ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 4, Issue, 01, pp.065-067, January, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

IN VITRO ADVENTITIOUS ROOT AND HAIRY ROOT CULTURES IN *Boerhaavia diffusa L.*

Jenifer, U., Francina Cecilia, K. and Ravindhran, R*

Department of Plant Biology and Biotechnology, Loyola College (Autonomous), Chennai - 600 034, Tamil Nadu, India

ARTICLE INFO

Article History:
Received 29th October, 2011
Received in revised form
24th November, 2011
Accepted 25th December, 2011
Published online 31st January, 2012

Key words:
Adventitious roots,
Auxins, *Boerhaavia diffusa*,
Hairy roots,
MS medium.

ABSTRACT

Boerhaavia diffusa L. is a valuable medicinal plant indigenous to India commonly called Punarnava. Adventitious roots were initiated from the leaf explants on a media containing Murashige and Skoog media supplemented with various concentrations (0.25, 0.5, 1.0, 2.0 & 4.0 mg/L) of NAA and IBA. Maximum number of roots were obtained on MS media supplemented with 1.0 mg/L NAA, while profuse rooting was also found in media supplemented with 2.0 mg/L and 4.0 mg/L NAA. *In vitro* grown roots were transferred to liquid culture media containing NAA for root proliferation. The cultured roots were harvested after 40 days. *Agrobacterium rhizogenes* mediated transformation was carried out with *in vivo* leaf explants of *B. diffusa*. Hairy roots were obtained on a medium containing hormone free MS medium.

Copy Right, IJCR, 2012, Academic Journals. All rights reserved.

INTRODUCTION

Boerhaavia diffusa L. commonly called Punarnava is an herbaceous member of the Nyctaginaceae family. It is widely used in the traditional medicine in treating numerous ailments (Chofee *et al*., 2000; Chopra *et al*., 1923). *B. diffusa* contains an important principle compound "punarnavine" which is the active principle compound in the herb (Srivastava *et al*., 1995; Wahi *et al*., 1997) and it has been effective against helminth infection (Singh and Udupa, 1972) and also used as diuretic, laxative, expectorant and against respiratory tract infections (Rawat *et al*., 1998) The roots and leaves have been found to be highly potent with antimicrobial activity (CSIR, 1988) and anti-inflammatory activity (Sangameswaran *et al*., 2008). Plant roots often serve as resource of bioactive molecules for therapeutic use as well as a source of agrochemicals, flavors, dyes, and fragrances (Fulzele *et al*., 2002). *In vitro* root culture has become an alternative method for the production of secondary metabolites with pharmaceutical interest (Ramachandra Rao and Ravishankar 2002; Verpoorte *et al*., 2002). Adventitious root formation is a key step in vegetative propagation (Baskaran and Jayabalan, 2009). Genetic transformation with *Agrobacterium rhizogenes* is for a means for hairy root induction, and hairy root cultures are used for the production of novel metabolites (Flores and Bolivar, 1995). Hairy roots are genetically stable and capable of synthesizing metabolites in abundance that are normally found in roots and other organs (Li *et al*., 2000). Moreover, *A. rhizogenes*-derived hairy roots and plants have application for many areas of research. Hairy root cultures have been used extensively in root nodule research (Diaz *et al*., 1989; Quandt *et al*., 1993) and for artificial seed production (Mary *et al*., 2004). Here, the first report of the adventitious and hairy roots of *B. diffusa* cultured in solid and suspension cultures is presented.

***Corresponding author:** raviloyola1998@gmail.com

MATERIALS AND METHODS

Adventitious root cultures

Five weeks old leaf explants of *B. diffusa* were collected from medicinal plant garden of Loyola College, Chennai, India. Leaves were surface sterilized for 3 min with 0.1% (w/v) aqueous $HgCl_2$, and then rinsed three times with sterile distilled water. The disinfected leaves of 0.3 cm were used for regeneration of adventitious or hairy roots. MS medium supplemented with various concentrations (0.25, 0.5, 1.0, 2.0, 4.0 mg/L) of NAA and IBA were used for adventitious root initiation of *B. diffusa*. After 20 days of incubation the *in vitro* roots of fresh weight (0.1 g) were transferred to 50 ml of full strength and half strength liquid MS medium supplemented with 1.0 mg/L NAA and kept in an orbital shaker at 80 rpm.

Induction of hairy roots

Agrobacterium rhizogenes strain A4 was obtained from MSSRF (M.S. Swaminathan Research Foundation), Chennai, Tamil Nadu, India. The culture was maintained in LB (Luria Bertani) agar medium. *A. rhizogenes* of about 0.1 ml from mother culture was inoculated in 50 ml of Luria –Bertaini broth and incubated at 28^0 C shaking at 80 rpm in an orbital shaker. The bacterial cells were collected at OD_{600} 0.5 (Jong *et al*., 2008) after 3 days by centrifugation at 3000 rpm. The supernatant was discarded and the pellet was dissolved in half-

strength MS medium. The bacterial suspension thus obtained was used for infection. The disinfected leaf discs (0.3 cm) were inoculated in the bacterial suspension for 30 min. The leaf discs were then blot dried on a sterile filter paper .The leaf discs were co-cultivated in hormone free MS media and kept in darkness for 72 h, then they were washed in half-strength MS medium containing 500 mg/L cefotaxime, blotted and inoculated into MS media containing 500 mg/L cefotaxime. Hairy roots were sub cultured every three weeks on MS medium supplemented with cefotaxime and in subsequent passages the antibiotic was eliminated. The plates were incubated in the dark at 28°C and the results were observed. The formation of hairy roots were analyzed by transferring it to a media without phytohormone and observed (Tepfer, 1984).

Statistical analysis

All the experiments were repeated thrice with 25 explants per treatment in experiment. An ANOVA using Duncan's multiple range test (DMRT) was used to compare the means of all treatments (Software SPSS 11.5.0)

RESULTS

Adventitious root initiation

MS medium supplemented with various concentration of NAA and IBA were individually tested for adventitious root production. Various frequencies of adventitious rooting and number of roots per explants were obtained in different concentrations of NAA and IBA (Table1).

Table 1. Effect of IBA and NAA on rooting of *Boerhaavia diffusa* cultured on MS medium supplemented with 3% (w/v) sucrose

Growth regulator (mg/l)		Adventitious rooting status	
IBA	NAA	% rooting	No. of roots
0.25	0	70	3.5 g
0.50	0	60	7.2 b
1.0	0	40	3.7 g
2.0	0	50	3.0 h
4.0	0	20	4.0 f
0	0.25	50	6.3 d
0	0.5	60	5.4 e
0	1.0	80	12.5 a
0	2.0	60	7.0 b
0	4.0	50	6.7 c

Values are mean of 25 explants per treatment and repeated thrice. Mean values within a column followed by different letters are significantly different from each other at 5% level comparison by Duncan's multiple range test (DMRT).

Higher frequency of roots (80 %) and number of roots (12.5 roots / explant) was observed in MS medium supplemented with NAA (1.0 mg/L). IBA induced rooting lesser than NAA at a frequency of (70 %) and number of roots (7.2 roots / explant) was observed in 0.25 mg/L IBA concentration. Higher concentrations of NAA also resulted in rooting but to a lesser extent. This is in line with the fact that a particular type of auxin is effective in enhancing rooting in a particular species (Singh and Bhansal, 1984; Puri and Shamet, 1988).

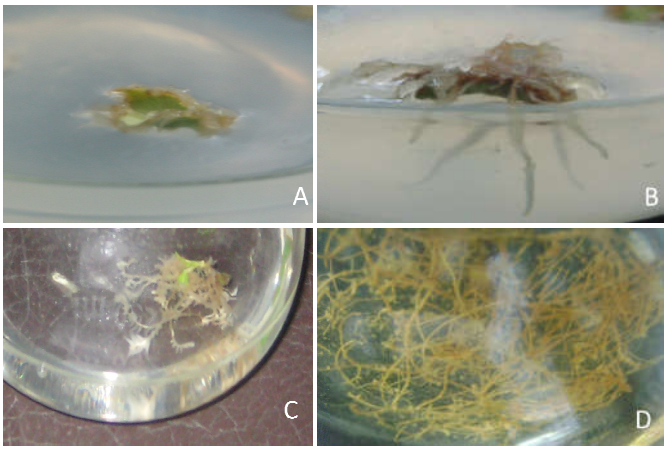

Fig. 1. Initiation and Establishment of Adventitious Root Cultures of *Boerhaavia diffusa:* A - Root initiation on leaf segment; B- Root growth after 4 weeks; C- Root multiplication in suspension culture and D- Root growth after 4 weeks.

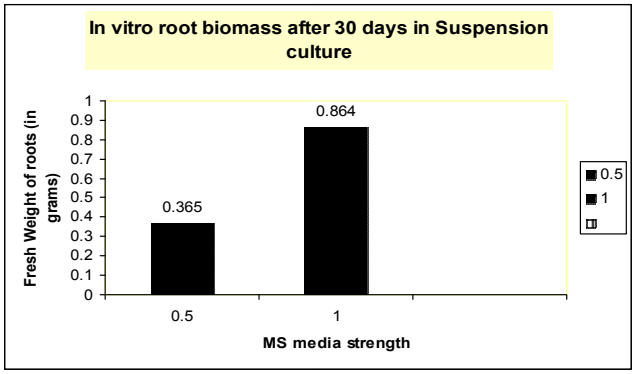

Fig. 2. *In vitro* root biomass production in different strength of MS media

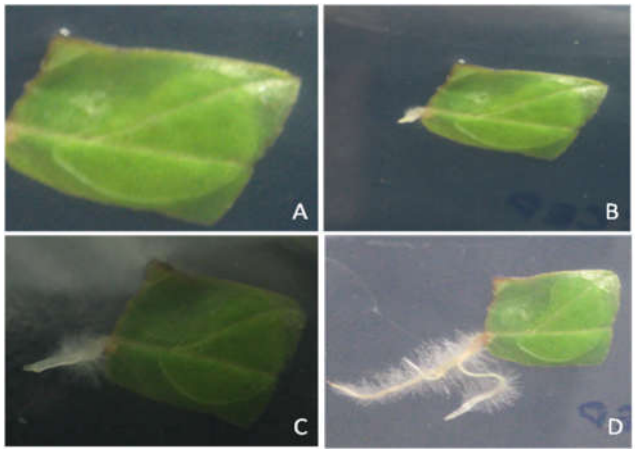

Fig. 3. Induction of Hairy Roots: A – Co cultivated leaf explants; B - Initiation of hairy roots; C - Elongation of hairy roots; D - Initiation of secondary roots

Full-strength and half-strength MS liquid medium were studied for adventitious root proliferation. Adventitious roots in full-strength MS liquid with 1.0 mg/L NAA (Fig. 1) enhanced rooting, however the rooting was scanty in half-strength MS medium. Adventitious roots were harvested and the fresh weight was measured after 30 days of cultures. Roots in full strength MS, recorded the maximum of 0.864 g fresh weight per culture whereas 0.365 g fresh weight per culture was measured in half strength medium (Fig. 2).

Hairy root culture

Hairy roots were induced at the site of infection within 12 days. No hairy roots were formed on control explants, which also remained green. The secondary hairy roots started developing after 22 days (Fig. 3). The leaf explant developed a slight callus at the wound site after 10 days. Roots began to emerge from the callus 1-2 days later and by 14 days, root development was clearly visible.

DISCUSSION

Adventitious roots were observed in various concentrations of IBA and NAA. In the present work NAA was found more potent in triggering adventitious roots from the *Boerhaavia diffusa* explant. Similar results were also observed in *Robinia psuedoacacia* adventitious rooting where NAA was found to induce rooting (Swamy, 2001). This is in line with the fact that a particular type of auxin is effective in enhancing rooting in a particular species (Singh and Bhansal 1984; Puri and Shamet, 1988). Root induction was suppressed markedly with higher concentrations of NAA and IBA. Similar phenomenon was also observed by Choffe *et al.,* 2000. *A. rhizogenes* has been used to obtain transgenic plants in several systems. Hairy roots were induced at the site of infection in *B. diffusa*. To the best of our knowledge this is the first report on *Agrobacterium rhizogenes* mediated transformation of *Boerhaavia diffusa* using A4 strain. The present investigations revealed that the frequency of induction was high with the leaf explants**.** The 72 h co-cultivation was effective in the production of hairy roots. However longer co-cultivation resulted in excess growth for the bacteria. Thus the current study concludes that adventitious root cultures and hairy root cultures are highly effective in producing highly valuable roots in *Boerhaavia diffusa.* This work highlights the production of adventitious roots from *B. diffusa* using exogenous auxin and induction of hairy roots by *Agrobacterium rhizogenes* mediated transformation. These methods will be highly rewarding in industrial production of the useful drug Punarnava.

REFERENCES

Baskaran, P and Jayabalan, N. 2009. Psoralen production in hairy roots and adventitious roots cultures of *Psoralea coryfolia*. *Biotechnology Letters*, 31: 1073–1077.

Choffe, K. L. J., Victor, M. R. S. J., Murch, and Saxena, P. K. 2000. *In vitro* regeneration of *Echinacea purpurea* L. Direct somatic embryogenesis and indirect shoot organogenesis in petiole culture. *In Vitro Cell. Dev. Biol. Plant* 36: 30–36.

Chopra, R.N., Ghosh, S., Dey, P and Gosh, B.N. 1923. Pharmacology and therapeutics of *Boerhavia diffusa* (punarnava). *Indian Medical Gazette*, 58: 203 - 208.

CSIR. 1988. The Wealth of India: Raw Materials Vol. VII B. CSIR, New Delhi, India. p. 174.

Diaz, C. L., Melchers, L. S., Hooykaas, P. J. J., Lugtenberg, B. J. J., and Kijne, J. W.1989. Root lectin as a determinant of host-plant specificity in the Rihizobium-legume symbiosis. *Nature* 338, 579-581.

Flores, H.E and Bolivar, M.F, 1995. Root culture and plant natural products: 'unearthing' the hidden half of plant metabolism. *Plant Tissue Culture Biotechnology*, 1: 59-74.

Fulzele, TB., Satdive, R.K and Pol, B.B. 2002. Untransformed root cultures of *Nothapodytes foetida* and production of camptothecin. *Plant Cell, Tissue and Organ Culture*, 69: 285-288.

Jong Se Kim., Sook Young Lee and Sang Un Park. 2008. Resveratrol production in hairy root culture of peanut, *Arachis hypogaea* L. transformed with different *Agrobacterium rhizogenes* strains. *African Journal of Biotechnology,* 7: 3788 -3790.

Li, W., Asada, Y., and Yoshikawa, T. 2000. Flavonoid constituents from *Gly - cyrrhiza glabra* hairy root cultures. *Phytochemistry*, 55: 447-456.

Mary, C. Christey., Robert, H. Braun. 2004. Production of Hairy Root Cultures and Transgenic Plants by *Agrobacterium rhizogenes*-Mediated Transformation. *Transgenic Plants: Methods and Protocols*, 286 - 47-60.

Puri, S and Shamet G.S. 1988. Rooting of some social forestry species. *International Tree Crops Journal*, 5: 63–70.

Puri, S and Swamy, S.L, 1999. Geographical variation in rooting ability of stem cuttings of *Azadirachta indica* and *Dalbergia sissoo*. *Genetic Resources and Crop Evolution,* 46: 29–36.

Quandt, H.J., Pühler, A. and Broer I. 1993. Transgenic root nodules of *Vicia hirsuta*: a fast and efficient system for the study of gene expression in indeterminate-type nodules. *Mol. Plant Microbe Interact.* 6: 699-706.

Ramachandra Rao, S and Ravishankar, G.A. 2002. Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnology Advances*, 20: 101-153.

Rawat, A.K.S, Mehrotra, S., Tripathi, S.C and Shome, U. 1997. Hepatoprotective activity of *Boerhaavia diffusa* L. roots – a popular Indian ethnomedicine. *Journal of Ethnopharmacology*, 56: 61–66.

Sangameswaran, B., Balakrishnan, N and Bhaskar, V.H. 2008. Anti-inflammatory and anti- bacterial activity of leaves of *Boerhaavia diffusa* L. *Pharmacognosy Magazine*, 65 – 68.

Srivastava, N., Padhya, M.A, 1995. 'Punarnavine' profile in the regenerated roots of *Boerhaavia diffusa* L. from leaf segments. *Current Science,* 68: 653-656.

Singh, K and Bansal, G.L. 1984. A note on rooting of stem cuttings of *P. deltoides* in relation to sex of the mother plant. *Journal of Tree Science*, 2: 92–93.

Singh, R.H. and Udupa, K.N. 1972. Studies on the Indian indigenous drug punarnava (*Boerhaavia diffusa* Linn.). Part IV: Preliminary controlled clinical trial in nephrotic syndrome. *Jouranl Res. Indian Med*., 7: 1-12.

Swamy, S.L., Puri, S. and Singh A.K. 2002. Effect of auxins (IBA and NAA) and season on rooting of juvenile and mature hardwood cuttings of *Robinia pseudoacacia* and *Grewia Optiva. New Forest,* 23: 143–157.

Tepfer, D 1984. Genetic transformation of several species of higher plants by *Agrobacterium rhizogenes*: Sexual transmission of the transformed genotype and phenotype. *Cell,* 37: 959–967.

Verpoorte, R., Contin, Aand Memelink, J., 2002. Biotechnology for the production of plant secondary metabolites. *Phytochemistry Reviews*, 1: 13-25.

Wahi, AK., Agrawal, V.K. and Gupta, R.C.. 1997. Phytochemical and pharmacological studies on *Boerhaavia diffusa*. Linn (Punarnava) alkaloids. *National Academy of Science Letters,* 20: 9-10.