Cey. J. Sci. (Bio. Sci.) 40 (1): 53-58, 2011

SHORT COMMUNICATION

CLONAL PROPAGATION OF *WATTAKAKA VOLUBILIS* THROUGH NODAL EXPLANT CULTURE

D. Vinothkumar[1]*, S. Murugavelh[2] and M. Senthilkumar[3]

[1]Faculty of Biotechnology, Adhiyamaan Engineering College, Hosur, Tamil Nadu, India
[2]Department of Chemical Engineering, Indian Institute of Technology, Guwahati, Assam, India
[3]Department of Botany, Jamal Mohammad College, Tiruchirappalli, Tamil Nadu, India
Accepted 22 May 2011

ABSTRACT

A protocol was developed for microporpagation of *Wattakaka volubils* (L.f) Stapf, an important medicinal plant in India. *In vitro* shoot multiplication nodal explants of *W. volubilis* was achieved on solid MS basal medium supplemented with BAP (6-Benzylaminopurine) and NAA (Naphthalene acetic acid). Presence of NAA in the culture medium along with BAP promoted multiplication of shoots than BAP alone. The highest shoot multiplication rate (23.4±0.48) was observed after 28 days of culture on MS basal medium supplemented with 0.6 mg/l BAP and 0.2 mg/l NAA. Elongated shoots produced roots within 6 weeks in half strength MS basal medium supplemented with 0.6 mg/l IBA (Indole-3-butyric acid) and 3% (w/v) sucrose. Rooted plantlets were acclimatized with a survival rate of 95% and transferred to the green-house and thereafter successfully transferred to the field with 90% survival rate.

Keywords: medicinal plants, micropropagation, nodal culture, survival rate

INTRODUCTION

Medicinal plants are an important health care and economic resource. India is one of the world's richest countries in medicinal plants comprising about 45000 species. Due to the unsustainable exploitation of eco-resources, several plant species of medicinal importance have become threatened or endangered and may become extinct if proper planning for their conversation is not emphasized (Rajani and Sharad, 2006). *Wattakaka volubilis* which is commonly known as 'Perukuruncha' in Tamil, is an important medicinal woody climber belonging to the family Asclepiadaceae. The whole plant is used for medicinal purposes and has been used for the treatment of various ailments since ancient times. Among various saponins obtained from the stem and flower of *W. volubilis*, two compounds are active against Ehrlich's ascites carcinoma (Pullaiah, 2002 and Yoshimura *et al*., 1983).

Plant tissue culture techniques for medicinal as well herbaceous plants have been well established. *In vitro* propagation technique is a powerful tool for plant germplasm conservation. Hence, tissue culture is the only rapid process for the mass propagation of plants. The ability to generate plants directly for explants is fundamental to clonal multiplication of elite germplasm via micropropagation (Ignacimuthu, 1997). Plant biotechnology is considered in a wide sense which comprises the various culture methods of plant organs and explants to facilitate experimental approaches with a large objective of developmental biology in grain legumes for crop modification (Ramawat, 2003). In the present study, to raise stock culture, nodal explants were taken from the green house plants.

Biotechnology offers several important tools for fast and efficient multiplication and production of plants with desired features. For genetic transformation efficient protocol for regeneration through microporpagation is a prerequisite. The main objective of the present study was to develop a successful protocol for regeneration of *W. volubilis in vitro* and optimum conditions for complete plantlet development.

MATERIALS AND METHODS

Explants of *W. volubilis* were collected from Cauvery River basin in Tiruchirappalli district of Tamil Nadu, India. The collected plant parts

*Corresponding author's email: vinoth.vinopaul79@gmail.com

were kept under green house conditions for further study. Well developed 3 year - old mature plants were cut into 2-3 cm nodal segments and used for induction of multiple shoots. Explants were washed thoroughly under running tap water for 25 min and treated with a surfactant, Tween 20 (15 drops per 100 ml of sterilized distilled water) for 10 min. Later these explants were surface sterilized with 70% ethanol (w/v) for 5 min and washed thrice using sterilized distilled water. Under aseptic conditions, explants were inoculated on basal MS (Murashige and Skoog, 1962) medium containing 3% (w/v) sucrose, supplemented with different concentrations and combinations of 6-Benzyleaminopurine (BAP:0.2-1.2 mg/l), Kinetin (KIN: 0.2-1.2 mg/l), Naphthalene acetic acid (NAA: 0.1-0.6 mg/l) or Gibberelic acid (GA_3: 0.1-0.6 mg/l) for direct plant regeneration and root induction. The pH of the media was adjusted to 5.8±0.1 prior to gelling with 0.8% (w/v) agar (Himedia-Mumbai®) and autoclaved at 1.05 kg/cm^2, 121 ºC for 15 min. Cultures were maintained at 25±2 ºC in 16/8 h photoperiod provided by fluorescent tubes (Philips® 40w) and 65-70% relative humidity was maintained in the growth room. Well developed multiple shoots were transferred to MS medium supplemented with different concentrations and combinations of GA_3 (0.1-0.6mg/l), BAP (0.1-0.6mg/l) and KIN (0.1-0.6mg/l) for shoot elongation.

For root induction, separated shoots were transferred to half strength MS basal medium supplemented with different concentrations of IBA 0.2-1.2 mg/l, IAA 0.2-1.2 mg/l and NAA 0.1-0.6 mg/l and 2% (w/v) sucrose. Rooted plantlets were thoroughly washed to remove the adhering gel and planted in specially made paper cups containing sand, garden soil and farmyard manure (1:2:1) and kept in the greenhouse for acclimatization. Twenty cultures were used per treatment and each experiment was replicated three times. Percentage of success was scored four weeks after culture. Data collected were statistically analyzed using standard deviation.

RESULTS AND DISCUSSION

Multiple shoots and buds originated from nodal explants, when MS medium was supplemented with different concentrations and combinations of BAP (0.2-1.2 mg/l), KIN (0.2-1.2 mg/l), NAA (0.2-1.2 mg/l) and GA_3 (0.2-1.2 mg/l). Fifteen days after inoculation, the nodal explants showed slight swelling prior to the emergence of shoot buds that develop from the pre-existing material. Initially two shoot buds per explant emerged 20 days after inoculation and gradually the number of shoot buds per explant increased up to 22-24 (Fig. 1 a-c and Table 1). Among the four different treatment combinations, after four weeks of incubation higher number of multiple shoots (23.4±0.48) along with 95% survival rate was observed in MS medium supplemented with 0.6 mg/l BAP combination of 0.2 mg/l NAA (Fig. 1c and Table 1). Many authors have reported that a combination of BAP and NAA are required for shoot multiplication in *Gentiana kurroo* (Sharma *et al*., 1993) *Pinellia ternate* (Tsay *et al*., 1989) *Psudarthria viscida* (Vinothkumar *et al*., 2009 and 2010) and *Momordica charantea* (Sultana *et al*., 2003). The healthy shoots (3-5 cm long) were transferred to MS medium supplemented with different concentrations and combinations of GA_3 (0.1-0.6 mg/l), BAP (0.1-0.6 mg/l) and KIN (0.1-0.6 mg/l). Among the three different concentrations, GA_3 along with BAP showed excellent shoot elongation. In this combination, the highest success rate (94%) with maximum shoot length (9.6±0.04) was observed with 0.4 mg/l GA_3 and 0.2 mg/l BAP after four weeks of inoculation (Table 1 and Fig. 1d).

Well developed shoot elongations were excised from culture tubes and cultured on half strength MS containing different concentrations of IBA (0.2-1.2 mg/l), IAA (0.2-1. 2 mg/l) and NAA (0.2-1.2 mg/l). The percentage of root frequency, number of roots per shoot and length of roots were recorded after 4-5 weeks of culture. The rooting response to different auxin treatments is shown in Table 2. Of the three types of auxins, IBA was found to be comparatively more effective than the other two auxins (IAA and NAA). IBA (0.6 mg/l) was found to be the best concentration of auxin for proper rooting where 95% of the shoots rooted within 6 weeks of culture. The highest average number of roots was (12.2±0.40) with root length (13.5±0.13 cm) (Table 2 and Fig. 1e). Many authors have reported IBA as the best rooting auxin for *Anethum graveolens* (Sharma *et al*., 2004), *Thapsia garganica* (Makunga *et al*., 2006), *Anisochilus carnosus* (Jeyachandran, 2004) *Annona sequamosa* (Roxana, 2005), *Psudarthria viscida* (Vinothkumar *et al*., 2009 and 2010) and *Quisqualis indica* (Poornima and Shivamurthy, 2005). At higher concentrations of auxins profuse callus was produced at the basal end of microshoots which inhibited the growth and elongation of roots.

The findings of this study would help in the conservation and propagation of *W. volubilis* and provide a possible lead to the synthesis and extraction of active compounds form the plant.

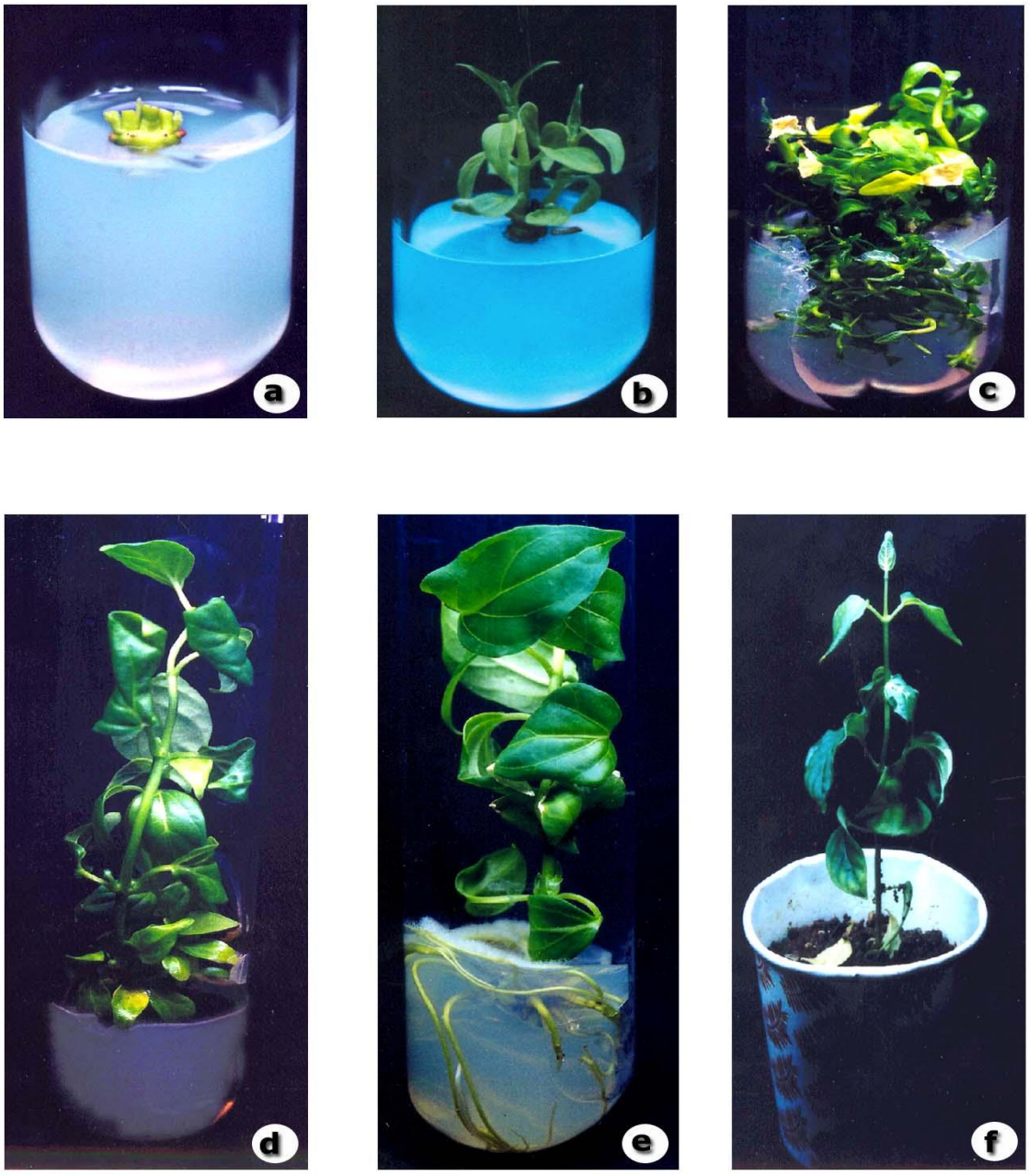

Figure 1. Plant regeneration from nodal explants of *Wattakaka volubilis*. (a) Initiation of shoots after 2 weeks (0.6 mg/l BAP) (b) Shoot bud formation (0.6 mg/l BAP) (c) Multiple shoots after 4 weeks (0.6 mg/l BAP+0.2 mg/l NAA) (d) Shoot elongation after 5 weeks (0.4 mg/l GA_3+0.2 mg/l BAP) (e) Rooting after 6 weeks (0.6 mg/l IBA) (f) Hardening (sand, garden soil and farmyard manure 1:2:1).

Table 1. Effect of different concentrations of growth regulators on multiple shoot induction in *W. volubilis* from nodal explants after four weeks. (20 replicates per treatment)

Plant Growth Regulators (mg/l)	Percentage of Responding cultures	Mean No. of shoots/explant	Mean shoots/length (cm)	Basal callus
BAP				
0.2	65	11.6±0.47	-	+++
0.4	80	12.6±0.48	-	++
0.6	**90**	**15.8**±0.74	-	+
0.8	75	13.4±0.48	-	++
1.0	50	6.6±0.48	-	++
1.2	45	4.8±0.74	-	+++
KIN				
0.2	60	4.8±0.80	-	++
0.4	**85**	**10.8**±0.97	-	+
0.6	75	6.8±0.74	-	++
0.8	65	5.0±0.63	-	++
1.0	55	4.4±0.48	-	+++
1.2	50	3.4±0.80	-	+++
BAP+NAA				
0.6+0.1	85	18.8±0.74	-	+
0.6+0.2	**95**	**23.4**±0.48	-	-
0.6+0.3	80	16.4±0.48	-	+
0.6+0.4	70	14.4±0.48	-	++
0.6+0.5	60	12.8±0.74	-	+++
0.6+0.6	50	11.4±0.48	-	+++
BAP+GA_3				
0.6+0.1	65	5.4±0.48	-	++
0.6+0.2	75	8.6±0.80	-	++
0.6+0.3	**85**	**13.8**±0.74	-	+
0.6+0.4	55	5.6±0.80	-	++
0.6+0.5	50	4.4±0.48	-	++
0.6+0.6	45	3.8±0.40	-	+++
KIN+NAA				
0.4+0.1	40	7.2±0.74	-	++
0.4+0.2	**80**	**10.6**±0.48	-	+
0.4+0.3	70	9.3±0.47	-	++
0.4+0.4	55	8.2±0.40	-	++
0.4+0.5	45	5.6±0.80	-	+++
0.4+0.6	35	4.2±0.40	-	+++
GA_3				
0.1	68	-	6.8±0.07	++
0.2	73	-	7.5±0.22	++
0.3	82	-	7.8±0.07	+
0.4	91	-	8.8±0.13	+
0.5	74	-	7.1±0.19	+
0.6	67	-	6.9±0.08	++
GA_3+BAP				
0.4+0.1	84	-	8.2±0.10	+
0.4+0.2	94	-	9.6±0.04	-
0.4+0.3	75	-	8.8±0.14	+
0.4+0.4	72	-	8.3±0.10	++
0.4+0.5	71	-	7.9±0.04	++
0.4+0.6	64	-	7.0±0.13	+++
GA_3+KIN				
0.4+0.1	66	-	1.6±0.14	+++
0.4+0.2	73	-	1.8±0.11	++
0.4+0.3	78	-	3.0±0.89	++
0.4+0.4	88	-	3.9±0.60	+
0.4+0.5	80	-	3.5±0.17	++
0.4+0.6	60	-	2.8±0.13	+++

- Callus not formed, + Little, ++ Moderate, +++ High

Table 2. Effect of different concentrations of IBA, IAA and NAA on *in vitro* rooting in *W. volubilis* after six weeks. (20 replicates per treatment)

Plant growth Regulators (mg/l)	Percentage of Responding cultures	Mean No. of Roots /explant	Average Root length (cm)
IBA			
0.2	60	9.3±0.47	10.0±0.12
0.4	90	11.6±0.80	11.4±0.28
0.6	**95**	**12.2±0.40**	**13.5**±0.13
0.8	80	10.4±0.48	11.0±0.19
1.0	75	9.4±0.48	10.2±0.25
1.2	50	8.0±0.63	9.4±0.37
IAA			
0.2	50	3.4±0.48	2.3±0.07
0.4	60	3.8±0.68	3.1±0.16
0.6	65	4.0±0.63	3.6±0.14
0.8	**80**	**4.8±0.74**	**3.7**±0.14
1.0	55	3.0±0.63	3.0±0.14
1.2	45	2.2±0.40	2.6±0.13
NAA			
0.2	60	1.8±0.74	2.8±0.10
0.4	**75**	**2.4±0.48**	**3.1**±0.13
0.6	55	2.2±0.40	2.9±0.10
0.8	40	1.8±0.74	2.7±0.04
1.0	30	1.4±0.48	2.4±0.06
1.2	25	1.2±0.40	2.0±0.13

- Callus not found, + Little, ++ Moderate, +++ High

ACKNOWLEDGEMENTS

Authors thank Mrs. A. Kethsy Prabavathy, Asst. Professor, Department of Computer Science, Karunya University, Coimbatore for editing the manuscript and other assistance.

REFERENCES

Ignacimuthu, S. (1997). Plant Biotechnology. Oxford and IBH Publishing Co. Pvt Ltd. 180 Pp.

Jeyachandran, R. (2004). *In vitro* culture root formation in *Anisochilus carnosus*. *J. Swamy Bot. Club*. **21**:27-30.

Makunga, N.P., Jeger, A.K. and Staden, J.V. (2006). Improved *in vitro* rooting and hyperhydricity in regenerating tissues of *Thapsia garganica* L. *Plant Cell Tiss. Org. Cult*. **86**:77-86.

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

Poornima, D. and Shivamurthy, G.R. (2005). Root formation in *Qulisqualis indica* L. *J. Swamy Bot. Club* **22**:37-38.

Pullaiah, T. (2002). In. Medicinal Plants in India, *Wattakaka volubilis* (L.f) stapf Daya Publishing House Co. Pvt Ltd. Delhi. Pp. 535-536.

Rajani Tomar, and Sharad Tiwari. (2006). *In vitro* Morphogenesis of *Rauwolfia serpentine* L. Through Cotyledons. *Plant Cell Biotechnology and Molecular Biology*.**7 (1&2)**: 53-58.

Ramawat, K.G. (2003) Plant Biotechnology, Publishing S. Chand and Co Ltd New Delhi. Pp. 1-37.

Roxana Ahmed, (2005). Best root formation in *Annona squamosa*. *Asian J. Microbial Biotech. Environment Science*.**7 (2)**: 191-194.

Sharma, N., Chandel, K.P.S. and Paul, A. (1993). *In vitro* propagation of *Gentiana kurroo*: an indigenous threatened plant of medicinal importance. *Plant Cell Tiss. Org. Cult*. **34**:307-309.

Sharma, R.K., Wakhlu, A.K. and Boleria, M. (2004). Micropropagation of *Anethum graveolens* L. through axillary shoots proliferation. *J. Plant Biochem & Biotech*. **13**: 157-159.

Sultana, R.S., Bari, A.S and Miah, M.A. (2003). *In vitro* propagation of Karalla (*Momordica charantea* Linn.) from nodal segment and shoot tip. *Journal of Biological Science* **3**:1134-1139.

Tsay, H.S., Gau, T.G. and Chen, C.C. (1989). Rapid clonal propagation of *Pinellia ternate* by tissue culture. *Plant Cell Reports* **8**:450-454.

Vinothkumar, D., Senthil Kumar, S. and Murugavel, S. (2010). Micropropagation of *Pseudarthira viscid* (L) from nodal explants – an medicinal plant. *Journal of Advanced Biotechnology* **10 (4)**:16-18.

Vinothkumar, D., John Britto, S., Sebastin raj, S., Philip Robinson, J. and Senthil Kumar, S. (2009). Callus regeneration from stem explants of *Pseudarthira viscida* (L.) Wight and Arn. –a Vulnerable medicinal plant. *African Journal of Biotechnology* **8 (17)**: 4048-4051.

Yoshimura, S., Narita, H., Hayashi, K. and Mitsuhashi. H. (1983). Studies on the constituents of Asclepiadaceae plants. LVI. Isolation of new antitumor-active glycosides from *Dregea volubilis* (L.) BENTH. *Chem. Pharm. Bull.* (Tokyo): 3971-3983.