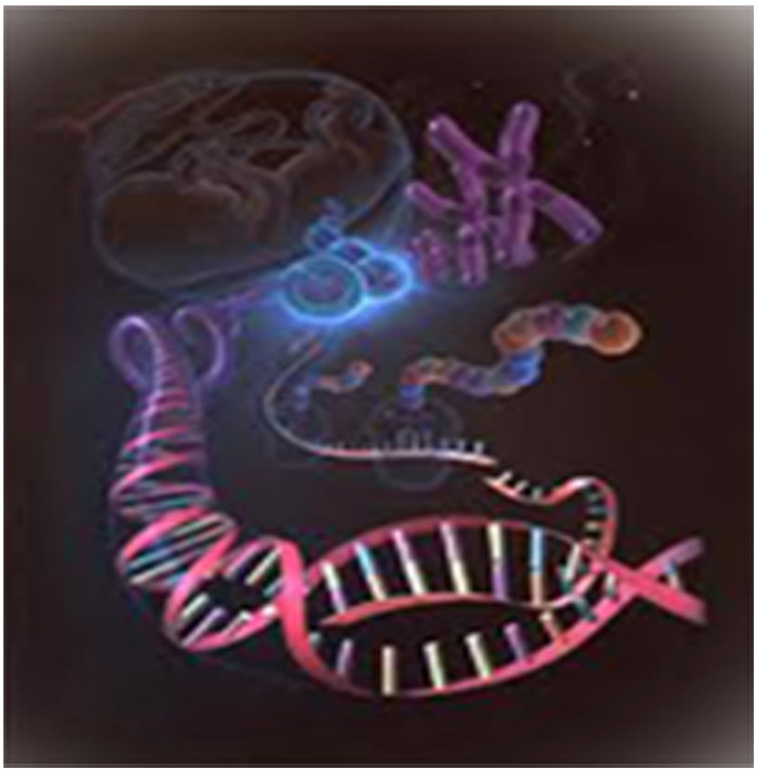
IJLBPR

ISSN 2250 - 3137
Vo1. 3, No. 2, April 2014

International Journal of

Life Sciences Biotechnology and Pharma Research



International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

www.ijlbpr.com

Email : editorijlbpr@gmail.com or editor@ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol. 3, No. 2, April 2014

Research Paper

IN VITRO PROPAGATION OF *ACALYPHA INDICA* LINN.: A MEDICINALLY IMPORTANT PLANT

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An *in vitro* regeneration protocol for *Acalypha indica* was developed using nodal explants on MS medium augmented with different concentrations and combinations of plant growth regulators for direct and indirect regeneration. The highest frequency (63.2%) of multiple shoot regeneration with maximum number of shoots (4.1shoots per explants) was noticed on ½ strength MS medium supplemented with BAP (1.0 mg/L) in combination with KIN (0.5 mg/L). Highest percentage of callus induction (81.5%) from nodal segments was observed on ½ strength MS medium supplemented with 2, 4-D (1.5 mg/L). The *in vitro* derived calli were sub-cultured for shoot regeneration. ½ strength MS medium fortified with BAP (1.0 mg/L) in combination with NAA (0.5 mg/L) showed the highest percentage (62.8%) shoot proliferation from the nodal segments derived calli. Elongated shoots were rooted best on ½ strength MS medium containing IBA (1.0 mg/L) producing maximum number of roots with 70% response. The plantlets were gradually acclimatized and successfully transferred to field condition with 100% survival rate within 6 weeks after rooting. The standardized protocol reported in this study may help in large scale propagation of this plant species which is currently exploited from the nature.

Keywords: *Acalypha indica, In vitro* propagation, Calli, Nodal segment, Regeneration

INTRODUCTION

Acalypha indica L. of the family *Euphorbiaceae* is an annual herb found wild throughout Southern Asia, including India, to Philippines and tropical Africa. The plant has been used as drug in traditional medicine since ancient times. It contains an active compound named 'acalyphine' which is used in the treatment of sore gums. The roots of the plant is used as laxative and leaves for scabies and other cutaneous diseases (Perry, 1980). The plant extract showed antibacterial activity against *Aeromonas hydrophila* and *Bacillus cereus* (Samy et al., 1999), post-coital antifertility effect in female albino rats (Hiremath et al., 1999), wound healing activity in rats (Reddy et al., 2002), *Viper russelli russelli* venom neutralization potential (Shirwaikar et al., 2004), antifungal activity against *T. mentagrophyte, A. flavus* and *C. albican* (Sanseera et al., 2010), antioxidant and anticancer activities (Sanseera et al., 2012). The whole plant possesses diuretic, purgative and anthelmintic properties which are

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used in the treatment of asthma, bronchitis, pneumonia, scabies and other cutaneous diseases (Kirtikar and Basu, 1999). Their pharmacological effects are due to the chemical constituents like cyanogenic glucoside, acalyphine, â-sitosterol, flavonoids, tannins, aurantiamide, acalyphamide, succinimide, four Kaempferol glycosides (mauritianin, clitorin, nicotiflorin and biorobin) and the pyranoquinolinone alkaloid flindersin (Talapatra et al., 1981, Nahrstedt et al., 1982, Nahrstedt et al., 2006).

Due to their medicinal importance, many scientific studies have been carried out on the phytochemical and pharmacological values of *A. indica.* Conventional propagation of this species is limited to seasonal means, which is difficult and slow in meeting the commercial quantities required. The application of tissue culture techniques has been routinely practiced for the multiplication of the medicinal plants to meet the demands of pharmaceutical firms and to protect the natural population of rare and endangered plant species. There are no reports to date on micropropagation in this species. Considering the medicinal importance of population of *A. indica*, this programmed of study was designed to develop a protocol for the large scale multiplication of the economically and medicinally important plant *A. indica* through nodal segments and nodal segments derived calli as explants for better exploitation.

MATERIALS AND METHODS

Plant Material and Explants

The plants collected from forest edges near Guwahati city, Assam were maintained in green house of Department of Biotechnology, Gauhati University and used as the explants source. Nodal explants collected from the young sprouts of the stock plants were washed thoroughly with running tap water for 30 minutes followed by 8 minutes wash in 0.1% (w/v) bavistin with 2–3 drops of Tween 20 (v/v). The explants were then washed thoroughly with sterilized double distilled water several times and kept in a laminar air flow chamber. They were then surface sterilized with 0.1% (w/v) aqueous $HgCl_2$ solution for 3 minutes, and subsequently rinsed with sterile double distilled water to remove traces of $HgCl_2$ before inoculation.

Culture Media and Conditions

MS (Murashige and Skoog 1962) medium full and half strength containing 3% sucrose was used in all the experiments. The pH of the medium was adjusted to 5.8 and gelled with 0.8% agar (w/v) agar agar (Hi-Media, Mumbai, India) before autoclaving at 1.1 kg/cm^2 pressure and 121 °C temperature for 15 min. All the cultures were maintained in aseptic culture rooms at 25±2°C temperature and 55 to 65% RH under 12 h photoperiod of 50-60 µmol m^{-2} s^{-1} light intensity provided by cool white fluorescent tubes.

Shoot Proliferation and Multiplication

The nodal explants were inoculated in MS basal medium and ½ MS medium fortified with different concentrations of BAP (0.5-2.0 mg/L), KIN (0.5-2.0 mg/L), 2, 4-D (0.5-2.0 mg/L) and NAA (0.5-2.0 mg/L) individually or in combination for shoot bud induction, callus induction and multiplication of shoots. All the cultures were transferred to fresh medium after 2-3 wks duration. The mean number of shoots and their lengths were evaluated after 6 wks of inoculation.

Rooting and Plant Regeneration

For rooting, the *in vitro* raised shoots were transferred to ½ strength MS medium augmented with various concentrations of auxin, namely, IAA (0.5-1.5 mg/L) and IBA (0.5-1.5 mg/L). Observations were taken at the end of the 4th week. Well rooted shoots were washed in sterile water and transferred to plastic cups containing sterilized mixture of sand, vermi and garden soil (1:1:1) covered with plastic cover and then kept in open diffused light for hardening. After one month, the surviving plants were transferred to pots containing garden soil and maintained in greenhouse for acclimatization.

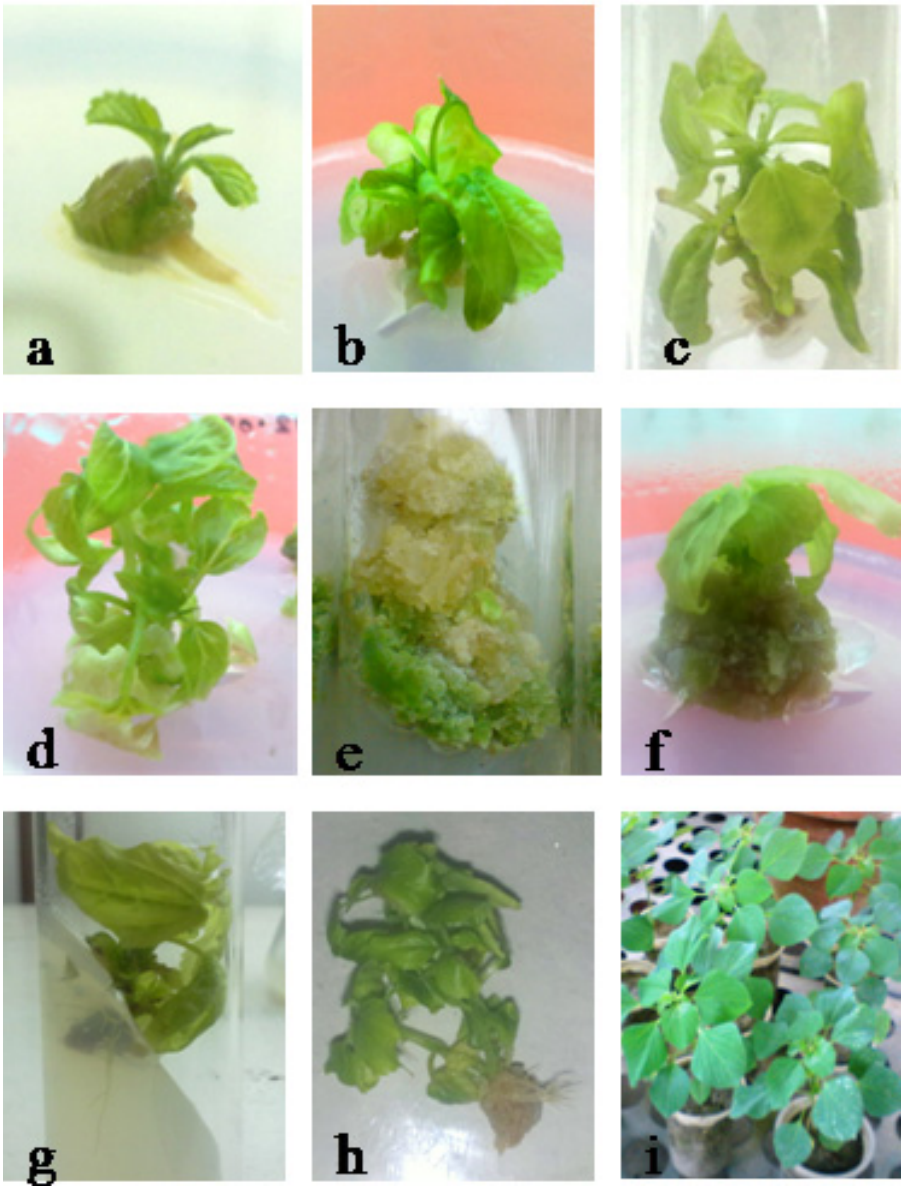
Statistical Analysis

The experiments were conducted in a completely randomized design and repeated three times with 25 replicates in each repeat. The data were analyzed using one way analysis of variance (ANOVA) and the difference between the mean of sample was analyzed by the least significant difference (LSD) test at a probability level of 0.05, using SPSS software version-16.

RESULTS AND DISCUSSION

The present study revealed that ½ strength MS medium was superior for in vitro growth and proliferation of *A. indica* shoots in comparison with MS full strength media. The nodal explants from mature *A. indica* plants were placed on ½ strength MS medium supplemented with different concentrations of BAP (0.5–2.0 mg/L) and KIN (0.5–2.0 mg/L) for shoot initiation (Figure 1). Among the individual cytokinin concentrations tested highest frequency of shoot regeneration was observed on ½ MS medium containing 1.0 mg/L BAP (59.5%) with 3.2 shoots per explants and 3.4 cm shoot length. KIN showed highest frequency of shoot regeneration (56.8%) at 0.5 mg/L, with 2.4 shoots per explants and 3.2 cm shoot length. At higher concentrations of cytokinins the shoot bud induction was found to be suppressed. Cytokinins play a significant role to induce bud break and shoot proliferation from nodal explants. Of the two cytokinins used, BAP was found to be more effective than KIN for shoot bud development from nodal explants. The percent of shoot proliferation increased with increasing the concentrations of BAP up to 1.0 mg/L, thereafter, shoot induction was gradually decreased with increasing concentration of BAP. On the other hand, when KIN concentration was increased beyond 0.5 mg/L, shoot regeneration was gradually suppressed. This result was similar to the results found in *Stevia rebaudiana* (Thiyagarajan and Venkatachalam 2012). An inhibitory effect of higher concentrations of BAP on shoot formation has also been reported earlier in *Pterocarpus marsupium* (Anis et al., 2005). The superiority of BAP over other cytokinin in shoot bud regeneration has been well documented in *Syzygium alternifolium* (Sha Valli Khan et al., 1997) and in *P. marsupium* (Chand and Singh, 2004). Similarly the influence of BAP on induction of multiple shoot buds from nodal explants has been reported in various plant species, including *Quercus euboica* (Kartsonas and Papafotiou, 2007), *Eclipta alba* (Dhaka and Kothari, 2005), *Ulmus parvifolia* (Thakur and Karnosky, 2007) and *Sarcostemma brevistigma* (Thomas and Shankar, 2009). Among the combinations used, BAP (1.0 mg/L) + KIN (0.5 mg/L) combination was found to be the best for regeneration frequency (63.2%) and multiple shoot induction with 4.1 shoots per explants and 5.3 cm shoot length (Table 1). Similar synergistic effect of two cytokinins for enhanced shoot multiplication was

Figure 1: *In vitro* Propagation of *Acalypha indica* from Nodal Explants (a) Shoot bud initiation from nodal segment on ½ MS medium containing 1.0 mg/L BAP, (b-d) shoot initiation and multiplication on ½ MS medium containing 1.0 mg/L BAP and 0.5 mg/L KN, (e-f) callus induction on nodal explants supplemented with 1.5 mg/L of 2, 4-D



reported in *Linum usitatissiumum* (Das et al., 1996 and Sharma, 2008).

The nodal segments cultured on ½ strength MS medium supplemented with various concentration of auxins (2, 4-D and NAA) and cytokinin (BAP) induce callus formation followed by proliferation of multiple shoots through indirect organogenesis with varied percentage. The nodal explants showed highest percentage of callus induction (81%) on ½ strength MS medium supplemented with 1.5 mg/L of 2, 4-D. Compact and friable calli were observed from the nodal

segments. The effect of 2, 4-D in the induction of callus was reported previously in *Withania somnifera* (Manickam *et al*., 2000), *Rhinacanthus nasutus* (Johnson *et al*., 2005) and in *Phyllanthus amarus* (Johnson, 2007). Highest frequency of shoot proliferation (62%) was observed in ½ strength MS medium augmented with BAP (1.0 mg/L) + NAA (0.5 mg/L) with 2.8 shoots per explants and 3.9 cm shoot length from the nodal segments derived calli. Multiple shoot proliferation induced directly from nodal explants cultured on MS basal medium supplemented with different concentrations of BAP + NAA was reported in *Aristolochia tagala* (Biswas *et al.,* 2007) and *A. bracteolata* (Sebastinraj and Sidique, 2011). The *in vitro* derived calli were sub-cultured for shoot regeneration. According to the plant growth regulators supplementation in the medium explants showed different types callus induction and varied frequency shoot proliferation, the results of the various combinations and concentrations of plant growth regulators were tabulated in Tables 2 and 3. Multiple shoot formation from nodal segments derived calli on MS medium augmented with different PGRs was reported in *Gymnema sylvestris* (Roy *et al*., 2008), *Vitex leucoxylon* (Chordia *et al.,* 2010) and *Cralluma stalagmifera* (Sreelatha and Pullaiah, 2010).

The *in vitro* raised shoots were subcultured on ½ strength MS medium augmented with various concentrations of IAA (0.5-1.5 mg/L) and IBA (0.5-1.5 mg/L) for root formation. Shoots produced roots within two weeks of culture and the data were recorded. Highest frequency of rooting (74%) was observed in ½ strength MS

Table 1: Effect of Cytokinin on Shoot Induction of *A. indica* from Nodal Explants

Hormones		Nodal Explants		
BAP	KIN	% of Response	Average Number of Shoots	Average Length of Shoots
0.5	0.0	47.6 ± 0.24^{jk}	1.4 ± 0.10^{ij}	1.8 ± 0.06^{i}
1.0	0.0	59.5 ± 0.37^{b}	3.2 ± 0.06^{c}	3.4 ± 0.10^{d}
1.5	0.0	57.3 ± 0.32^{bc}	2.8 ± 0.06^{d}	2.9 ± 0.10^{g}
2.0	0.0	45.4 ± 0.23^{klm}	1.2 ± 0.09^{jkl}	2.3 ± 0.12^{h}
0.0	0.5	56.8 ± 0.41^{cd}	2.4 ± 0.11^{ef}	3.2 ± 0.10^{def}
0.0	1.0	55.1 ± 0.34^{cdf}	2.3 ± 0.10^{fg}	3.3 ± 0.12^{de}
0.0	1.5	50.4 ± 0.26^{hi}	1.6 ± 0.12^{i}	2.9 ± 0.10^{g}
0.0	2.0	46.8 ± 0.42^{kl}	1.3 ± 0.10^{jk}	2.3 ± 0.12^{h}
1.0	0.5	63.2 ± 0.26^{a}	4.1 ± 0.11^{a}	5.3 ± 0.04^{a}
1.5	0.5	50.5 ± 0.43^{h}	3.7 ± 0.11^{b}	4.0 ± 0.10^{b}
0.5	1.0	49.1 ± 0.23^{hij}	2.6 ± 0.12^{de}	3.9 ± 0.10^{bc}
0.5	1.5	53.7 ± 0.41^{fg}	2.2 ± 0.11^{fgh}	3.3 ± 0.12^{de}

Note: Values represent means ± SE of 25 replicates per treatment. Means within a column followed by different letters in superscript differ significantly at Pd"0.05 level.

Table 2: Effect of Auxin and Cytokinin in Callus Production of *A. indica* from Nodal Explants

Growth Hormones			Nodal Explants	
2, 4-D	NAA	BAP	% of Callus	Types of Callus
0.5	0.0	0.0	63.6 ± 0.42^{de}	G,W
1.0	0.0	0.0	74.3 ± 0.53^{b}	G,W
1.5	0.0	0.0	81.5 ± 0.69^{a}	G
0.0	0.5	0.0	57.6 ± 0.50^{g}	Y,F
0.0	1.0	0.0	68.8 ± 0.41^{c}	Y,F
0.0	1.5	0.0	62.3 ± 0.41^{ef}	Y,F
0.0	0.0	1.0	52.1 ± 0.23^{i}	S,W
0.0	0.0	2.0	54.9 ± 0.38^{h}	S,W
0.0	0.0	3.0	65.2 ± 0.37^{d}	S,G

Note: Values represent means ± SE of 25 replicates per treatment. Means within a column followed by different letters in superscript differ significantly at Pd"0.05 level.S= induced shoot, G= green compact callus, W= white compact callus, Y= light yellowish and F= soft friable callus.

Table 3: Effect of Plant Growth Regulators on Indirect Organogenesis of *A. indica*

BAP	NAA	% of Callus	% of Shoot Formation	Mean Number of Shoots	Mean Length of Shoots
0.5	0.5	20.6 ± 0.36^{f}	41.2 ± 0.44^{f}	1.7 ± 0.11^{c}	2.0 ± 0.06^{d}
1.0	0.5	24.8 ± 0.25^{de}	62.8 ± 0.25^{a}	2.8 ± 0.09^{a}	3.9 ± 0.10^{a}
1.5	0.5	37.3 ± 0.42^{c}	57.6 ± 0.24^{b}	2.2 ± 0.05^{b}	2.4 ± 0.10^{b}
0.5	1.0	25.4 ± 0.47^{d}	55.8 ± 0.25^{bcd}	1.7 ± 0.08^{c}	2.3 ± 0.12^{bc}
1.0	1.0	46.8 ± 0.15^{b}	56.4 ± 0.42^{bc}	1.6 ± 0.12^{cd}	1.7 ± 0.12^{ef}
1.5	1.0	54.1 ± 0.27^{a}	45.1 ± 0.34^{e}	1.3 ± 0.10^{e}	1.8 ± 0.12^{de}

Note: Values represent means ± SE of 25 replicates per treatment. Means within a column followed by different letters in superscript differ significantly at Pd"0.05 level.S= induced shoot, G= green compact callus, W= white compact callus, Y= light yellowish and F= soft friable callus.

medium augmented with IBA (0.5 mg/L). Maximum number of roots (23.3) and mean length of roots (4.5) were observed in ½ strength MS medium supplemented with IBA (1.0 mg/L) (Table 4). The results showed consistency with other studies where the addition of IBA promoted the induction of roots in several systems including *Dioscorea zingiberensis* (Chen *et al*., 2003), *Woodfordia fruticosa* (Islam *et al*., 2009) and *Ophiorrhiza eriantha* (Jaimsha *et al*., 2010). The *in vitro* raised plantlets were hardened in polycups containing a mixture of sterile garden soil: vermin: sand (1:1:1) covered with polypropylene bags. The plants were kept in a culture room for 15 days for acclimatization and further transferred into greenhouse condition.

Table 4: Effect of IAA and IBA on Rooting of Microshoots of *A. indica*

Growth Hormones			Rooting	
IAA	IBA	% of Response	Average Number of Roots	Average Length of Roots
0.5	0.0	65.2 ± 0.34^{de}	12.4 ± 0.10^{ef}	2.1 ± 0.06^{f}
1.0	0.0	57.9 ± 0.34^{f}	13.9 ± 0.06^{e}	2.4 ± 0.09^{e}
1.5	0.0	66.6 ± 0.42^{cd}	19.1 ± 0.06^{c}	3.4 ± 0.11^{d}
0.0	0.5	74.4 ± 0.57^{a}	15.5 ± 0.06^{d}	4.0 ± 0.06^{b}
0.0	1.0	70.5 ± 0.46^{b}	23.3 ± 0.12^{a}	4.5 ± 0.10^{a}
0.0	1.5	68.1 ± 0.21^{bc}	21.6 ± 0.11^{b}	3.8 ± 0.10^{bc}

Note: Values represent means ± SE of 25 replicates per treatment. Means within a column followed by different letters in superscript differ significantly at Pd"0.05 level.

CONCLUSION

This report describes a rapid protocol for direct and indirect shoot regeneration from nodal explants of *A. indica.* This protocol can be utilized for commercial scale propagation and conservation of this important medicinal plant species.

ACKNOWLEDGMENT

Department of Biotechnology, Gauhati University is duly acknowledged for permission of carrying out the work.

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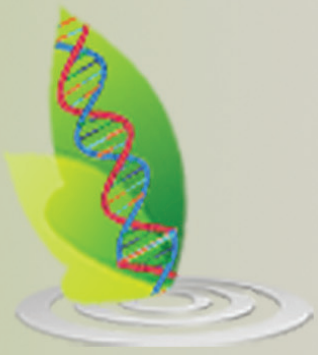

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ISSN 2250-3137