ISSN 0975-6299 Vol 2/Issue 1/ Jan-Mar 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

BIOTECHNOLOGY

HIGH FREQUENCY *IN VITRO* RHIZOGENESIS IN *BRYONOPSIS LACINIOSA* (L.) NAUD. A HIGHLY VALUABLE MEDICINAL CUCURBIT.

Corresponding Author

V.J.E CAROLINE

Plant Biotechnology Research Laboratory, Dept.of Botany, Kakatiya University, Warangal - 506009, A.P., India

Co Author

B.MALLAIAH

Plant Biotechnology Research Laboratory, Dept.of Botany, Kakatiya University, Warangal - 506009, A.P., India

ABSTRACT

Bryonopsis laciniosa (L.) Naud. is a hjghly valuable medicinal plant belongs to the family Cucurbitaceae. It is used as an operient medicine and tonic. The objective of the present study was to achieve high frequency *in – vitro* rooting from explants like leaf,stem,nodal and cotyledon, explants derived calli and regenerated shoots on MS medium. Among the explants tested leaf and cotyledon cultures showed high frequency rooting on MS medium augmented with 2.0 mg/l BAP + 2.0 mg/l NAA and MS + 2.0 mg/l BAP + 2.0 mg/l IBA. The auxins2,4-D, NAA and IBA are used for rooting but NAA had found as the most effective hormone compared to IBA on root induction and elongation.

KEYWORDS

Bryonopsis laciniosa, Goniothalamin , High frequency rhizongenesis, NAAa – naphthalene acetic acid, IBA indole – 3 – butyric acid, BAP 6- benzylaminopurine.

INTRODUCTION

Bryonopsis laciniosa is a highly valuable medicinal cucurbit commonly known as lollipop climber and it is called as "Shivlingi" in India. It is widely distributed through out the world. Goniothalamin, a bioactive molecule isolated from this medicinal plant was highly effective against the larvae of the mosquito – culex *quinquefasciatus*[20].

Cytotoxic activity of (S) goniothalamin and analogues isolated from *Bryonopsis* were evaluated against eight human cancer cells[10]. This medicinal plant has a wide range of anti – bacterial activity. The anti – inflammatory effect of the leaves of *Bryonopsis laciniosa* was evaluated in experimental animal models[12].

Bryonopsis laciniosa is used to treat asthma, bronchitis, cholera, colic, fever, megalospleny, paralysis, pluritic and pulmonic disorders, snake – bite, tuberculosis, rheumatoid arthritis etc.

Medicinal plants constitute the basic resource for the various traditional medicines. Developed countries are importing seventy five percent of their required medicinal raw material from India [21].

Plant tissue culture is the most useful tool which helps in the conservation and rapid propagation of plants [26].

Rhizogenesis is an important phase in the formation of complete plantlets. Root initiation is the type of organogenesis which occurred in culture tissues more frequently[28]. In *in vitro* conditions, the initiation of roots can be developed in root initiating medium which is quite different from the shoot medium. In many cases auxin alone or in combination with a low level of cytokinin will enhance the root premordia formation. Optimal root formation occurred in presence of auxins and cytokinins in many plant species[8, 9, 11]. Phenolic compounds may act with auxin to promote rooting[31].

Auxins play a vital role for the initiation of roots in many species. Among the auxins, NAA is most effective auxin for the induction of root[1, 22]. Generally natural auxin IAA and synthetic auxin NAA and IBA are used for rooting. Auxin alone or with cytokinin, GA_3, ABA and phenolics effect mainly during the root induction and initiation. The role of auxins in root development is well established and reviewed[27]. The frequency of root initiation is quite high despite the concentration of auxins and cytokinins only root initiation was observed in attempts to obtain plant regeneration for *Psophocarpus tetragonolobus*[7]; *Phaseolus vulgaris*[15]. Root formation occurs prior to shoot regeneration with *Stylosanthes hamata* [29].

MATERIALS AND METHODS

The plants and seeds of *Bryonopsis laciniosa* (L) Naud were collected from local forest area of Khammam and Warangal Dist. A.P. and were grown in university campus garden.

The healthy and young explants leaf, stem, nodal and cotyledon were selected and washed thoroughly in running tap water for 20 minutes, then in 70% alcohol for 5 minutes and surface sterilized with 1.0% mercuric chloride solution and then explants were rinsed in sterile distilled water for 3 – 4 times to discard the traces of mercuric chloride. The explants were cultured on MS Medium[25] supplemented with different concentrations of auxins and cytokinins (0.5 mg/l to 2.0 mg/l). The PH of the medium was adjusted to 5.7 to 5.8 using 0.1 NaoH or Nacl before autoclaving. 15 ml of the medium were dispensed in each culture tube and autoclaved at15 Psi for 20 minutes. All the cultures were maintained at 16 hr photoperiod with 2000 lux light. The root and shoot length and percentage of rooting was recorded from each explant and explant derived callus.

RESULTS AND DISCUSSION

During the present study different combination of cytokinins with various concentrations of auxins were tried for rhizogenesis.

Leaf Explants Cultures

Leaf explants when cultured on MS medium fortified with 1.0 mg/l 2, 4 – D and 2.0 mg/l NAA induced callus and rhizogenesis. (Plate 1, Fig 1).

A brown callus was observed after 4 weeks of cultures on the same composition of medium(plate1, fig 2). The brown callus when sub-cultured on MS medium supplemented with 1.0 mg/l 2,4 – D + 2.0 mg/l BAP and 2.0 mg/l NAA produced green callus(plate1, fig 3). When leaf derived callus was cultured on MS medium fortified with 2.0 mg/l BAP and 2.0 mg/l NAA produced multiple fibrous roots with root caps (plate 1, fig 4).

When leaf cultures cultured on MS medium fortified with 1.0mg/l BAP and 2.0NAA induced two to five roots(plate1, fig 5) and when sub-cultured on MS medium fortified with 2.0mg/l BAP +2.0NAA mg/l produced direct multiple roots (plate1, fig6).

Cotyledon explant cultures

The cotyledon explants when they were cultured on MS medium supplemented with 2.0 mg/l 2,4-D and 2.0 mg/l IBA induced multiple roots (plate2, fig 1) when cotyledon derived callus was sub-cultured on MS medium containing 2.0 mg/l BAP and 2.0 mg/l IBA produced direct multiple roots (plate2,fig 2).

Two to three roots were induced from stem derived callus on MS medium fortified with 2.0mg/l NAA and 0.5mg/l TDZ (plate2, fig3).

Single root was produced directly from the callus of nodal explant on MS medium fortified with 1.0 mg/l BAP and 1.0 mg/l IBA (plate2, fig4).

.

Plate 1

High Frequency rhizogenesis from leaf explant cultures of Bryonopsis laciniosa (L.). Naud.

Fig 1: Induction of callus and rhizogenesis from leaf explants cultures on MS + 1.0 mg/l, 2,4 – D + 2.0 mg/l NAA.
Fig 2: Brown callus after 4 weeks of cultures on the same medium as in Fig.1.
Fig 3: Green callus on MS + 1.0 mg/l 2,4 – D + 2.0 mg/l BAP + 2.0 mg/l NAA
Fig 4: Multiple fibrous root production with root caps from callus derived from leaf explants on MS = 2.0 mg/l BAP + 2.0 mg/l NAA.
Fig 5: Two to five root induction on MS + 1.0 mg/l BAP + 2.0 mg/l NAA.
Fig 6: High frequency direct multiple root production from leaf cultures on MS + 2.0 mg/l BAP + 2.0 mg/l NAA

Plate 2

Rhizogenesis from different explant cultures of Bryonopsis laciniosa (L.) Naud.

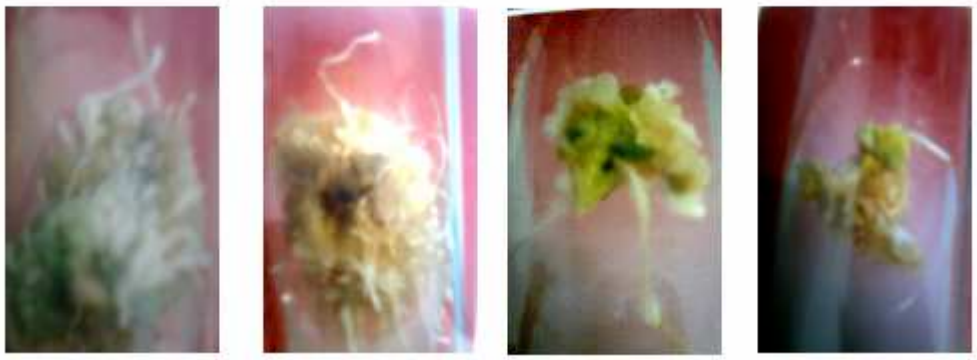

Fig 1: Induction of multiple root production from cotyledon explant culture on MS + 2.0 mg/l 2,4 – D + 2.0 mg/l IBA.
Fig 2: Direct multiple roots production from cotyledon derived callus on MS + 2.0 mg/l BAP + 2.0 mg/l IBA.
Fig 3 Induction of two to three roots from callus derived stem explant on MS + 2.0 mg/l NAA + 0.5 mg/l TDZ..
Fig 4: Induction of single roots from the callus of nodal explant on MS + 1.0 mg/l BAP + 1.0 mg/l IBA.

From the present study it was proved that the NAA alone or combination with BAP can induce high frequency rhizogenesis .Among the auxins applied NAA displayed high percentage of rooting and root length(table1).The other growth regulator IBA also shown good

response to rhizogenesis on half strength MS medium(table2). It was observed that leaf and cotyledon explants were potential to induce high percentage of rhizogenesis than other explants tested thus all the explants do not have equal potential to induce roots.

Table 1

Effect of IBA, NAA and 2,4 – D (AUXINS) in various concentrations on rhizogenesis from leaf explant in Bryonopsis laciniosa (L.) Naud.

Auxin type	Auxin concentration mg/l							
	0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0
	Percentage of rooting				No. of roots per shoot			
IBA	22.0	34.0	38.0	81.0	1.8	2.0	2.2	2.4
NAA	28.0	38.0	52.0	92.0	2 .6	2.8	3.6	6.1
2,4 – D	8.0	8.0	9.0	8.0	0.2	0.4	1.0	1.5
	Root length				Shoot length			
IBA	11.0	12.5	13.2	16.0	18.2	18.6	19.1	20.1
NAA	15.0	16.5	18.2	20.0	24.1	25.6	26.4	28.2
2, 4 – D	5.8	6.1	6.3	6.5	2.8	3.6	5.2	6.1

After six weeks of culturing the regenerates before transfer to soil

Table – 2

Rhizogenesis from in vitro raised shoots on half strength MS medium containing IBA in combination with BAP, kinetin and 2, 4 – D in *Bryonopsis laciniosa* (L.) Naud.

Growth regulators (mg/l)		% of response	Average no.of roots/shoots (Mean ± S.D.)
IBA	BAP		
0.5	0.5	40.5	1.35 ± 0.45
1.0	0.5	55.0	1.10 ± 0.27
2.0	0.5	62.0	1.00 ± 0.18
0.5	1.0	67.0	1.80 ± 0.31
0.5	2.0	82.0	2.6 ± 0.38
IBA	Kn		
0.5	0.5	60.5	1.25 ± 0.40
1.0	0.5	72.5	1.40 ± 0.35
2.0	0.5	80.5	1.70 ± 0.43
0.5	1.0	89.0	1.85 ± 0.55
0.5	2.0	92.0	2.50 ± 0.47
IBA	2,4 -D		
0.5	2.0	60.5	1.40 ± 0.45
0.5	2.0	65.0	1.26 ± 0.58
1.0	2.0	70.5	1.80 ± 0.50
2.0	2.0	82.5	2.35 ± 0.47
3.0	2.0	60.5	2.45 ± 0.42

The production of roots from various explants depend on various concentrations of growth regulators. Different growth regulators influence in different way the root induction and elongation[4, 5, 6, 14, 32]. The *in vitro* root inducing capacity of phenolics was demonstrated in apple root stocks[17], and in *Prunus insititia*[19]. Specifically phenolics acts on the middle phase, the "initiation phase" of rhizogenesis[24]. The presence of phloroglucinal (PG) during the shoot proliferation stage significantly promoted root formation[18]. During the study it was noticed that the auxin in right and appropriate concentration induces rooting. The similar results were noticed in carrot explants[13]. IAA, IBA and NAA are most suitable auxins which can promote high percentage of rhizogenesis thus it is widely accepted that auxins play a vital role in the production of adventitious roots in different plants [16,33]. IBA showed that, it promotes and initiate the root but can not produce the roots where as NAA had the most positive effect on induction and elongation of roots. Many workers reported similar results: tomato [30] *Porteresia coaretata* [23] *Decalepis hamiltonni*[3],*Zehneriascabra*[2].

REFERENCES

1. Ancora, G., Belli - Donini, M.L. and Cuozzo, L. (1981). Globe atichoke plants obtained from shoot apices through rapid *in vitro* micropropagation. *Sci.Hort.,* 14: 207-213.
2. Anand, S.P. and Jeyachandran, R. (2004). In vitro multiple shoot regeneration from nodal explants of *Zehneria scabra* (L.F.) Sonder an important medicinal climber.*Plant Tissue Cult. 14(2):* 101-106.
3. Anitha, S. and Pullaiah, T. (2002). *In vitro* propagation of *Decalepis hamiltonii. J. Trop. Med. Plants,* 3: 227-232.
4. Balestri, E. and Bertini, S. (2003). Growth and development of *Posidonia oceanica* seedlings treated with plant growth regulators: Possible implications for meadow restoration. *Aquat. Bot.,* 76: 291 -297.
5. Balvanyos. I. Kursinszki, L. and Szoke, E. (2001). The effect of plant growth regulators on biomass formation and lobeline production of *Lobelia inflate* L. hairy root cultures. *Plant Growth Reg.,* 34: 339-345.
6. Biondi, S., Lenzi, C., Baraldi, R. and Bagni, N. (1997). Hormonal effects on growth and morphology of normal and hairy roots of *Hyoscyamus muticus. J. Plant Growth Regul.* 16: 159 -167.
7. Bottino, P.J., Maire, C.E. and Goff, L.M. (1979). Tissue Culture and organogenesis in the Winged bean. *Can. J. Bot*., 57:1773 – 1776.
8. Chlyah, A and Tran Thanh Van, M. (1975). Differential reactivity in epidermal cells of *Begonia rex* excised and grown *in vitro*. *Physiol. Plant,* 35:16-20.
9. Dudits, D., Nemeth, G. and Haydu, Z. (1975). Study of callus growth and organ formation in wheat *Triticum aestivum* tissue culture. *Can. J. Bot.,* 53: 957-963.
10. Fatima A., Kohn, L.K., Carvalho, J.E. Pili, R. A.,Cytotoxic activity of (S) – goniothalamin and analogues against human cancer cells. *Bioorg med chem*. 2006 Feb, 14(3): 622-31, Epub 2005 Oct, 3.
11. Gamborg,O.L., Constabel,F and Shyluk,J.P.(1974).Organogenesis in callus from shoot apices of *Pisum sativum.Physiol.Plant*.30:125-128.
12. Gupta,M.,Mazumdar,U.K.,SivakumarT., Vamsi,M.L.,Karki,S.S.,Sambath

Kumar,R.,Manikandan,L.,Evaluation of anti-inflammatory activity.*Biol pharm Bull.*2003.Sep.26(9):1342-4.

13. Gautheret, R.J. (1945). Use vaie Nauvelle in Biologie Vegetable. In: Culture de Tissues, Gallimard, Paris.
14. Gyulai, G., Kiss, J. and Heszky, L.E. (1993). Hormone selective bioassay for auxins and cytokinins *in vitro. Hung Agric. Res*., 2: 13-17.
15. Haddon, L. and Northcote, D.H. (1976). The influence of gibberellic acid and abscisic acid on cell and tissue differentiation of bean callus. *J. Cell Sci.*, 20: 47 – 55.
16. Henselova M (2002). Synergistic effect of benzolinine with IBA and fungicides on the vegetative propagation of ornamental plants, park, and fruit woody species, *zahradnnictri*, 29(2): 41-50.
17. James and Turbon, I.J. (1979). Rapid *in vitro* rooting of the apple root stock M – 9. *J. Hort. Sci.,* 54: 309-311.
18. James and Thurbon, I.J. (1981). Shoot and root initiation *in vitro* in apple root stock M – 9 and the promotive effects of phloroglucinol. *J. Hort. Sci.,* 56: 15-20.
19. Jones, O.P. and Hopgood, M.E. (1979). The successful propagation in vitro of two root stocks of *Prunus.* The plum root stock Pixy *(P. insititia)* and the every root stock F12/1 *(P. avium)*. J. Hort. Sci., 54: 63-66.
20. Kabir,K.E.,Khan,A.R.,Mosaddik,M.A.,Gonioth alamin-a potent mosquito larvicide from *Bryonopsis laciniosa .Journal of Applied Entomology Volume* 127,Issue:2,p.112,March 2003.
21. Kamboj,V.P.(2000).Herbalmedicine.*Curr.Sci*. 78(1):35-39.
22. Kitto, S.L. and Young, M.J. (1981). *In vitro* propagation of *Carrizo citrange*. Hort. Science 16: 305-306.
23. Latha, R. Ajitha, A., Srinivasa, R.C, Eganathan, P., and Balakrishna, P. (1998). *In vitro* propagation of salt – tolerant wild rice relative, *porteresia coarctata Tateoa*. J. Plant Growth Regul. 17: 231-235.
24. Mosella Chancel,L., Mactteix,J.J. and Jonard, R.(1980). Les conditions du microbouturage *in vitro* du pecher(*Prunus persica* Batsch):Influences combines des substances d croissance et de divers composes phenoliques. *Physiol.Veg*., 18:597-608.
25. Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco cultures. *Plant physiol*., 15: 473 - 497.
26. Prakash E., Sha, Valli Khan, P.S. Sairam, P.Reddy and R.Rao, 1999. Regeneration of plants from seed - derived callus of *Hybanthus enneaspermus* L.Muell., a rare ethnobotanical herb Plant Cell Rep., 18: 873 – 878.
27. Scott TK (1972) auxins and roots. Ann Res., *Plant Physiol* 23: 235-258.
28. Street, H.E. (1977). Cell suspension cultures techniques. In: Street, H.E. (e d.). Plant Tissue and Cell Culture, University California Press, Berkley, California, pp. 61-102.
29. Scowcroft, W.R. and Adamson, J.A. (1976). Organogenesis from callus cultures of the legume, *Stylosanthes hamata. Plant Sci.Lett 7: 39-42*
30. Taylor, J.L.S. and Van Staden, J. (1998). Plant – derived smoke solutions stimulate the growth of *Lycopersicon esculentum roots in vitro*. *Plant Growth Reg.,* 26: 77-83.
31. Thorpe, T.A. (1980). "Organogenesis *in vitro:* Structural, physiological and biochemical aspects". *Int. Rev. Cytol. Suppl*. IIA" 71 -112.
32. Venhala, L., Eeva. M., Lapinjoki, S., Hiltunen, R. and Oksman Caldentey, K.M.

(1998). Effect of growth regulators on transformed root cultures of *Hyoscyamus muticus. J. Plant Physiol.,* 153: 475-481.

33. WanX, Landhausser SM, Lieffers VJ, Zwiazek JJ (2006). Signals controlling root suckering and adventitious shoot formation in aspen *(Populus tremuloide), Tree,Physiol*,26:681-687.