Asian Journal of Agriculture & Life Sciences Vol. 3(1), January 2018: 12-18

Website: www.crsdindia.com/ajals.html e-ISSN: 2455-6149

ORIGINAL ARTICLE

In vitro Regeneration of *Tylophora indica* (Burm.f.) Merr. from Nodal Explants

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Received: 11th Oct. 2017, Revised: 28th Oct. 2017, Accepted: 2nd Nov. 2017

ABSTRACT

Antamul is a commercially important high value medicinal plant. Due to habitat lost, slow growth and over exploitation for medicinal industry, its conservation strategy is very important. This paper is an attempt to develop an in vitro technique for conservation and large scale production of this plant. For the purpose two media (MS medium and B_5 medium) were tested supplemented with various concentrations and combinations of four Plant Growth Regulators- Kinetin, 6-Benzyle Adenine (BA), Naphthalene Acetic Acid (NAA) and Indol-3-butyric Acid (IBA). Nodes with axillary bud were selected as explants. Best regeneration was obtained in MS medium supplemented with 2.0+0.5 mg/l of BA plus IBA on an average 8.33 number of shoots of 7.37 cm length in 120 days. Roots developed with good growth in ½ strength MS medium with 1.0 mg/l of IBA in 15 days.

***Key words**: Regeneration, MS medium, B_5 medium, kinetin, 6-Benzyle Adenine (BA), Indol-3-butyric Acid (IBA), Naphthalene Acetic Acid (NAA)*

INTRODUCTION

Tylophora indica (Burm.f.) Merr. syn. *T. asthmatica* Wt. & Arn. (common name Antamul) is a twining plant of family Ascelpiadeaceae. Leaves in opposite pairs, ovat with pointed tip. Flowers large, dull yellow purple within, in short clusters. Plant is with many, long, fleshy roots.

The dried roots of the plant constitute the drug, which is a good substitute for Ipecac, a well known drug. It is useful drug of dysentery and an infusion of the drug is given in asthma and bronchitis to bring about vomiting and give relief (Jain1968). This plant is one of threatened plant reported from Assam and for conservation purpose their cultivation is recommended in Assam (Jain1968). In addition to that *Tylophora indica* has a great market potential as substitute of *Cephaelis ipecacuanha*. For successful tissue culture, type of explants is an important aspect. Different plant organs like seed, seedling parts, axillary and apical meristem, leaf etc. are used for the purpose. Many medicinal plants are propagated through *in vitro* organogenesis from nodal axillary bud meristem eg. *Withania somnifera* (Sen & Sharma 1991), *Simmondsia chinensis* (Sardana & Batra 1998), *Rauvolfia serpentina* (Deka, *et al.*, 2000), *Adenophylla triphylla* (Chen, *et al.*, 2001), *Bacopa monnieri* (L.) Penn. (Devi & Sarma 2004; Devi & Sarma 2009; Devi & Sarma 2013; Devi 2017). Attempt of this paper is to get fruitful regenerative media from node with axillary bud explants culture in MS and B_5 media supplemented with various combination and concentration of kinetin, BA, NAA and IBA are presented.

MATERIAL AND METHOD

Two media, Murashige & Skoog medium (1962) and Gamborg B_5 medium (1968) were supplemented with following combinations and concentrations of plant growth regulators and 100mg/ml of CH-

a. BA (0.1, 0.5, 1.0, 1.5, 2.0, 2.5 mg/l) + IBA (0.1, 0.5 mg/l)
b. Kinetin (0.5, 1.0, 1.5, 2.0, 2.5 mg/l) + NAA(0.1,0.5,1.0,2.0,3.0,4.0 mg/l)
c. Kinetin (0.5, 1.0, 1.5, 2.0, 2.5 mg/l) + NAA (0.5, 1.0 mg/l) + IBA (0.1, 0.5 mg/l)

1cm long nodes with axillary bud were used for the purpose. Materials were first cleaned under tap water. Then surface sterilized first with 70 per cent alcohol followed by 0.01 per cent $HgCl_2$ solution. Single explant, one in each flask was inoculated on sterilized media under aseptic

condition. The pH of media was adjusted to 5.7 and media were sterilized under 16 lb pressure at 121°C. Culture tubes were incubated at the temperature of 25 ±1°C under 12hr. photoperiods /day (2500-3000 lux). During the whole procedure aseptic condition was maintained. The three repetitions were done and mean and standard error calculated by standard formula.

RESULTS AND DISCUSSION

The experiments have shown that the node with axillary buds of *Tylophora* was successful explant in both MS and B_5 media for regeneration in both the media at higher cytokinin concentrations, but B_5 proved to be better as callusing medium.

Table 1: Variation in development from nodal explants of *Tylophora indica* (Burm.f.) Merr in MS and B_5 media with different concentrations of BA plus IBA

PGRs (mg l^{-1})		Observation after 120 days					
		Node with axillary bud explant					
		MS medium			B_5 medium		
BA	IBA	Callus	Shoot	Root	Callus	Shoot	Root
0.1	0.1	-	-	-	-	-	-
0.5	0.1	-	-	-	-	-	-
0.5	0.5	-	+	-	-	+	-
1.0	0.1	-	+	-	-	+	-
1.0	0.5	-	+	-	-	+	-
1.5	0.1	-	+	-	-	-	-
1.5	0.5	-	+++	+	-	++	+
2.0	0.1	+++	+	-	+++	+	-
2.0	0.5	-	+++	++	-	++	+++
2.5	0.5	+++	+	-	+++	-	-

Table 2: Variation in development from nodal explants of *Tylophora indica* (Burm.f.) Merr in MS and B_5 media with different combinations and concentrations of Kinetin + NAA + IBA

PGRs (mg l^{-1})			Observation after 120 days					
			Node with axillary bud explant					
			MS medium			B_5 medium		
Kn	NAA	IBA	Callus	Shoot	Root	Callus	Shoot	Root
0.5	0.1	-	-	-	-	-	-	-
0.5	0.5	-	+	-	-	+	-	-
0.5	1.0	-	+	-	-	+	-	-
0.5	2.0	-	++	-	-	++	-	-
0.5	3.0	-	++	-	-	++	-	-
0.5	4.0	-	+++	-	-	+++	-	-
1.0	0.1	-	-	-	-	-	-	-
1.0	0.5	-	+	+	-	+	+	-
1.5	0.1	-	+	+	-	+	+	-
1.5	0.5	-	++	++	-	++	++	-
2.0	0.1	-	-	-	-	-	-	-
2.0	0.5	-	+++	-	-	+++	-	-
2.5	0.1	-	-	-	-	-	-	-
2.5	0.5	-	+++	+	-	+++	-	-
0.5	0.1	0.1	-	+	-	-	+	-
1.5	0.5	0.1	+++	+	-	+++	+	-
1.5	0.5	0.5	+++	+	-	+++	+	-
2.0	0.5	0.1	+++	-	-	+++	-	-
2.0	0.1	0.5	-	++	+++	-	++	-
2.0	0.5	0.5	+++	-	-	+++	-	-
2.5	0.5	0.1	+++	++	-	+++	++	-
2.5	0.5	0.5	+++	++	-	+++	-	-

In lower concentrations of BA plus IBA explants did not survive long in any of the tried media. With increase in concentration the regeneration was noted (Table 1). Similarly, in both MS and B_5 media

supplemented with lower concentrations of kinetin (0.1, 0.5 mg/l) along with lower concentration of NAA (0.1 mg/l) nodal explants were not generative and explants died within 30 days. These findings are similar as reported in *Clerodendrum colebrookianum* (L.) Walp (Devi & Sarma 2009) where explants died in lower concentration of cytokinin.

Table 3: Mean number of shoots, shoot length, number of roots and root length regenerated from nodal explants of *Tylophora indica* (Burm.f.) Merr in two media with different PGRs (Mean of 3±SE)

PGRs (mg l^{-1})				Mean no. of shoots and root per explants and Mean length of regenerated shoots and roots in cm							
				Observation after 120 days							
				Node with axillary bud explant							
				MS medium				B_5 medium			
BA	Kn	NAA	IBA	No. of Shoot	Shoot length	No. of root	Root length	No.of Shoot	Shoot length	No. of root	Root length
0.5	-	-	0.5	1.33 ± 0.33	6.60 ± 0.20	-	-	1.00 ± 0.00	5.97 ± 0.17	-	-
1.0	-	-	0.1	1.33 ± 0.33	3.27 ± 0.14	-	-	1.00 ± 0.57	3.47 ± 0.88	-	-
1.0	-	-	0.5	1.67 ± 0.33	7.37 ± 0.24	-	-	1.67 ± 0.33	6.17 ± 0.13	-	-
1.5	-	-	0.1	0.33 ± 0.33	3.53 ± 0.31	-	-	0.00 ± 0.00	0.00 ± 0.00	-	-
1.5	-	-	0.5	5.00 ± 0.57	4.90 ± 0.23	3.33 ± 0.21	6.33± 0.27.	2.33 ± 0.33	4.73 ± 0.14	-	-
2.0	-	-	0.1	1.03 ± 0.57.	3.03 ± 0.26	-	-	1.00 ± 0.00	3.37 ± 0.12	-	-
2.0	-	-	0.5	8.33 ± 0.33.	7.37 ± 0.12	6.00 ± 0.24.	7.10 ± 0.21.	3.67 ± 0.33	5.60 ± 0.15	-	-
2.5	-	-	0.5	0.67 ± 0.33	2.90 ± 0.11	-	-	0.00 ± 0.00	0.00 ± 0.00	-	-
-	1.0	0.5	-	1.00 ± 0.00	10.20 ± 0.21	-	-	1.33 ± 0.33	7.37 ± 0.29	-	-
-	1.5	0.1	-	1.00 ± 0.00	9.33 ± 0.17	-	-	1.67 ± 0.33	5.37 ± 0.14	-	-
-	1.5	0.5	-	3.67 ± 0.66	10.00 ± 0.15	-	-	3.33 ± 0.33	9.01 ± 0.11	-	-
-	2.5	0.5	-	0.67 ± 0.33	4.57 ± 0.53	-	-	0.00 ± 0.00	0.00 ± 0.00	-	-
-	0.5	0.1	0.1	1.33 ± 0.33	10.57 ± 0.17	-	-	2.33 ± 0.33	8.67 ± 0.23	-	-
-	1.5	0.5	0.1	1.33 ± 0.33	4.47 ± 0.09	-	-	1.33 ± 0.33	4.17 ± 0.09	-	-
-	1.5	0.5	0.5	1.33 ± 0.33	4.20 ± 0.15	-	-	3.33 ± 0.33	4.00 ± 0.15	-	-
-	2.0	0.1	0.5	1.33 ± 0.33	6.57 ± 0.31	10.40± 0.13	11.67± 0.21	2.00 ± 0.57	5.37 ± 0.18	-	-
-	2.5	0.5	0.1	3.67 ± 0.66	4.00 ± 0.11	-	-	3.33 ± 0.33	3.30 ± 0.11	-	-
-	2.5	0.5	0.5	2.67 ± 0.33	3.13 ± 0.23	-	-	0.00 ± 0.00	0.00 ± 0.00	-	-

(Mean of three repetitions taken and Standard Error calculated)

Table 4: Rooting of regenerated plantlets of *Tylophora indica* (Burm.f.) Merr in ½ strength MS medium

MS + PGRs (mg/l)			Observation after 15 days	No. of roots per plantlet	Root length (cm.)
Kn	NAA	IBA			
2.0	0.1	0.5	Good rooting	11.00 ± 0.54	9.67 ± 0.17
-	-	0.1	Few roots come out	2.33 ± 0.15	2.27 ± 0.07
-	-	0.5	Good roots at base	5.33 ± 0.41	2.63 ± 0.05
-	-	1.0	Good roots at base	8.00 ± 0.27	4.77 ± 0.09
-	0.1	-	Small callus at base	-	-
-	0.5	-	Small callus at base	-	-
-	1.0	-	Branched roots	-	-

In media supplemented with PGRs, BA and IBA direct regeneration from axillary bud of explants took place in four different combinations tried. On an average 5.00 number of rootless shoots of 4.90cm length were regenerated in the medium supplemented with 1.5mg/l BA plus 0.5mg/l IBA in MS medium. In B_5 medium the same concentration and combination of PGRs showed 2.33 numbers of shoots of in an average 4.73cm length. In the medium with 2.0+0.5mg/l of BA plus IBA on an average 8.33 number of shoots of 7.37 cm length were generated in MS medium and in an average 3.67 number of shoot of 5.60 generated in B_5 medium (Table 3). In 1.5 mg/l of Kinetin and 0.5mg/l of NAA in an average 3.67 shoots of 10.00cm length were obtained without roots in MS medium. In B_5 medium results were similar but quality of regenerated shoot was little inferior (Table 3). If the

results of MS and B_5 media are compared in terms of multiple shoots regenerated (Fig 1), then MS appears to be better medium then B_5. Again in terms of length of regenerated shoots, superior results have been observed in MS medium in comparison to B_5 medium (Fig 2). Similar reports of superiority of MS medium over B_5 medium have been reported in *Beta* sp. (Detrez *et al.* 1988), sweet potato (Bordoloi & Sarma 1997), Indian spinach (Mitra & Mukharjee 2001), *Clerodendrum colebrookianum* (L.) Walp (Devi & Sarma 2009), *Bacopa monnieri* (Devi 2017).

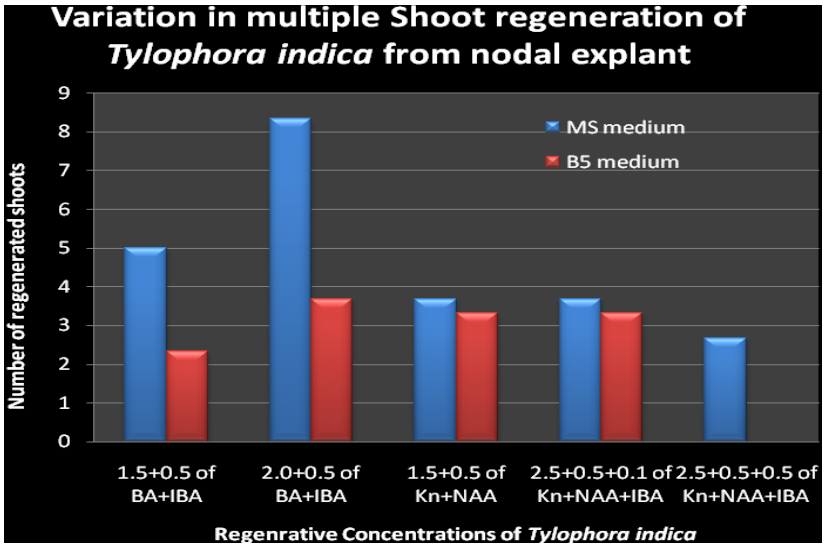

Fig. 1: Graphical comparison of multiple shoot regeneration from nodal explants in two media

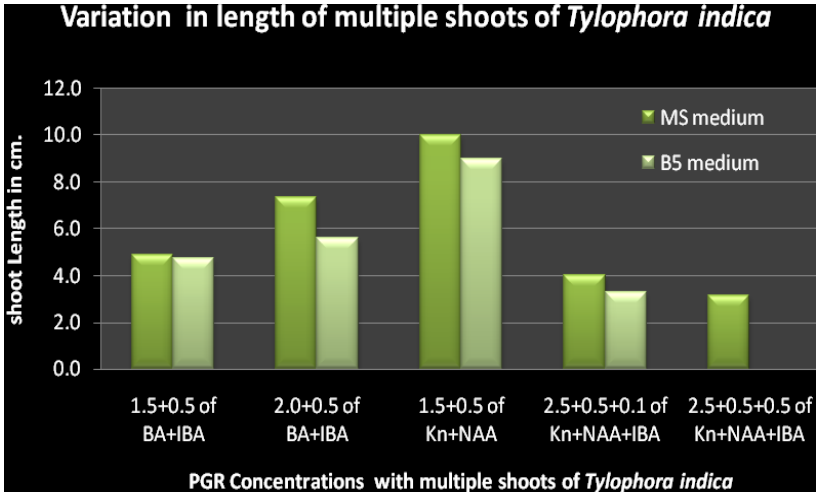

Fig. 2: Graphical comparison of length of multiple shoots regenerated in two media

Tiwari, *et al.* (2001) reported that number of adventitious shoot buds per explant increased up to certain concentrations then decreased in presence of kinetin. Kinetin (0.5mg/l) plus higher concentrations of NAA (1.0, 2.0, 3.0, 4.0mg/l) gave good callus from the whole explants in both the media (Table 2). In *Tylophora* too number of regenerated shoots increased gradually with increase in kinetin concentration then decreased. Higher concentrations of kinetin (1.0, 1.5, 2.0 mg/l) plus 0.5 g/ml NAA induced direct regeneration from bud and callus from the explant. Further increase in kinetin 2.5mg/l with 0.5 mg/l of NAA resulted in good callusing. Shoot regeneration decreased (Table 3). But same concentrations of kinetin with 0.1 mg/l of NAA were not fruitful as the explants died shortly. The ratio of cytokinin to auxin is a critical determinant of organogenesis in plant tissue culture (Xu, *et al*., 2008). In the present study too proportion of auxin-cytokinin in the media is vital in

determining the fate of the culture. The observations are in line of the findings of many different workers (Sankhla, *et al.*, 1995; Rao, *et al.*, 2003; Pawar, *et al.*, 2004; Devi & Sarma 2009; Devi 2017).

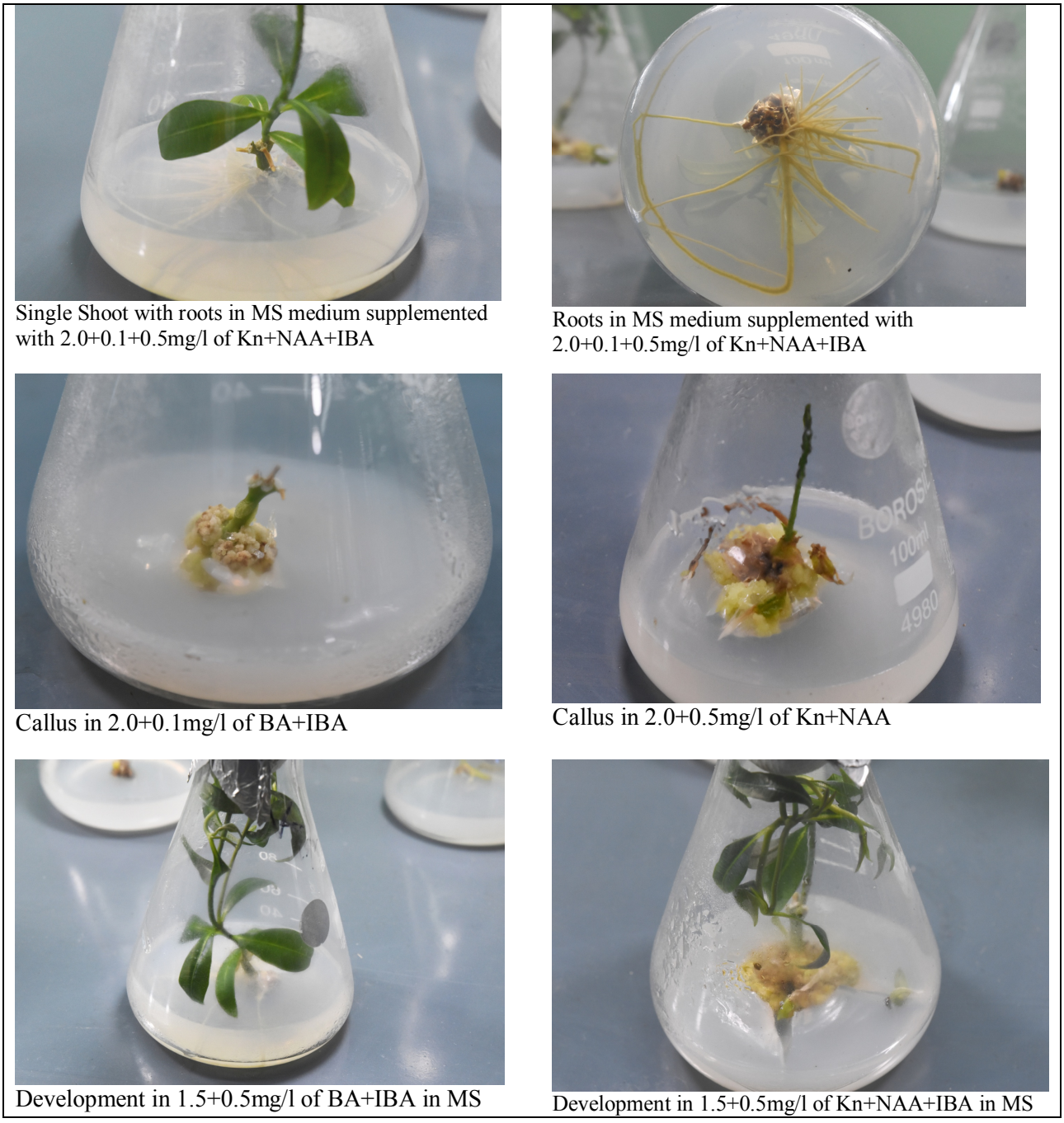

Fig. 3: In vitro culture of *Tylophora indica* (Burm.f.) Merr

Su, *et al.* (2011) found that a low concentration of auxin combined with cytokinin aid in shoot initiation. Lower concentration of auxin in the culture media nullifies the effect of the higher concentration of cytokinin on axillary shoot elongation (Hu & Wang 1983). A lower concentration of auxin along with a higher concentration of cytokinins induced multiplication of shoots in *W. somnifera* (Fatima & Anis 2012). Auxin exerts an effect on DNA replication, while cytokinin seems to exert some control over the events leading to mitosis (Pasternak, *et al.*, 2000). Therefore, auxins might be considered as "inducers" of the all cycle (Fatima & Anis 2012) while cytokinins might behave more as its "promoter" (Wood, *et al.*, 1990). The presence of axillary bud, the high

concentration of cytokinin and lower ratio of auxin in the media might have induced formation of more shoot primordial instead of growth of single axillary bud in node with axillary bud explants and the xylem transport of the cytokinin helps in quick transport of PGRs in the explants to get quicker results.

Some workers reported that auxin-cytokinin ratio may be responsible for ethylene to methane level in culture media (Sankhla, *et al.*, 1995; Rao, *et al.*, 2003). Increase in ethylene production resulted in the development of light green callus with further decrease in shoot induction (Rao, *et al.*, 2003). There are reports of *in vitro* culture of pigeon pea cotyledonary nodes, where ethylene-methane ratio more than 1.0 resulted in development of elongated shoots, near 1.0 resulted in multiple shoots and gradual reduction resulted in regeneration capacity of callus (Rao, *et al.*, 2003). This may be true for *Tylophora* too, where the same concentration of cytokinin produced multiple shoot with higher amount of auxin (2.0+0.5mg/l of BA plus IBA, 1.5 + 0.5 mg/l of Kinetin plus NAA) but with lower amount of auxin (2.0+0.1mg/l of BA plus IBA, 1.5 + 0.1 mg/l of Kinetin plus NAA) explants died. Further change in ratio again resulted in callusing only.

On addition of IBA along with kinetin and NAA slight variation in results were seen. Callus at base and shoots developed in 2.0+0.5+0.1mg/l (3shoots), 2.5+0.5+0.1mg/l (3-4 shoots) in MS medium but length of shoot decreased in 2.5+0.5+0.1mg/l. Results are similar in B_5 medium but the number of shoot decreased and shoot length was also less as growth of shoot was slow (Table 3).

In all other regenerative concentration only the axillary buds developed into one or two shoots. No callus but single shoot with good roots developed at the base of explants with 1.5+0.1mg/l, 2.0+0.1mg/l of BA plus IBA and with kinetin plus NAA plus IBA in concentrations 2.0+0.1+0.5mg/l in MS medium only. No roots found in B_5 medium with these PGR combinations tried.

Rooting is a problem for regenerated shoots of *Tylophora indica* (Burm.f.) Merr. The media supplemented with 2.0mg/l of kinetin, 0.1mg/l of NAA and 0.5mg/l of IBA resulted in single shoot with good roots (Table 3). In addition to that different concentrations of IBA (0.1, 0.5, 1.0 mg/l) and NAA (0.1, 0.5 mg/l) were tried in ½ strength MS medium. B_5 medium was not tried as rooting was not favourable in any of the concentrations tried for regeneration. In ½ strength MS medium also the combination of PGR 2.0mg/l of kinetin, 0.1mg/l of NAA and 0.5mg/l of IBA showed best results (Table 4).

CONCLUSION

In vitro culture of *Tylophora indica* (Burm.f.) Merr node with axillary bud is a fruitful explant in both the media tried with different PGRs. Of the different concentration and combination tried, the best regeneration was obtained in MS medium supplemented with 2.0+0.5 mg/l of BA plus IBA on an average 8.33 number of shoots of 7.37 cm length followed by 2.0+0.5+0.1mg/l (3shoots), 2.5+0.5+0.1mg/l (3-4 shoots) of Kinetin plus NAA plus IBA. Roots developed with good growth in MS medium with 2.0+0.1+0.5mg/l (3-4 shoots) of Kinetin plus NAA plus IBA or 1.5+0.1 mg/l and 2.0+0.1 mg/l of BA and IBA or ½ strength MS medium with 1.0 mg/l of IBA.

ACKNOWLEDGEMENT

Grateful thanks are due to the University Grant Commission, New Delhi, India for funding this work under their Minor Research Project scheme.

REFERENCES

1. Bordoloi N.D. and Sarma C.M. (1997): *In vitro* regeneration of sweet potato and their establishment under natural environment. Neo Botanica. 5(1&2):11-14.
2. Chen C.C., Chen S.J., Sagare A.P. and Tsay H.S. (2001): Adventitious shoot regeneration from stem internode explants of *Adenophora triphylla* (Thunb.) A. DC. (Campanulaceae)-an important medicinal herb. Bot. Bull. Acad. Sinica 42:1-7.
3. Deka A.C., Talukdar A., Chakraborty M. and Kalita M.C. (2000): *In vitro* clonal propagation and tuber formation of *Rauvolfia serpentina* L. Benth. Ex Kurz. J. Phytol. Res., 13(2):135-138.
4. Detrez C., Tetu T., Sangwan R.S. and Sangwan B.S. (1988): Direct organogenesis from petiole 5 and thin cell layer explants in sugarbeet cultured *in vitro*. J. Exp. Bot., 39(204): 997-926.
5. Devi P. (2017): Micropropagation of Brahmi for large scale production and conservation in BA and IBA supplemented media. Asian Journal of Agriculture & Life Sciences, 2(4): 9-14.
6. Devi P. and Sarma C.M. (2004): Effect of kinetin, NAA & IBA on regeneration of *Bacopa monnieri* (L.) Penn. Environmental Biology and Conservation, 9: 67-72.

7. Devi P. and Sarma C.M. (2009): Micropropagation of *Clerodendrum coleobrookianum* (L) Walp from nodal explants. Advanced Plant Sciences, 4 (3&4): 62-65.

8. Devi P. and Sarma C.M. (2013): Effect of kinetin, NAA & IBA on regeneration of *Costus speciosus* (Koen. Ex Retz.) Sm from aerial axillary bud explants. Advanced Plant Sciences, 6(3&4): 32-37.

9. Fatima N. and Anis Md. (2012): Role of growth regulators on *in vitro* regeneration and histological analysis in Indian ginseng (*Withania somnifera* L.) Dunal. Physiol Mol Biol Plants, 18(1): 59–67. Published online 2011 Dec 30. doi: 10.1007/s12298-011-0099-x. PMCID: PMC3550525.

10. Gamborg O.L., Miller R.A. and Ojima K. (1968): Nutrient requirement of suspension culture of soybean root cells. Exp. Cell Res., 50: 148-151.

11. Hu C.Y. and Wang P.J. (1983): Meristem, shoot tip, and bud cultures. In: Evans DA, Sharp W.R., Ammirato P.V. and Yamada Y. (eds.). Handbook of Plant Cell Culture: Techniques for Propagation and Breeding. McMillan Publishing Co., New York, 1: 177-227.

12. Jain S.K. (1968): Medicinal Plants. National Book Trust, India. (3rd edition 1996).

13. Mitra S.K. and Mukherjee K.K. (2001): Direct organogenesis in Indian Spinach. Plant Cell Tiss. Org. Cult., 67:191-194.

14. Murashige T. and Skoog F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant, 15: 473-497.

15. Pasternak T., Miskolczi P., Ayaydin F., Meszaros T., Dudits D. and Feher A. (2000): Exogenous auxin and cytokinin dependent activation of CDKs and cell division in leaf protoplast-derived cells of alfalfa. Plant Growth Regul., 32: 129–141.

16. Pawar S.V., Patil S.C., Mehetre S.S. and Jambhale V.M. (2004): Micropropagation studies in Kartoli (*Momordica dioica* Roxb.). Ad. Plant Sci., 17(1): 275-278.

17. Rao G.V.S., Khetarpal S., Chandra R., Raj A., Kanth S. and Polisetty R. (2003): Interactive effect of photoperiod and plant growth regulators on *in vitro* regeneration from cotyledonary node explants of pigeonpea (*Cajanus cajan* (L.) MillSP.). Indian J. Plant Physiol., (Special Issue): 689-692.

18. Sankhla D., Sankhla N. and Davis T.D. (1995): Promotion of *in vitro* shoot formation from excised roots of silk tree by an oxime ether derivative and other ethylene inhibitors. Plant Cell Rep., 15: 143-146.

19. Sardana J. and Batra A. (1998): *In vitro* regeneration of Jojoba (*Simmondsia chinensis*): A plant of high potential. Adv. Plant Sci., 11(1): 143-146.

20. Sen J. and Sharma A.K. (1991): Micropropagation of *Withania somnifera* from germinating seeds and shoot tips. Plant Cell Tiss. Org. Cult., 26: 71-73.

21. Su Y.H., Liu Y.B. and Zhang X.S. (2011): Auxin-cytokinin interaction regulates meristem development. Mol Plant, 4(4): 616–625.

22. Tiwari V., Tiwari K.N. and Singh B.D. (2001): Comparative study of Cytokinin on in vitro propagation of *Bacopa monniera*. Plant Cell, Tissue and organ Culture, 66:9-16.

23. Wood H.N., Sterner R., Alves L.M. and Basile D.V. (1990): Auxin-phorbol ester: an example of two stage-stage initiation promotion system mediating cell proliferation in plants. Vitro Cell Dev Biol Plant, 26:1125–1127.

24. Xu Z., Um Y.C., Kim C.H., Lu G., Guo D.P., Liu H.L., Bah A.A. and Mao A. (2008): Effect of plant growth regulators, temperature and sucrose on shoot proliferation from the stem disc of Chinese jiaotou (*Allium chinense*) and in vitro bulblet formation. Acta Physiol Plant, 30(4): 521–528.