Progress. Agric. 19(1) : 39-44, 2008 ISSN 1017-8139

STUDIES ON *In vitro* CALLUS INDUCTION AND REGENERATION FROM NODAL EXPLANTS OF *Cassia alata* L.

M. F. Hasan, M. S. Rahman, M. S. Hossain and M. Rahman[1]
Department of Genetic Engineering and Biotechnology, University of Rajshahi
Rajshahi-6205, Bangladesh

ABSTRACT

A successful protocol for adventitious shoot regeneration was developed from the nodal explant derived callus tissue of *Cassia alata*. Greenish friable callus was induced from the cut surface of the nodal explants on MS medium supplemented with 1.5 mgl^{-1} 2,4-D within twenty days of inoculation. The calli differentiated into adventitious shoots when they were cultured on MS medium supplemented with 1.5 mgl^{-1} 2,4-D and 0.5 mgl^{-1} Kn. In this treatment, the highest number of shoot induction per callus was 6.0 ±1.0. The callus derived shoots rooted on MS medium containing 1.0 mgl^{-1} IBA within ten days of culture. The *in vitro* grown plantlets were acclimatized and successfully transferred to natural condition with 80% survival.

Key words : Callus induction, Shoot regeneration, Nodal explants, *Cassia alata*

INTRODUCTION

Cassia alata is an endangered tropical medicinal plant belonging to the family of Caesalpiniaceae. In Bangladesh, this plant is known as "Dadmardan" or "Dadmari" (Kirtikar and Basu, 1994). This plant is erect, annual large spreading shrub with irregular, angled, glabrous branches (Sarma, 2003) with yellow flowers of short pedicels, spiciform, pedunculate raceme (Kirtikar and Basu, 1994) and fruit is flat, terete, winged, hirsute (Boonkerd *et al*., 2005). Different parts of this plant contain various kinds of alkaloid such as rhein, β-sitosterol, chrysophenol and kaempferol (Sarma, 2003). The plant also contains anthraquinone derivatives such as aloe-emodin, chrysophanol, emodin and physcin that are well known to exhibit a variety of biological activities, such as, antimicrobial, antifungal, antitumar, antioxident, cytotoxin and hypoglycemic activities (Fernand *et al.,* 2008). The leaves of this plant are regarded as an excellent medicine for ringworm disease, itching, cough, asthma, snake-bites, eczema, herpes and skin diseases (Kirtikar and Basu, 1994). This species is of economic interest for its wide ranging pharmacological activity and one of the major constraints in utilizing natural populations is the existence of plant to plant chemovariability. That is why, there is an increasing awareness in people for the use of this herbal medicinal plant day by day. The market and public demand has been so great that there is a great risk of this medicinal plant that may face either extinction or loss of genetic diversity (Siddique, 2005).

[1] Correspondence: *E-mail*: motiur7@yahoo.com

In nature, the propagation of *C. alata* through seeds is difficult because of their hard seed coat – a trait which explains its sparse distribution. It is, therefore important to develop a protocol for *in vitro* propagation to save this medicinally important taxon from further depletion of its population, at the same time to meet up the demand of the traditional medicine industry. Micropropagation offers a great potential for large scale multiplication of such useful species and subsequent exploitation (Boro *et al.*, 1998). There are many reports on media compositions and protocol establishment for the *in vitro* propagation of several medicinal plants, such as *Cassia fistula, C. occidentalis*, *C. hirsuta, C. obtusifolia, C. angustifolia, C. siamea* (Mondal *et al*., 1998*)* and other medicinal plants (Sudhersan and Hussain, 2002). But there are no reports on *in vitro* propagation in this valuable medicinal plant. The present investigation reports the *in vitro* propagation technique that can be used as a potential tool for large scale production and preservation of this valuable germplasm.

MATERIALS AND METHODS

For this investigation, mature seeds of *Cassia alata* were collected from the Campus of Rajshahi University, Bangladesh. The seeds were washed thoroughly in running tap water for 1 hour, then in 1% tween-80 for 10 minutes and washed for several times with autoclave distilled water. For surface sterilization, the pretreated seeds were rinsed in 90% ethyl alcohol for 1 minute and washed with autoclave distilled water for several times to remove traces of alcohol. Seeds were germinated on MS (Murashige and Skoog, 1962) medium.

The nodal explants were aseptically excised (appropriate size 1 cm) from ten days old *in vitro* grown seedlings and inoculated on MS medium containing different concentrations of 2,4-D for callus induction. The calli were cultured on MS medium containing different combinations and concentrations of 2,4-D and Kn for shoot proliferation. Callus derived shoots were isolated and cultured in different concentrations of IBA for root induction. Well rooted plantlets were acclimatized and transplanted in the field condition. Throughout the experiments full strength MS medium with 3% sucrose (W/V) and 0.8% (W/V) of agar was used. The pH of the medium was adjusted to 5.7 before addition of agar. The media were autoclaved at 121^{o}C for 21 minutes. All cultures were maintained at 16 hour photoperiod with 3000 lux light in intensity at 25±2°C. After an interval of 30 days, the experiment was terminated, and the data with respect to cultures producing shoots, number of shoots in each culture, height of the shoots, number of shoots rooted and root lengths were recorded.

RESULTS AND DISCUSSION

Callus induction

MS medium supplemented with different concentrations of 2,4-D were used for callus induction. Within ten to twenty days of culture, callus induction started at the cut surface of the nodal explants, when cultured on MS medium supplemented with 0.1-2.5 mgl^{-1} 2,4-D (Table 1). The highest percentage of callus induction from nodal explants was 93.3% on

the medium containing 1.5 mgl^{-1} 2,4-D and the lowest percentage of callus induction (20.0%) was on MS medium having 0.1 mgl^{-1} 2,4-D. In all the treatments, the induced calli were greenish in color and nodular in nature (Fig. A).

Shoot regeneration

For shoot regeneration, the nodal segment derived calli were subcultured in MS medium supplemented with different combinations and concentrations of 2,4-D and Kn. As per the data recorded (Table 2), the highest mean number of shoots per callus was 6.0±1.0 in MS medium consisting of 1.5 mgl^{-1} 2,4-D and 0.5 mgl^{-1} Kn (Fig. B-C). On the contrary, MS medium supplemented with 0.1 mgl^{-1} 2,4-D and 0.5 mgl^{-1} Kn showed lowest mean number of shoot induction (2.0±1.0). However, an increase or decrease in the concentrations and combinations of 2,4-D and Kn showed a negative trend in shoot proliferation.

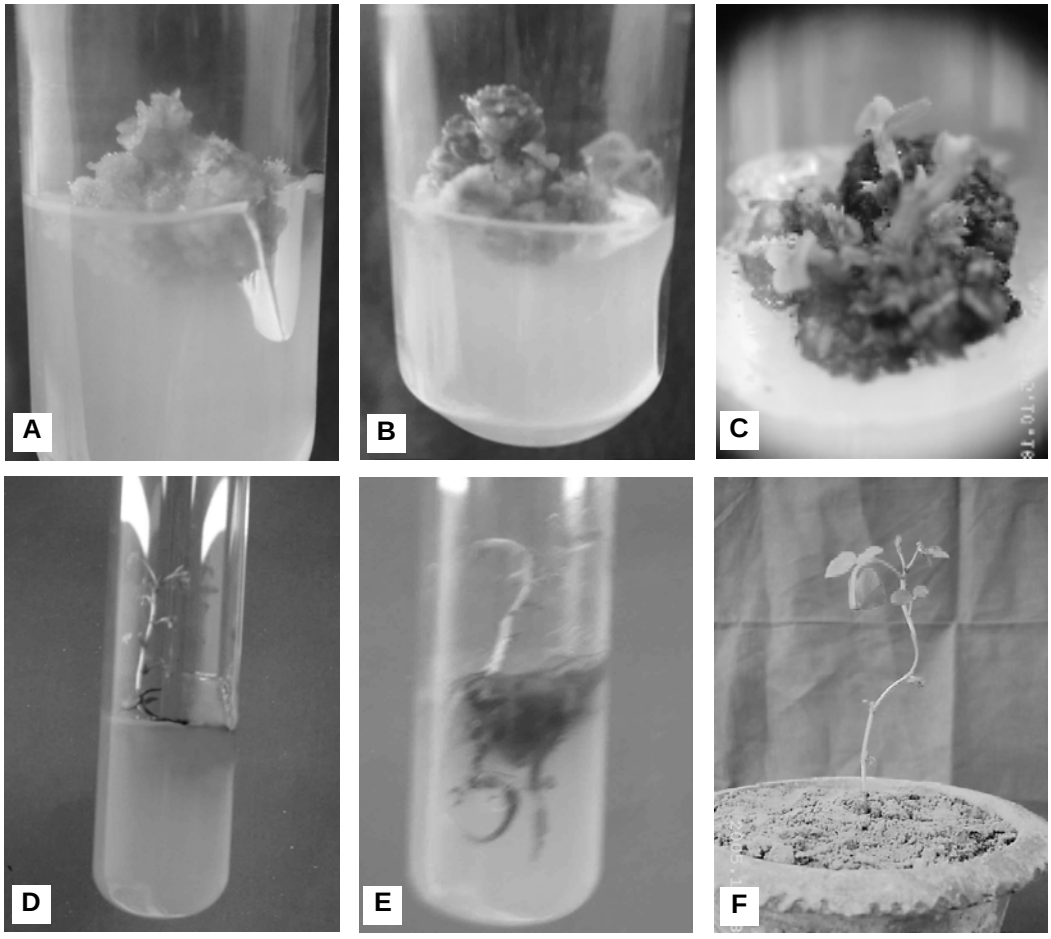

Fig. 1. Callus induction and plant regeneration from nodal explants of *Cassia alata*; A. Callus initiation, B-C. Shoot induction and elongation; D. The inoculated single shoot for root induction; E. Root induction and elongation, F. Acclimatization

In this investigation, shoots were developed from the callus and the regenerated shoots were elongated and rooted. There were significant differences in regeneration frequencies, number of shoots/callus and average length of shoots. As stated by Martin (2002) the high morphogenic efficiency of nodal segment derived callus may be due to the presence of some internal components from the pre-existing axillary buds that are essential for induction of callogenesis. Shoots regeneration *via* a callus phase was the simplest way to induce somaclonal variation and thus pave the way for improvement of the species (Thorpe *et al.*, 1991). Such types of plant regeneration was also reported in many medicinal plant species including *Abrus precatorius* (Biswas *et al.*, 2007), *Acmella calva* (Senthilkumar *et al.*, 2007), *Phellodendron amurense* (Azad *et al.*, 2005), *Plumbago zeylanica* (Das and Rout, 2002) and *Holosma ada-kodien* (Martin, 2002). Callus derived shoots were isolated (Fig. D) and cultured in different concentrations of IBA (0.1-2.0 mgl^{-1}) for root induction. The highest mean number of roots per shoot was 5.0±1.0 in MS medium consisting of 1.0 mgl^{-1} IBA (Table 3; Fig. E). On the other hand, MS medium having 2.5 mgl^{-1} IBA showed lowest mean number of root induction and that was 2.0±1.0. Thus, 1.0 mgl^{-1}IBA was found to be an ideal treatment for root induction. Similar root induction was reported by Rahman *et al*. (2008) in *Vanda tessellata*, Biswas *et al*. (2007) in *Abrus precatorius,* Karuppusamy *et al.* (2006) in *Vanasushava pedata,* Chaplot *et al.* (2006) in *Plumbaga zelanica* and Thiruvengadam and Jayabalan (2000) in *Vitex negundo.* After 30 days, well rooted plantlets were achieved in the present study. Subsequently, the plantlets were removed from agar medium and planted in small pots containing sterile sand, soil and humus in the ration of 1:2:2 (Fig. F). The potted plantlets were covered by transparent polythene sheet to maintain high humidity and within 15-20 days new leaves were emerged from the plantlets that resumed new growth. After 50-55 days, the plants were transplanted in the field condition, where 80% plants survived and their growth was satisfactory.

Table 1. Effect of different concentrations of 2,4-D on callus induction from nodal explants of *Cassia alata*

2,4-D con. (mgl^{-1})	Days to callus initiation	% of explants producing callus (M ± SE)	Nature of callus
0.1	10-18	20.0 ± 0.6	Greenish
0.5	11-19	33.3 ± 1.9	Greenish
1.0	10-17	63.3 ± 2.6	Greenish
1.5	**10-20**	**93.3 ± 1.3**	**Greenish**
2.0	10-19	36.6 ± 1.5	Greenish
2.5	10-19	30.0 ± 0.9	Greenish

N.B. Each value represents an average of 10 replicates and each experiment was repeated at least thrice

Table 2. Effect of different combinations of 2,4-D and Kn in MS medium for shoot regeneration

Conc. (mgl^{-1}) of		Regeneration (%) (M ± SE)	Mean No. of shoots/callus (M ± SE)	Length of shoots (cm) (M ± SE)
2,4-D	Kn			
0.1	0.5	26.6 ± 2.2	2.0 ± 1.0	2.0 ± 0.5
0.5	0.5	43.3 ± 1.9	2.6 ± 0.5	3.0 ± 0.5
1.0	0.5	50.0 ± 1.4	3.0 ± 1.0	3.6 ± 1.0
1.5	0.5	**70.0 ± 1.2**	**6.0 ± 1.0**	**4.0 ± 1.0**
2.0	0.5	56.6 ± 1.7	3.5 ± 1.0	3.2 ± 0.5
2.5	0.5	33.3 ± 2.7	2.5 ± 0.5	1.5 ± 1.0

N.B. Each value represents an average of 10 replicates and each experiment was repeated at least thrice

Table 3. Effect of IBA on root induction in callus derived elongated shoots

IBA (mgl^{-1})	Root induction (%)	No. of roots/shoot (M±SE)	Root length (cm) (M±SE)
0.1	30.0 ± 1.2	2.5 ± 0.5	2.5 ± 0.5
0.5	56.6 ± 0.4	3.0 ± 1.0	3.5 ± 0.5
1.0	**83.3 ± 0.6**	**5.0 ± 1.0**	**4.0 ± 1.0**
1.5	50.0 ± 0.0	3.5 ± 0.5	3.0 ± 1.0
2.0	36.6 ± 1.7	2.5 ± 0.5	2.0 ± 1.0
2.5	23.3 ± 1.4	2.0 ± 1.0	1.5 ± 0.5

N.B. Each value represents an average of 10 replicates and each experiment was repeated at least thrice

In this investigation, a reproducible protocol for plant regeneration was established through callus induction from nodal explants in *C. alata*. It is expected that a standard protocol to induce callus and rapid proliferation of shoots through *in vitro* culture would provide a more homogeneous source of medicine.

ACKNOWLEDGEMENT

The authors wish to thank the Department of Genetic Engineering and Biotechnology, University of Rajshahi, Bangladesh for providing financial support and laboratory facilities to carry out this investigation.

REFERENCES

Azad, M. A. K., Yokota, S., Ohkubo, T., Andoh, Y., Yahara, S. and Yoshizawa, N. 2005. *In vitro* regeneration of medicinal woody plant *Phellodendron amurense* Rupr. through excised leaves. *Plant Cell Tiss. Org. Cult*. 80: 43-50.

Biswas, A., Roy, M., Bari Miah, M. A. and Bhadra, S. K. 2007. *In vitro* propagation of *Abrus precatorius* L. - A Rare Medicinal Plant of Chittagong Hill Tracts. *Plant Tiss. Cult. Biotechnol*. 17(1): 59-64.

Boonkerd, T., Pechsri, S. and Baum, B. R. 2005. A phenetic study of *Cassia sensu* lata (Leguminosae–Caesalpinioideae:: Cassieae : cassiinae) in Thailand. *Plant Syst. Evol*. 252: 153-165.

Boro, P. S., Shrma Deka, A. C. and Kalita, M. C. 1998. Clonal Propagation of *Alternathera Sessilis*: A Biopharmaceutically Potent Herbal Medicinal Plant. *J. Phytol. Res*. 11: 103-106.

Chaplot, B. B., Dave, A. M. and Jasrai, Y. T. 2006. A valued medicinal plant-Chitrak (*Plumbaga zeylanica* Linn.) Successful plant regeneration through various explants and field performance. *Plant Tiss. Cult. Biotechnol.* 16(2): 77-84.

Das, G. and Rout, G. R. 2002. Plant regeneration through somatic embryogenesis in leaf derived callus of *Plumbago indica. Biologia Plantarum.* 45(2): 299-302.

Fernand, V. E., Dinh, D. T., Washigton, S. J., Fakaode, S. O., Losso, J. N., Ravensway, R. O. and Wanner, I. M. 2008. Determination of Pharmacologically Active Compounds in Root Extracts of *Cassia alata* L. by Use of High Performance Liquid hromatography. *Talanta*. 74(4): 896-902.

Karuppusamy, S., Kiranmai, C., Aruna, V. and Pullaiah, T. 2006. Micropropagation of *Vanasusha pedata* – An endangered medicinal plant of South India. *Plant Tiss. Cult. Biotechnol*. 16(2): 85-94.

Kirtikar, K. R. and Basu, B. D. 1994. Indian Medicinal Plants. Voll. II. Jayyed Press. New Delhi. pp. 870-872.

Martin, K. P. 2002. Rapid propagation of *Holostema ada-kodien* Schult. a rare medicinal plant, through axillary bud multiplication and indirect organogenesis. *Plant Cell Rep.* 21: 112-117.

Mondal, A. K., Sanjukta, P. and Mandal, S. 1998. Biochemical analysis of four species of *Cassia* L. pollen. *Aerobiologia*. 14: 45-50.

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

Rahman, M. S., Hasan, M. F., Hossain, M. S., Mandal, A. and Rahman, M. 2008. Plant Regeneration of Orchid (*Vanda tessellata* L.) Through Meristem Culture. *Intl. J. Bio. Res*. 4(1): 8-16.

Sarma, R. 2003. Medicinal plants of India-An encyclopedia. Daya publishing house. Delhi-110035. pp. 46-47.

Senthilkumar, P., Paulsamy, S., Vijayakumar, K. K. and Kalmuthu, K. 2007. *In vitro* Regeneration of the Medicinal Herb of Nilgiri shola, *Acmella calva* L. from leaf Derived callus. *Plant Tiss. Cult. Biotechnol*. 17(2): 109-114.

Siddique, N. A. 2005. *Endangered Medicinal Plants in the Barind Tract and Biotechnological Approaches for Their Conservation*. Ph.D. Dissertation. I. B. Sc. Rajshahi University, Rajshahi. Bangladesh. pp. 1-227.

Sudhersan, C. and Hussain, J. 2002. Development of *In vitro* Protocol for Mass Propagation and Conservation of Some Medicinal Plants. International Association of Plant Tissue Culture and Biotechnology. pp. 217-224.

Thiruvengadam, M. and Jayabalan, N. 2000. Mass Propagation of *Vitex negundo* L. *In vitro J. Plant Biotech*. 2: 151-155.

Thorpe, T. A., Harvey, I. S. and Kumar, P. P. 1991. Application of micropropagation in forest. ***In***: Debergh PC, Zimmerman RH (eds) Micropropagation, technique and application. Kluwer, Dordrecht, pp. 311-336.