Journal of Medicinal Plants Research Vol. 5(20), pp. 5044-5049, 30 September, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

High frequency regeneration and shoot multiplication in *Andrographis lineata wall. ex. nees*: an endemic medicinal plant of south India

V. Sudarshana Deepa, K. Rajaram, M. Anis Kumar, Soni Das and P. Suresh kumar*

Department of Biotechnology, Anna University of Technology, Tiruchirappalli-620024, Tamilnadu, India.

Accepted 26 August, 2011

An attempt was made to micropropagate the potential medicinal plant *Andrographis lineata Wall. ex. Nees* using shoot tip and nodal explants. Maximum number of shoots were produced from shoot tip explants (25.7 ± 0.11 shoots /explants) in the optimized concentration of the MS medium containing 1.5 mg/l BAP in combination with 30.0 mg/l AdS compared to the nodal explants (15.0 ± 0.19 shoots/explants). The juvenile *in vitro* regenerated shoots were elongated (7.0 ± 0.6 cm) in 0.3 mg/l GA_3. Rooting was best induced in MS medium supplemented with 1.0 mg/l IBA. The plantlets were successfully hardened in the plant growth chamber and later transferred to greenhouse at 70% survival rate.

Key words: *Andrographis lineata*, shoot tip explants, nodal explants.

INTRODUCTION

In recent years there has been an increased interest for *in vitro* culture techniques which offer a viable tool for mass multiplication and germplasm preservation of rare, endemic and endangered medicinal plants (Villalobos and Engelmann, 1995).These technologies could be a cost effective means of high volume production of the elite planting material throughout the year, without any seasonal constraints (Cassell et al., 1999). *Andrographis lineata Wall.ex.Nees* (AL) is a member of Acanthaeceae family which is an erect perennial herb widely distributed throughout Deccan and Carnatic regions of south India (Gamble, 1956). All the parts of AL are important in the traditional system of medicine in India which has been extensively used for snake bites, antipyretics, cancer and inflammation (Alagesaboopathi and Balu, 1995; Alagesaboopathi, 2000). The methanolic leaf extract of AL was reported for its antimicrobial (Ignacimuthu et al., 2006), diuretic, hepatoprotective (Sangameswaran et al., 2007a, b), insecticidal activity (Elango et al., 2010) and detoxification action on Indian cobra *Naja naja* venom (Dinesh et al., 2010). The ethanolic stem extract of AL was reported for its significant scavenging activity in the *in vitro* models (DPPH, Nitric oxide, lipid peroxidation, reducing power and superoxide assays). Chemical composition of the ethanolic stem extract revealed the presence of omega fatty acids which might be responsible for the antioxidant activity (Sudarshana and Suresh, 2010). Flavanoids such as 5, 7, 2′, 3′, 4′-pentamethoxyflavone (1), 2′-hydroxy-2, 4′, 6′-tri-methoxychalcone (2) dihydroskullcapflavone (3), together with 17,19,20-trihydroxy-5β, 8α H, 9β H,10β-labd-13-en-16,15-olactone (4), a known diterpenoid and six known flavonoids,5-hydroxy-7,8-dimethoxyflavanone (5), 5-hydroxy- 7, 8, 2′, 3′, 4′-pentamethoxyflavone (6), 5, 2′-dihydroxy-7-methoxyflavanone (7), 5,20-dihydroxy-7,8-dimethoxyflavone (8), 5, 2′-dihydroxy-7-methoxyflavone (9) and 5,2′-dihydroxy-7-methoxyflavone 2′-O-β-d-glucopyranoside (10) were isolated from the methanolic whole plant of AL (Kishore et al., 2003). The high demand of the flavone glycoside andrographolide which is originally extracted from *Andrographis paniculata* by the pharmaceutical industry is largely met by extraction of the compound from wild; however the commercial exploitation of this compound is hampered due to its limited availability. AL is a potential alternative source of andrographolide (Alagesaboopathi, 2000). The heavy demand of andrographolide in Indian as well as international markets has motivated Indian farmers to start commercial cultivation of this medicinal plant

*Correspondingauthor.E-mail: sureshbiotech2003@yahoo.co.in. Tel: +91-9786075353. Fax: 431- 2407333.

(Purkayastha et al., 2008; Balu and Alagesaboopathi, 1995).

The conventional propagation of *A. lineata* is limited to vegetative means, which is difficult and slow (Balu and Alagesaboopathi, 1995). Seed germination studies in *A. lineate* recorded 38% response (Alagesaboopathi and Senthilkumaran, 2006). Therefore, measures to develop micropropogation protocols for elite stocks of AL are warranted. Hence an attempt was made to develop an *in vitro* multiplication protocol through high frequency axillary shoot proliferation from shoot tip and nodal explants supplemented with polyamine (Adenine sulphate).

MATERIALS AND METHODS

Plant material

The medicinal plant AL *was* collected in the Kolli Hills of Namakkal (Dt), Tamilnadu, India. The plant was identified by the Botanical Survey of India (BSI). A voucher specimen (BSI/SC/5/23/07-08/Tech.1803) was deposited in the Rappinart Herbarium, St.Joseph's College, Tiruchirappalli, Tamilnadu, India. The plants were established in the Medicinal Garden at Anna University of Technology, Tiruchirappalli, Tamilnadu, India.

Explant source and surface sterilization

The explants shoot tips (1.5 cm) and nodal explants (1.0 cm) of approximate size (0.5 to 1.0 cm) were washed thoroughly under running tap water for 30 min followed by treatment with the fungicide Bavastin (0.05% w/v in water) for 5 min and then washed with sterile distilled water 3 to 4 times to remove the surface debris. Explants were then washed in sterile distilled water containing 2 to 3 drops of Tween-20 for 10 min, rinsed in sterile distilled water 3 to 4 times, disinfected with 70% alcohol for 30 s, and finally rinsed with sterile, double distilled water under aseptic condition 3 to 4 times.

A final surface sterilization with 0.1% w/v aqueous $HgCl_2$ solution for 3 min was then performed, followed by rinsing with sterile double-distilled water.

Media and culture conditions

The basal medium used in this investigation consists of MS (Murashige and Skoog, 1962) supplemented with 30 g/l sucrose (Himedia, India) and 0.8% agar (Himedia, India) was used as the gelling agent on respective media. The pH of all media were adjusted between 5.7 to 5.8 using 0.1 N NaOH or 0.1N HCl before solidification. The media was autoclaved at 121°C for 15 min for sterilization.

The medium was distributed to the test tubes and plugged with cotton wrapped in gauss cloth. The tubes were autoclaved and used. All the cultures were incubated in culture room at 25 ± 2°C under 16/8 h (light/dark) cycle with a light intensity of 50 μmol^{-2} s^{-1} supplied by cool white fluorescent lamps and with 60 to 65% relative humidity.

Multiple shoot induction

For multiple shoot induction shoot tips and nodal explants were cultured in Murashige and Skoog (MS) medium (15 ml) in culture tubes (25X150 mm, Borosil, India) with various cytokines: 6-benzylaminopurine (BAP), kinetin (KN), thidiazuron (TDZ), and 2-isopentenyl adenine (2-iP) at different concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) in combination with (5.0 to 50.0 mg/l) AdS to improve the efficiency of shoot multiplication. All the growth regulators were procured from Sigma, St.Louis, USA. The growth regulator TDZ and GA_3 (Gibberallic acid) was filter sterilized (0.2 µm pore size, Pall Gelman Sciences, USA) and added to warm autoclaved media. Data on percentage of responding explants were recorded after four weeks of culture initiation.

Elongation of shoots

The juvenile *in vitro* regenerated shoots were transferred to MS medium supplemented with GA_3 (0.5 to 2.5 mg/l) for shoot elongation and growth. The data were recorded after two weeks of culture initiation.

Rooting and acclimatization

Individual elongated shoots (5.0 to 7.0 cm) were transferred to MS medium containing various concentrations of auxins indole-acetic acid (IAA), indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA) at different concentrations (0.5, 1.0 and 2.0 mg/l) for rooting. Data's were recorded on percentage of rooting, number and length of roots. Hardening of rooted plants was performed in paper cups containing autoclaved sand, soil and vermiculate mixture (2:1:1.v/v/v). Plants were covered with polythene bags to maintain adequate moisture content and grown in the growth chamber (Caltan, India) at (28°C day, 20°C nights, 16 h day-length with 70% relative humidity) for 2 weeks and then transferred to the greenhouse. After a week the plants were transferred to the nursery.

Statistical analysis

All experiments were set up as randomized complete block designs with atleast 50 explants. Each experiment was repeated thrice. The percentages of explants displaying multiple shoot initiation responses were determined and the number of shoots were noted and tabulated for every subculture. Data's were analysed using one-way ANOVA.

The mean values of treatments were subjected to Duncan Multiple Range Test (DMRT). Significance was determined at $p<0.05$ (Gomez and Gomez, 1976) by using the software SPSS 11.09 for Windows operating system.

RESULTS AND DISCUSSION

Multiple shoot induction

The shoot buds that developed on medium containing BAP, KN, TDZ and 2-iP showed stunted growth which is also been reported in the same genus *A. paniculata* (Purkayastha et al., 2008) and *Andrographis echoides (L) Nees* (Hemalatha and Vadivel, 2010). Hence the shoot proliferation efficiency was improved by supplementation of AdS for promotion of adventious shoot formation. There was quick emergence of shoot bud from the shoot tip explants after the 5th day of inoculation whereas the

Table 1. Effect of various concentrations of cytokinins supplemented with 30.0 mg/l AdS for multiple shoot induction in shoot tip and nodal explants of *Andrographis lineate*.

Growth regulators (mg/l)	Sources of explants					
	Shoot tip			Nodal segments		
	Percentage of explants produced shoots	Average number of shoot per explants	Mean shoot length (cm)	Percentage of explants produced shoots	Average number of shoot per explants	Mean shoot length (cm)
BAP						
0.5	85^{cd}	8.5±0.13^{ef}	2.4±0.02^{a}	60^{ij}	6.3±0.63^{d}	2.0±0.03^{a}
1.0	88^{bc}	11.3±0.22^{c}	2.2±0.01^{bc}	72de	8.1±0.58^{b}	1.9±0.07^{b}
1.5	95^{a}	25.7 ±0.11^{a}	2.1±0.01^{cd}	85^{a}	15.0 ±0.19^{a}	1.7±0.04^{bc}
2.0	82^{ef}	18.5±0.26^{b}	2.0±0.06^{de}	80^{bc}	4.2±0.25^{g}	1.4±0.06^{de}
2.5	80^{fg}	7.8±0.21^{h}	1.9±0.03^{f}	71^{ef}	3.0±0.21^{lm}	0.9±0.05^{gh}
KN						
0.5	75^{hi}	4.8±0.23^{l}	2.3±0.^{05ab}	52no	2.01±0.54^{n}	1.7±0.04^{bc}
1.0	82^{ef}	8.1±0.17^{fg}	2.0±0.^{08de}	59^{k}	3.12±0.26^{kl}	1.5±0.03^{cd}
1.5	83de	8.5±0.30^{ef}	1.3±0.03^{hi}	60^{ij}	3.0±0.17^{lm}	0.8±0.07^{hi}
2.0	89^{b}	11.8±0.26^{c}	1.6±0.04^{gh}	68^{g}	5.6±0.14^{f}	1.2±0.06^{ef}
2.5	74^{ij}	5.2±0.34^{j}	1.0±0.02^{ij}	55^{lm}	3.0±0.32^{lm}	0.7±0.07^{ij}
TDZ						
0.5	55^{q}	3.4±0.33^{mn}	1.8±0.04^{fg}	54^{mn}	2.0±0.34^{n}	1.7±0.04^{bc}
1.0	60no	5.2±0.23^{j}	1.0±0.07^{ij}	62^{hi}	4.0±0.^{12gh}	0.8±0.09^{hi}
1.5	57^{p}	3.6±0.45^{m}	1.3±0.09^{hi}	57^{kl}	3.5±0.45^{ij}	1.2±0.03^{ef}
2.0	39	3.0±0.34^{no}	0.8±0.03^{kl}	48^{p}	3.3±0.23^{jk}	0.7±0.02^{ij}
2.5	28	3.0±0.45^{no}	0.7±0.01^{lm}	40^{pq}	3.0±0.34^{lm}	0.7±0.01^{ij}
2-iP						
0.5	72^{jk}	4.7±0.44	2.0±0.02^{e}	68^{g}	3.6±0.23^{i}	1.9±0.04^{b}
1.0	70^{kl}	8.7±0.23^{e}	1.9±0.06^{f}	77^{d}	4.2±0.34^{g}	1.5±0.06^{cd}
1.5	78^{h}	9.2±0.26^{d}	1.3±0.05^{hi}	82^{ab}	7.6±0.21^{c}	1.0±0.04^{fg}
2.0	63^{m}	7.4±0.27^{hi}	0.9±0.07^{k}	71^{ef}	6.0±0.22^{de}	0.8±0.03^{hi}
2.5	62^{mn}	5.0±0.34^{jk}	0.7±0.08^{lm}	65^{gh}	3.1±0.22^{kl}	0.7±0.02^{ij}

Each experiment was repeated thrice with 50 explants. Values with the same letter within the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

nodal segments responded by an initial enlargement of the dormant axillary buds followed by bud break shoot of adventious shoot formation was observed within 4 weeks in the shoot tip explants at an optimised concentration of 1.5 mg/l BAP along with 30 mg/l (AdS) (Table 1) with 25.7 ± 0.11 per explant (Figure 1a and b) compared to the nodal explants which showed 15.0 ± 0.19 shoots per explants formation (Figure 1c and d).

Similar strategy for using AdS as an adjuvant has also been adopted effectively for many other plant species such as *Curcuma angustifolia Roxb*. and *Holarrhena antidysenterica* Wall, *Bacopa monnieri* (Shukla et al., 2007; Raha and Roy, 2001; Ramesh et al., 2006). Thus, BAP when added along with AdS exhibited synergetic effect and improved the cell proliferation efficiency (Ramesh et al., 2006).

Shoot elongation

The stimulative effect of GA_3 on elongation of shoots is well known as it has been found to promote cell division and elongation in the apical zone of shoots (George et al., 1993). GA_3 at 0.3 mg/l induced maximum percentage (87.0%) of shoot elongation in 2 weeks with shoot length (7.0 ± 0.18 cm) and number of nodes 4.0 nodes/shoot (Table 2 and Figure 1e). Similar results were reported in *A. paniculata* (Purkayastha et al., 2008), *Graptophyllum pictum* L. (Justin and Wilson, 2010) and *Andrographis echoides (L.) Nees* (Hemalatha and Vadivel, 2010).

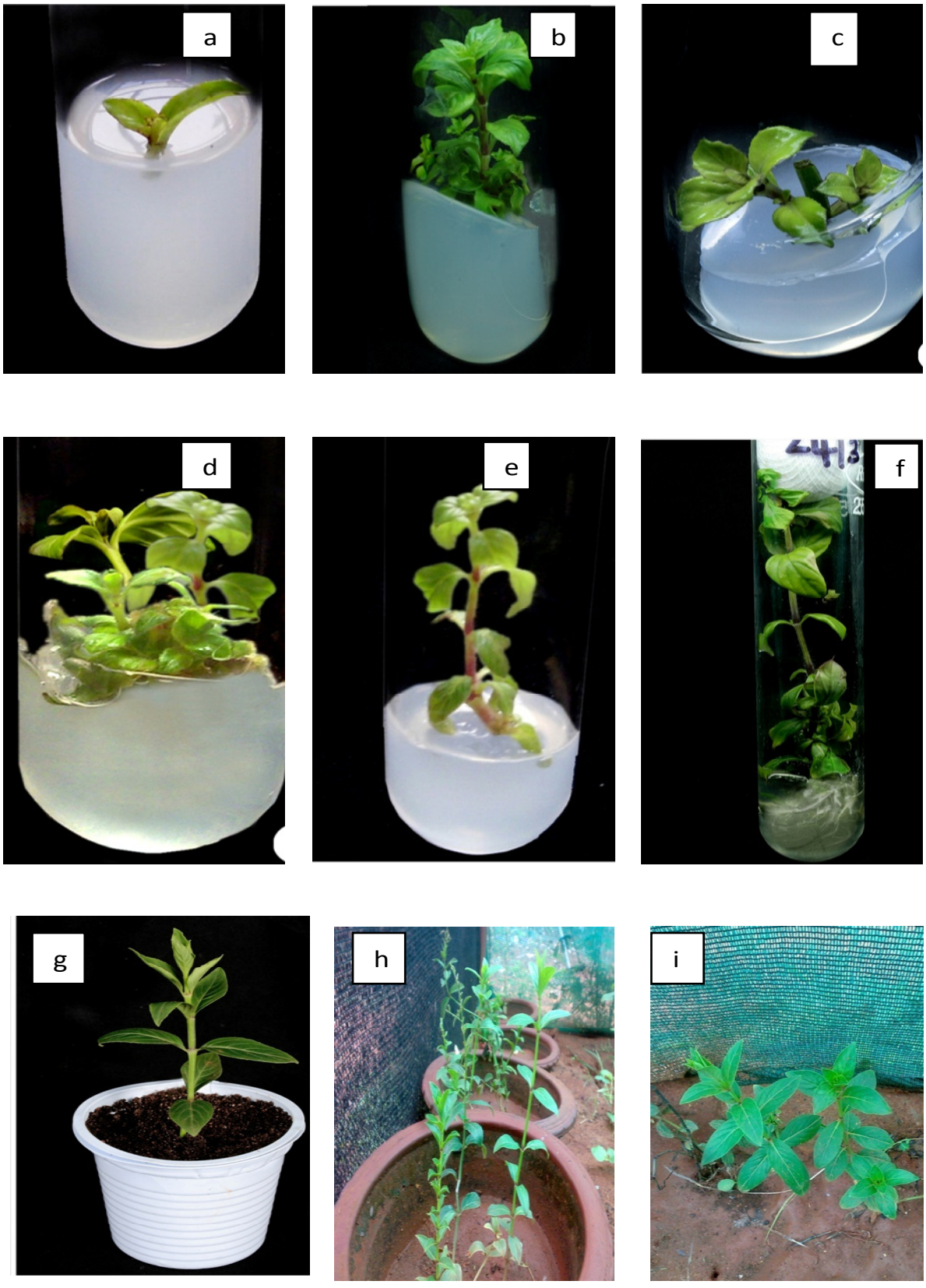

Figure 1. (a) Shoot tip explant supplemented in MS+30.0 mg/l AdS + 1.5 mg/l BAP. (b) Multiple shoot induction in shoot tip explant. (c) Nodal explant supplemented in MS+30.0 mg/l + 1.5 mg/l BAP.d) Multiple shoor induction in nodal explant. (e) Elongation of regenerated shoots in GA_3 0.3 mg/l. (f) Root induction of elongated shoots in MS +1.0 mg/l IBA. (g) Hardening. (h) and (I) Acclimatized plant in the nursery.

Table 2. Effect of GA_3 on elongation of shoots of *Andrographis lineata.*

Concentration of GA_3 (mg/l)	Percentage of response	Maximum elongation of shoots (cm)	Mean no of nodes
0.1	55^{e}	3.3±0.2^{e}	2.5±1.1^{de}
0.2	72^{b}	4.8±0.4^{d}	3.0±0.3^{cd}
0.3	87^{a}	7.0±0.6^{a}	4.0±0.1^{a}
0.5	68^{c}	6.5±0.8^{b}	3.8±0.2^{b}
1.0	60^{cd}	5.1±0.2^{c}	3.5±0.4^{bc}

Each experiment was repeated thrice with 50 explants. Values with the same letter within the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

Table 3. Effect of different auxins on rooting of the *in vitro* raised shoots of *Andrographis lineata*.

Growth regulators (mg/l)	Percentage of root induction	Mean no. of shoots per explants	Mean root length (cm)	Days to emergence of roots
IBA				
0.5	68^{c}	8.22 ±0.12^{b}	2.81±0.81^{b}	15-18
1.0	85^{a}	11.94±0.45^{a}	3.02±0.56^{a}	07-10
2.0	75^{b}	6.11±0.45^{c}	2.62±0.88^{d}	13-17
IAA				
0.5	65^{cd}	3.61±0.67^{d}	2.7±0.45^{c}	14-8
1.0	51^{ef}	2.41±0.44^{f}	1.73±0.78	14-20
2.0	57^{e}	3.29±0.32^{de}	2.11±0.79^{f}	15-21
NAA				
0.5	45^{gh}	2.11±0.14^{hi}	2.22±0.23^{e}	17-25
1.0	51^{ef}	2.34±0.56^{fg}	2.10±0.88^{g}	18-23
2.0	47^{g}	2.17±0.99^{gh}	2.05±0.22^{h}	18-25

Each experiment was repeated thrice with 50 explants. Values with the same letter within the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

Rooting

Although the promotive effect of auxins was achieved in eliciting rooting response (D'Silva and D'Souza, 1992) their type and level in the nutrient medium were found to vary from tissue to tissue and species to species (Rao and Padmaja, 1996).

The elongated shoots regenerated from shoot tip and nodal explants failed to produce roots when cultured on media containing half or full strength MS medium without any growth regulator even after 35 d of culture. Among the various concentrations of auxins tested IBA at 1.0 mg/l produced maximum no of roots/explants (11.94±0.45) within a week compared to IAA or NAA (Table 3 and Figure 1f). The roots were induced directly from the shoot base without an intervening callus phase on media supplemented with IBA. In contrast rooting of shoots occurred through an intervening callus phase on IAA and NAA. Similarly, *Beloperone plumbaginifolia* (Shameer et al., 2009), *Adhatoda vasica* (Khalekuzzaman et al., 2008) and *A. paniculata* (Purkayastha et al., 2008) showed maximum root formation in IBA.

Acclimatization

For acclimatization, well rooted plants were transferred to pots containing a mixture of sterilized sand, soil and vermiculate (2:1:1,v/v/v), covered with a clear plastic bag and grown (28°C day, 20°C night, 16 h day-length with 70% relative humidity) (Figure 1g) (2 weeks). Plants were misted manually with sterilized water once a day during this period to avoid desiccation. At the end of 2nd week the acclimatized plantlets were successfully established in the greenhouse with 70% survival rate and transferred to the nursery (Figure 1 h and i). The present study demonstrates a simple and efficient method for high

frequency direct shoot regeneration from shoot tip and nodal explants of AL.

Conclusion

The system is rapid: starting with the initiation of tissue culture and ending with the transplanting of regenerants to soil takes 3 to 4 months duration. Such a high regeneration frequency would be useful for mass propagation and multiplication of this valuable medicinal plant. Commercial exploitation of this developed protocol is possible, as the shoot tips and nodal segments from *in vitro* raised shoots can be employed as propagules for further multiplication, obviating the dependence on field material. In our laboratory studies on clonal fidelity and phytochemical aspects using target based approach, are in progress to scientifically document the traditional claims of this endemic medicinal plant.

ACKNOWLEDGEMENTS

The authors thank DST-SERC and UGC, Govt of India, New Delhi for providing financial assistances.

REFERENCES

Alagesaboopathi C (2000). *Andrographis spp*: A source of bitter compounds for medicinal use. Ancient Sci. Life, 19: 1-4.

Alagesaboopathi C, Balu S (1995). Antivenom activities of some *Andrographis species.* Ancient. Sci. Life, 14: 187-190.

Alagesaboopathi C, Senthilkumaran G (2006). Seed germination studies on *Andrographis lineata Nees* – An endemic species of medicinal importance. Res. Crop, 7: 353-354.

Balu S, Alagesaboopathi C (1995). Propagation of *Andrographis lineata Nees* by stem cuttings. Ancient Sci. Life, 24: 1-5.

Cassell AC, Walsh C, Belin M, Cambornac M, Rohit JR, Lubrano C (1999). Establishment of plantation from micropropagated *Arnica chamissonsis* a pharmaceutical substitute for the endangered *A.montana*. Plant. Cell. Tiss. Organ., 156: 139-144.

D'silva I, D'souza L (1992). *In vitro* propogation of *Anacardium occidentale L*. Plant. Cell. Tiss. Org., 29: 1-6.

Dinesh KP, Mohanraj S, Gopi K, Baskaran MB (2010). Detoxification action of *Andrographis lineata* leaf extracts on Indian cobra Naja naja Venom. J. Pharm. Res., 3: 2548-2551.

Elango G, Abdul RA, Bagavan A, Kamaraj C, Abduz ZA, Rajakumar G, Marimuthu S, Santhoshkumar T (2010). Studies on effects of indigenous plant extracts on malarial vector, Anopheles subpictus Grassi (*Diptera: Culicidae*). Trop. Biomed., 27: 143-154.

Gamble JS (1956). Flora of the Presidency of Madras. Botanical Survey of India. Calcutta, 2: 1052.

George PS, Ravishankar KA, Venkataraman IV (1993). Clonal multiplication of *Gardenia jesmineides* Ellis through axillary buds culture. Plant. Cell. Rep., 13: 59-62

Gomez KA, Gomez AA (1976). Statistical procedures for agricultural research with emphasis of rice. International Rice Research Institute, Los Banos, Phillippines.

Hemalatha P, Vadivel E (2010). Evaluation of *in vitro* seed germination and micropropogation techniques in *Andrographis echioides* (L.) Nees. Int. J. Agric. Sci., 6: 209-212.

Ignacimuthu S, Ayyanar M, Sharasivaraman K (2006). Ethanobotanical investigations among the tribes in Madurai district of Tamilnadu (India). J. Ethanobiol. Ethanomed., 2: 1-20.

Justin KY, Wilson S (2010). *In vitro* Propagation of *Graptophyllum pictum* L. (Acanthaceae) - A Medicinal plant. J. Pharm. Res., 3: 2201-2202.

Khalekuzzaman M, Rahman MS, Rashid MH, Hossain MS (2008). High *frequency in vitro* propogation of *Adhatoda vasica Nees* through shoot tip and nodal explants. J. Biosci., 16: 35-39.

Kishore PH, Reddy MV, Reddy MK, Gunasekar D (2003). Flavanoids from *Andrographis lineata*. Phytochem., 63: 457-461.

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

Purkayastha J, Sugla T, Paul A, Solleti S, Sahoo L (2008). Rapid *in vitro* multiplication and plant regeneration from nodal explants of *Andrographis paniculata*: a valuable medicinal plant. *In vitro*. Cell. Dev-Pl., 44: 442-447.

Raha S, Roy SC (2001). *In vitro* plant regeneration in *Holarrhena antidysenterica Wall.* through high frequency axillary shoot proliferation. *In Vitro*. Cell. Dev-Pl., 37: 232-236.

Ramesh M, Saravanakumar RM, Pandian SK (2006). Benzyl amino purine and adenine sulphate induced multiple shoot and root induction from nodal explants of Brahmi, *Bacopa monnieri* (Linn.) Penn. Nat. Prod. Rep., 5: 44-51.

Rao S, Padmaja M (1996). Genotypic and explant differences in establishing of callus and shoot regeneration in chickpea (*Cicer arietinum L.*) In: A.I. Khan and A.S.A Farook (eds). Role of Biotechnology in Pulse Crops.pp.159-170.Ukaaz Publication, Hyderabad, India.

Sangameswaran B, Chandraganth RT, Jayakar B (2007a). Hepatoprotective effects of ethanolic leaf extracts of *Andrographis lineata* on liver damage caused by carbon tetrachloride rats. Phytother. Res., 22: 124-126.

Sangameswaran B, Reddy TC, Jayakar B (2007b). Diuretic activity of leaf extracts of *Andrographis lineata*. Nig. J. Nat. Prod. Med., 11: 61-63.

Shameer MC, Madusodanan VP, Sailas BPV (2009). Direct organogensis and somatic embryogenesis in *Beloperone plumbaginifolia (Jacq.) Nees*. Indian. J. Biotechnol., 8: 132-135.

Shukla SK, Shukla S, Koche V, Mishra SK (2007). *In vitro* propagation of *Tikhur Curcuma angustifolia Roxb:* a starch yielding plant. Indian. J. Biotechnol. 6: 274-276.

Sudarshana DV, Suresh KP (2010).Preliminary phytochemical investigations and *in vitro* antioxidant studies on *Andrographis SPP*. J. Pharm. Res., 9: 1-5.

Villalobos VM, Engelmann F (1995). *Ex situ* conservation of plant germplasm using biotechnology. World J. Microb. Biotechnol., 11: 375-382.