev. Biology–Plant. 2005;41(3):333-7.
18. Gupta, SC, Handra N. Control of organogenesis in culture of leaf, multilayerd strips and segments of stem and root in *Solanum surattense* Burm. Indian j. Exp. Biol. 1982;20:126-31. Available from :http://agris.fao.org/agris-search/search.do?recordID=US201301988383
19. Gharyl PK, Maheswari SC. In vitro differentiation pantlets, from tissues culture of *Albizzia lebbeck* L. Plant cell tissue org.cult. 1982;2(1):49-53. Availablefrom:https://link.springer.com/article/10.1007/BF00033552#citeas
20. Sudhershan L, Aboel MN, Hussian J. In Vitro propagation of *Ziziphus mauritiana* cultivar Umran by shoots tip and nodal multiplication. Curr. Sci. 2001Jan;80(2):290-2.
21. Geetha S, Shetty SA.In vitro Propagation of *Vanilla Planifolia*- a tropical orchid. Curr. Sci. 2000 Jan;79(6):886-9.
22. Zaman A, Islam R, Joarder OI Barman AC. Clonal propagation of Mulberry plants through *in vitro* techniques. In: plant tissue cultures (Ed.) A.S.Islam Oxford and BH Publishing Co., pp 1996;71-5.
23. Ugandhar T. *In Vitro* Micropropagation studies on shoot tip explants of *Solanum torvum*-An Important medicinal plant. Int. J. Sci. Res. 2017;6(1):697-701.
24. Hasan, SMZ PCM. Jasen. *Solanum* L.. In J.S. Siemonsma and K. Piluek, eds. Prosea: Plant Resources of South-East Asia No 8 Vegetables. Prosea Foundation, Bogor, Indonesia. pp. 1994; 249-52.