Plant Tissue Cult. & Biotech. **19**(2): 177-184, 2009 (December)

PTC&B

In vitro Micropropagation of *Abutilon indicum* L. through Leaf Explants

Jyoti Ranjan Rout, Manorama Mishra, Ritarani Das and Santi Lata Sahoo*

Biochemistry and Molecular Biology Laboratory, P.G Department of Botany, Utkal University, Vani Vihar, Bhubaneswar, Orissa- 751004. India.

Key words: Abutilon indicum, Callus induction, Leaf explants, Micropropagation

Abstract

The present investigation was conducted to develop a protocol for rapid callus induction and plant regeneration from leaf explant of *Abutilon indicum* L. Callus induction and plantlet regeneration at various frequencies were observed on MS using different concentrations of 2,4-D alone or in combination with BAP and Kn. The highest percentage of callus induction was observed with 2.5 mg/l 2,4-D (90) and with combination of 0.5 mg/l Kn (85). Optimum shoot formation was observed on same medium but supplemented with 2.0 mg/l Kn and 1.0 mg/l NAA (11.2). Rooting experiments with half strength of MS revealed that NAA was more suitable for root induction compared to IBA and IAA. The healthy *in vitro* rooting plantlets were successfully transferred to the field. The survival of the plantlets under *ex vitro* condition was 87%.

Introduction

Abutilon indicum L. (Kanghi) belongs to Malvaceae. It is an important medicinal plant. It is grown as an erect velvety-pubescent shrub in the hotter parts of India and also distributed in tropical and subtropical regions of the world. It can grow up to 90 cm in height and flowers are orange-yellow in colour (Chopra et al. 1992, Saxena and Brahmam 1994). It has been reported in the Siddha system of medicine as a remedy for jaundice, piles, ulcer and leprosy (Yoganarasimhan 2000). In some cases, juice from the leaves of the plant is used in combination with the liquid extract of *Allium cepa* to treat hepatoprotective effect (Porchezhian and Ansari 2000, 2005).

Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend on plants for the production of pharmaceutical compounds (Chand et al. 1997). Micropropagation

*Author for correspondence. <santi_bot_uu@yahoo.co.in>.

of elite plants is of importance and propagation of plants through leaf culture allows recovery of genetically stable and true-to-type progeny (Hu and Wang 1983, George and Sherrington 1984). Plant cell and tissue culture has become a major tool in the study of an increasing number of fundamental and applied programs in plant science. Tissue culture techniques are being used globally for the exist conservation of plants. The endeavor is to adopt the method to multiply the medicinal herbs and monitor their secondary metabolites. Conservation of endangered medicinal plants has also been achieved through tissue culture (Gupta et al. 1997, Verma and Kant 1996, Hogue et al. 2000, Nichol et al. 1991, Palai et al. 2000) and cell cultures with significant results (Rao et al. 1996). However, there is no report on *in vitro* regeneration of *Abutilon indicum* L. In this study, the aim was to establish an efficient protocol for regenerating large number of plantlets *in vitro* from the leaf derived callus cultures.

Materials and Methods

The young healthy leaves of *Abutilon indicum* L. were collected from Botanical Garden of Utkal University, Vani Vihar, Bhubaneswar, Orissa, India. They were washed first under running tap water (15 - 20 min) to remove surface adhered particles and then with 5% Teepol for 5 min followed by 70% ethanol for 30 sec. The inoculum was rinsed in distilled water (three - four times) and transferred to Laminarair flow cabinet. The inoculum was then surface sterilized by 0.1% (w/v) $HgCl_2$ for two - three minutes. Finally, the explants were washed in sterile distilled water for three - five times to remove the residual $HgCl_2$ and then cut into appropriate sizes for inoculation on to the sterile medium.

The callus induction medium composed of MS containing 100 mg/l myo-inositol, 3% (w/v) sucrose, 0.8% (w/v) agar with different concentrations of 2,4-D alone or in combination with BAP and Kn for callus induction. The calli were transferred to the fresh medium for further proliferation and maintenance. The well developed calli were selected and subcultured on regeneration media. MS was supplemented with different concentrations of Kn and BAP alone or in combinations with NAA for shoot regeneration. Individual regenerated shoots were excised and used for rooting. Root induction was carried out on half strength of MS supplemented with NAA, IBA and IAA at different concentrations. Medium without plant growth regulators was used as a control. The pH of the medium was adjusted to 5.8 before autoclaving for 15 min at 121ºC. All the cultures were incubated at 25 ± 2ºC with a 16 hr photoperiod (40 $\mu E/cm^2/min/sec$) provided by cool white fluorescent tubes (Phillips, India). Well developed rooted shoots were removed from the culture vessels, washed gently under running tap water and planted in pots containing sand, soil and farmyard manure(1 : 1 : 1). The plantlets were kept in the greenhouse for acclimation (two -

three weeks) before their subsequent transfer to the field. Humidity was maintained by sprinkling water regularly (Jasrai et al. 1999). Plants were gradually exposed to the normal conditions and finally transferred to the Botanical Garden of Utkal University.

Experiments were set in RCBD and each experiment was repeated twice times. Data were recorded on the percentage of response, number of shoots per explants, number of roots and root length per shoot. Means and standard errors were estimated for each treatment.

Results and Discussion

Leaf segments were cultured on MS with various levels of growth regulators, namely 2,4-D alone (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/l) or in combination with BAP or Kn (0.25, 0.5, 1.0, 1.5 and 2.0 mg/l) for callus induction. Morphogenic potentialities of the explant were found to differ depending upon growth regulator supplements (Table 1). After one week of inoculation with 2,4-D (2.5 mg/l) the tissue started swelling (Fig. 1A). Among different concentrations

Table 1. Effect of different concentrations of 2,4-D alone or in combination with BAP or Kn in MS for callus induction from leaf explants of *A. indicum.*

Growth regulators (mg/l)	Response (%)	Callus colour	Degree of callus formation
Hormone free MS	-	-	-
2,4-D			
0.5	55	C	*
1.0	60	CG	*
1.5	75	YG	**
2.0	75	YG	**
2.5	90	CG	***
3.0	80	CG	**
3.5	60	C	*
4.0	55	C	*
2,4-D + BAP			
2.5 + 0.25	40	C	*
2.5 + 0.5	65	CG	*
2.5 + 1.0	80	C	**
2.5 + 1.5	60	YG	*
2.5 + 2.0	45	YG	*
2,4-D + Kn			
2.5 + 0.25	70	YG	**
2.5 + 0.5	85	CG	***
2.5 + 1.0	75	YG	**
2.5 + 1.5	60	C	*
2.5 + 2.0	60	C	*

*Slight callusing. **Considerable callusing and ***Profuse callusing. C = Creamy. CG = Creamy-green. YG = Yellow-green. Data were taken after eight weeks of culture and each treatment consisted of 20 flasks.

of auxins, 2.5 mg/l 2,4-D alone was found to be most effective (90%) for callus induction (Fig. 1B). Callus induction gradually increased up to 2.5 mg/l 2,4-D and then declined. Where a combination of 2,4-D and Kn were applied, the highest callusing rate of 85% was observed for the leaf explant in the medium containing 2.5 mg/l 2,4-D + 0.5 mg/l Kn. When different concentrations of 2,4-D with BAP were tried, 2.5 mg/l 2,4-D + 1.0 mg/l BAP produced 80% of callus. Several researchers observed that 2,4-D was the best auxin for callus induction for monocot and dicot plants (Chee 1990, Malamug et al. 1991). In the present study 2,4-D alone showed better effect for callus induction in *A. indicum.* Similar results were also observed in leaf explants of sugarcane (Begum et al. 1995, Karim et al. 2002).

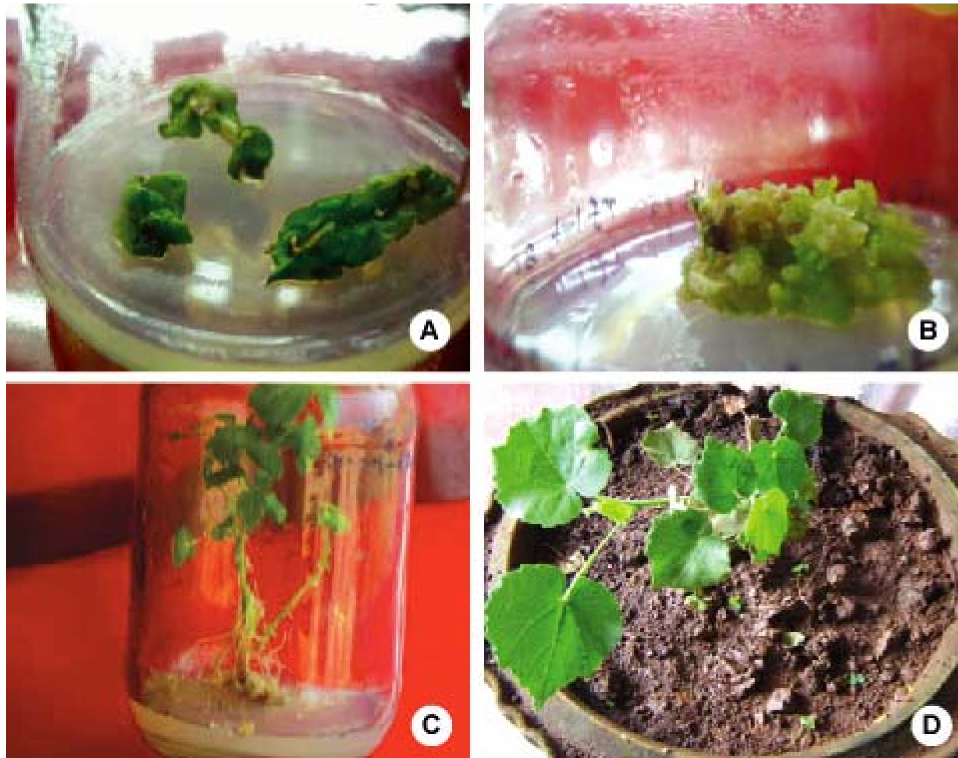

Fig. 1. Callus induction and organogenesis from leaf explants of *A. indicum*. A. Swelling of leaf explants. B. Callus development after eight weeks of culture. C. Root induction. D. *In vitro* plantlet hardened in pot.

Calli produced under light conditions and transferred to MS supplemented with various concentrations and combinations of BAP/ Kn (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l)] along with NAA (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) for shoot regeneration. Hormone free MS was used as a control, which induced shoots at a rate of 2.1 shoots per explant. Shoot formation was noted to be highly influenced by

concentrations and type of the growth regulators. Comparisons between the best individual concentrations for each growth regulator of cytokinins (Kn and BAP) revealed an average of 7.2 (2.0 mg/l Kn) and 5.8 (2.0 mg/l BAP) shoots, respectively. A lower number of shoot multiplication and elongation was observed at higher concentration of Kn and BAP (Table 2). The highest regeneration was achieved on MS medium supplemented with 2.0 mg/l Kn in combination with 1.0

Table 2. Effect of different concentrations of Kn/ BAP alone or in combination with NAA on shoot formation from leaf callus of *A. indicum*. There were 20 explants in each treatment and data (mean ± SEM) were recorded after 6 weeks of culture

Growth regulator (mg/l)			Response	No. of shoots/culture
Kn	BAP	NAA	(%)	(Mean ± SEM)
Hormone free MS			70	2.1 ± 0.2
1.0			70	4.2 ± 0.1
2.0			90	7.2 ± 0.1
3.0			90	6.4 ± 0.2
4.0			80	5.8 ± 0.2
5.0			80	4.0 ± 0.1
	1.0		65	4.2 ± 0.1
	2.0		85	5.8 ± 0.2
	3.0		80	5.1 ± 0.1
	4.0		70	4.5 ± 0.3
	5.0		50	3.0 ± 0.1
2.0		0.5	90	8.4 ± 0.2
2.0		1.0	95	11.2 ± 0.2
2.0		1.5	80	7.9 ± 0.2
2.0		2.0	70	3.2 ± 0.2
2.0		2.5	70	3.8 ± 0.2
	2.0	0.5	90	5.7 ± 0.3
	2.0	1.0	80	4.2 ± 0.2
	2.0	1.5	70	4.2 ± 0.1
	2.0	2.0	65	3.4 ± 0.1
	2.0	2.5	70	4.5 ± 0.3

mg/l NAA after six weeks of inoculation. On this combination, shoot organogenesis was 95% and the number of shoots per culture was 11.2 (data not shown). The synergistic effect of cytokinins in combination with a low concentration of auxin has been reported earlier for several medicinal plant species like *Curcuma longa* (Rahman et al. 2004) and *Withania somnifera* (Sen and Sharma 1991). The present study also exemplified the positive modification of shoot multiplication efficacy by low concentrations of auxin (NAA) in combination with Kn.

There were significant differences among treatments for root induction. Among different concentrations of NAA (0.1, 0.2, 0.5 and 1.0 mg/l) was found to give comparatively better response than IBA and IAA (Table 3). The highest

number of roots (7.2 per explant) was observed with half strength of MS supplemented with 0.2 mg/l NAA (Fig. 1C). The findings agreed with those

Table 3. Effect of different concentrations of auxins on root formation from the *in vitro* grown shoots on half strength of MS medium. Data represents the mean of 20 cultures. Data were recorded after four - six weeks of culture (mean ± SEM).

Growth regulators (mg/l)	Response (%)	No. of roots (Mean ± SEM)	Root length (cm) (Mean ± SEM)
Hormone free ½ MS	50	-	-
NAA			
0.1	75	4.0 ± 0.2	6.6 ± 0.1
0.2	85	7.2 ± 0.3	8.5 ± 0.1
0.5	60	3.9 ± 0.2	6.1 ± 0.2
1.0	60	3.1 ± 0.2	4.3 ± 0.1
IBA			
0.1	70	2.7 ± 0.2	3.3 ± 0.1
0.2	70	3.2 ± 0.8	5.0 ± 0.2
0.5	55	3.0 ± 0.2	5.7 ± 0.2
1.0	60	3.3 ± 0.2	6.3 ± 0.2
IAA			
0.1	60	2.4 ± 0.1	1.8 ± 0.2
0.2	70	4.6 ± 0.3	2.6 ± 0.2
0.5	70	4.1 ± 0.3	2.5 ± 0.2
1.0	50	2.2 ± 0.1	2.4 ± 0.1

observed in other plant species such as *Cephaelis ipecacuanha, Plantago ovata* (Jha and Jha 1989, Wakhlu and Barna 1989). Rooting did not occur in auxin free control. The *in vitro* developed plantlets were transferred to small pot containing sterilised mixture of sand, soil and farmyard manure (1 : 1 : 1) for acclimation. The hardened plants were transferred to the field after 45 days of good growth (Fig. 1D). The survival rate was 87% in the garden.

The present study describes an efficient and easy to handle protocol for *in vitro* regeneration of *A. indicum* which could be considered for large scale multiplication and propagation of this important medicinal plant.

Acknowledgements

The authors are grateful to Professor Manoranjan Kar, Head, Department of Botany, Utkal University for his encouragement and providing laboratory facilities and SAP-DRS Programme of UGC, New Delhi for the financial support.

References

Begum S, Hakim L and **Azam MA** (1995) Efficient regeneration of plants from leaf base callus in sugarcane. Plant Tissue Cult. **5**: 1-5.

Chand S, Sahrawat AK and **Prakash DVSSR** (1997) *In vitro* culture of *Pimpinella anisum* L (anise). J. Plant Biochem. Biotech. **6**: 1-5.

Chee PP (1990) High frequency of somatic embryogenesis and recovery of fertile cucumber plants. Hort. Sci. **25**: 792-793.

Chopra RN, Nayar SL and **Chopra IC** (1992) Glossary of Indian Medicinal Plants. CSIR, New Delhi. India. 2.

George EF and **Sherrington PD** (1984) Plant propagation by tissue culture. England, Eversley, Exegetics Ltd.

Gupta SK, Srivastara AK, Singh PK and **Tuli R** (1997) *In vitro* proliferation of shoots and regeneration of cotton. Plant cell Tiss. Org. Cult. **51**: 149-152.

Hogue A, Islam R and **Arima S** (2000) High frequency of plant regeneration form cotyledon derived callus of *Momordica dioicea* (Roxb) Wild. Phytomorphology **50**(384): 267-272.

Hu CY and **Wang J** (1983) Techniques for propagation and breeding. In: Evans.D.A., Sharp, W.R., Ammirato PV and Yamada Y (eds.) Handbook of plant cell culture. New York. MacMillan Inc. **1**: 177-277.

Jasrai YT, Kannan VR and **George MM** (1999) *Ex vitro* survival of *in vitro* derived banana plants without greenhouse facilities. Plant Tissue Cult. **9**: 127-132.

Jha S and **Jha TB** (1989) Micropropagation of *Cephaelis ipecacuanha.* Plant Cell Report **8**: 437-439.

Karim MZ, Amin MN, Hossain MA, Islam S, Hossin F and **Alam R** (2002) Micropropagation of two sugarcane (*Saccharum officinarum*) varieties from callus culture. Online J. Bio. Sci. **2**: 682-685.

Malamug JJF, Inden H and **Asahira T** (1991) Plantlets regeneration and propagation from ginger callus. Scientia-Hort. **48**: 89-97.

Nichol JW, Slade D, Viss P and **Stuart DA** (1991) Effect of organic acid pretreatment on the regeneration and development (Conversion) of whole plant from callus culture of alfalfa (*Madicago sativa* L.) Plant Sci. **79**: 181-192.

Palai SK, Rout GR, Samantary S and **Das P** (2000) Biochemical changes during *in vitro* organogenesis in *Zingiber officinale* Rosc. J. Plant Biol. **27(2)**: 153-160.

Porchezhian E and **Ansari SH** (2000) Effect of liquid extract from fresh *Abutilon indicum* `leaves and *Allium cepa* bulbs on paracetamol and carbon tetrachloride induced hepatotoxicity. Pharmazie **55**: 702-705.

Porchezhian E and **Ansari SH** (2005). Hepatoprotective activity of *Abutilon indicum* on experimental liver damage in rats. Phytomedicine **12**: 62-64.

Rao PS, Suprasanna P and **Ganapathi T** (1996) Plant biotechnology and agriculture: Prospects for crop improvement and increasing productivity. Sci. Cult. **62**(7-8): 185-191.

Rahman MM, Amin MN, Jahan HS and **Ahmed R** (2004) *In vitro* regeneration of plantlets of *Curcuma longa* Linn.: A valuable spice plant in Bangladesh. Asian J. Plant Sci. **3**: 306-309.

Saxena HO and **Brahmam M** (1994) The Flora of Orissa, Regional Research Laboratory (CSIR), Bhubaneswar, Orissa. **1**: 133-136.

Sen J and **Sharma AK** (1991) Micropropagation of *Withania somnifera* from germinating seeds and shoot tips. Plant Cell Tiss. Org. Cult. **26**: 71-73.

Verma B and **Kant U** (1996) Micropropagation of *Embilica officinale* Gaertz through mature nodel ex plant. J. Playtol. Res. **9**(2): 107-109.

Wakhlu AK and **Barna KS** (1989) Callus initiation, growth and plant regeneration in *Plantago ovata* Forsk. Cv. Gl-2, Plant Cell, Tiss. Cell and Org. Cult. **17**: 235-241.

Yoganarasimhan SW (2000) Medicinal plants of India: Cyber Media, Bangalore **2**:10-11.