CALLUS INDUCTION AND PLANT REGENERATION FROM SHOOT TIPS OF CHAKUNDA (*Cassia obtusifolia* L.)

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ABSTRACT

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The present investigation was conducted at Professor Ali Md. Eunus Laboratory, Depertment of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi, Bangladesh, during the period of April to December 2007. The experiment was carried out to develop an efficient protocol for regeneration of chakunda (*Cassia obtusifolia* L.) *via* callus culture from shoot tips, rooting of callus derived shoots and establishment onto the natural condition. Different concentrations and combinations of auxin and cytokinin were used in full strength ofmedium to observe the callus induction, shoot regeneration and root proliferation. The highest percentage of callus induction was 96.6% observed in the medium supplemented with 2.0 $mg1^{-1}$ 2,4-D. The highest number of shoots was 5.0 found in the medium having 2.0 $mg1^{-1}$ 2.4-D+0.2 $mg1^{-1}$ Kn. The highest percentage of root induction was 80.0% and the highest number of roots per shoot was 5.0 observed in MS medium consisting of 2.0 $mg1^{-1}$NAA. Well rooted plantlets were acclimatized and successfully established onto the natural condition with 70% survival. Regenerated plantlets were morphologically uniform having normal leaf shape and growth.

Key words: Callus induction, plant regeneration, shoot tips, chakunda.

INTRODUCTION

Chakunda (*Cassia obtusifolia* L. Syn. *Cassia tora*) is an important medicinal plant, belongs to the family of Caesalpiniaceae (alt. Fabaceae, Leguminosae).It is an annual, erect, shrub like herb, pinnate leaves, yellow flowers, maxillary stalks and blooming in the month of August to September (Sharma, 2003). The roots extract of this plant contains tannins, flovonoids, alkaloids (Olabiyi *et al*. 2008), betulinic acid, chrysophanol, physcion, stigmasterol, 1-hydroxy-7- methoxy -3- methyl- anthraquinone, 8-o- methylchrysophanol, 1-o- methylchrysophanol and aloe-emodin (Yang *et al*. 2006). The seeds of this plant are effective for insomnia, headache, constipation, oliguria, cough, opthalmia, dacryoliths, omblyopia, ocular congestion and hypertension (Purohit and Vyas, 2005). Leaf extracts of this plant were demonstrated a broad- spectrum of activities against both gram positive and gram negative bacteria and fungi that can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections including gonorrhea, pneumonia, eye infections and mycotic infections (Doughari *et al*. 2008). Joshi (2000) reported that the plant extract is antiviral, spasmolytic and diuretic used against epilepsy, scabies and sores.

In nature, the propagation of chakunda (*C. obtusifolia*) through seed is unreliable due to poor seed quality, erratic germination and seedling mortality, which explain its sparse distribution. It is therefore important to develop a protocol for *in vitro* propagation to conserve 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 has proven as a potential technology for mass scale production of medicinal plant species (Wawrosch *et al*. 2001; Martin, 2003; Faisal *et al*. 2003; Hassan and Roy, 2005). But there are no reports on *in vitro* propagation in this valuable medicinal plant. The present study was undertaken to examine the *in vitro* propagation technique that can be used as a potential tool for large-scale production of this valuable medicinal plant.

MATERIALS AND METHODS

The shoot tips of chakunda were collected from the Campus of Rajshahi University, Rajshahi, Bangladesh. They were washed first under running tap water for 30 minutes and treated with 1% tween-80 for 10 minutes followed by repeated rinsing with autoclave-distilled water. Further sterilization was done under aseptic conditions in a Laminar Airflow Hood. Explants were surface sterilized with 0.1% (W/V) $HgCl_2$ for 10 minutes. Finally, the explants were washed thoroughly with autoclave-distilled water for several times to remove traces of $HgCl_2$. The shoot tips were cut into convenient size (1cm) and cultured on MS medium (Murashige and Skoog, 1962) consisting of different concentrations and combinations of auxin and cytokinin. Throughout the experiments full strength MS medium with 3% (W/V) sucrose and gelled with 0.8% (W/V) agar was used. The pH of all media (supplemented with respective growth regulators) was adjusted to 5.8 with 1N NaOH or 1N HCl prior to autoclaving (21 min). The cultures were

incubated in a culture room at 25±2^{0}C with a photoperiod of 16 hour at 3000-lux light intensity provided by cool white fluorescent tubes.

In this investigation, the basal medium was supplemented with different concentrations and combinations of auxin and cytokinin for callus induction. Once the callus developed, they were further cultured for regeneration and elongation in the medium having different concentrations and combinations of auxin and cytokinin. Root induction from the callus-derived shoots was achieved on MS medium supplemented with NAA/IBA at different concentrations. Well developed plantlets were removed from the culture vessels, washed gently under running tap water and planted in plastic pots containing a potting mixture of sand, soil and farmyard manure in the ratio of 1:1:1. The potted plantlets were covered by polythene bag to maintain suitable humidity. After 30-35 days of bagging, the plantlets were transplanted in the natural condition, where 70% plants were survived.

RESULT AND DISCUSSION

Callus induction

For callus induction and shoot regeneration shoot tips were cultured on MS medium supplemented with different concentrations and combinations of 2,4-D and Kn. From seven to ten days of inoculation callus was formed at the cut surfaces of shoot tips. The highest percentage of callus induction was 96.6% onto the medium consisting of 2.0mg1^{-1} 2,4-D, followed by 90.0% on the medium having 2.0 gm1^{-1} 2,4-D+0.2 mg1^{-1} Kn (Table 1). On the other hand, the lowest percentage of callus induction was 23.3% on the medium supplemented with 0.5 mg1^{-1} 2,4-D+0.2 mg1^{-1} Kn. In these treatments, the induced calli were creamish in color and structurally friable (Figure 1A).

Shoot regeneration

The shoot tip derived calli were subculture in MS medium supplemented with different concentrations and combinations of 2,4-D and Kn for shoot regeneration. Shoot initiation appeared from shoot tip derived calli within ten days of subculture. The highest percentage of shoot induction was 90.0% on MS medium supplemented with 2.0 mg1^{-1} 2,4-D + 0.2 mg1^{-1} Kn followed by 63.3% on the medium consisting of 1.5 mg1^{-1} 2,4-D+0.2 mg1^{-1} Kn. The highest number of shoots per callus was 5.0 on the medium having 2.0 mg1^{-1} 2,4-D+0.2 mg1^{-1}Kn (Figure 1B) followed by 4.0 on the medium consisting of 2.5 mg1^{-1} 2,4-D+0.2 mg1^{-1} Kn. On the left hand, the lowest percentage of shoot induction was 13.3% on the medium supplemented with 2.5 mg1^{-1} 2,4-D and the lowest number of shoots per callus was 1.0 on the medium consisting of 0.5 mg1^{-1} 2,4-D+0. 2 mg1^{-1} Kn. Thus, 2.0 mg1^{-1} 2,4-D+0.2 mg1^{-1} Kn was found to be an ideal treatment for shoot induction as well as elongation. Such types of indirect organogenesis was also reported in many plant species including *Triticum aestivum* (Rahman *et al*. 2008), *Agave amaniensis* (Andrijany *et al.* 1999), *Rotula aquatica* (Martin, 2003), *Phellodendron amurense* (Azad *et al.* 2005) and *Acmella calva* (Senthilkumar *et al.* 2007).

Root induction, elongation and acclimatization

For root induction callus derived shoots were isolated (Figure 2A) and cultured in the MS medium supplemented with different concentrations of IBA/NAA (Figure 2B). The highest percentage of root induction was 80.0% in the medium supplemented with 2.0 mg1^{-1} NAA followed by 73.3% on the medium consisting of 2.0 mg1^{-1} IBA (Table 2). The highest number of roots per shoot was 6.0 on the medium supplemented with 2.0 mg1^{-1} NAA (Figure 2C) followed by 5.0 in the medium consisting of 1.5 mg1^{-1} NAA. On the contrary, the lowest percentage of root induction was 20.0% on the medium having 3.0 mg1^{-1} IBA and the lowest number of roots per shoot was 2.0 on the medium consisting of 1.0 mg1^{-1} IBA. Thus, 2.0 mg1^{-1} NAA was found to be an ideal treatment for root induction as well as elongation. Many other workers reported similar results for root induction in various types of plants species including, *Dendrobium candidum* (Zhao *et al*. 2008), *Zehneria scabra* (Anand and Jeyachandran, 2004), *Decalepis hamiltonii* (Anitha and Pulalaiah, 2002), and *Porteresia coaretata* (Latha *et al*. 1998).

After 35 days well-rooted plantlets were obtained. Subsequently, the plantlets were removed from agar medium and planted in plastic pots containing sterile sand, soil and farmyard manure in the ration of 1:1:1 (Figure 3A-B). The potted plantlets were covered by polythene bag to maintain suitable humidity. After proper acclimatization, the plantlets were transplanted in the natural condition with 70% survival.

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.

Table 1. Effect of different concentrations and combinations of 2,4-D and Kn in MS medium for callus induction and shoot regeneration

Growth regulators ($mg1^{-1}$)	Days to callus initiation	Percentage of callusing (M±SE)	Cultures producing shoots (%) (M±SE)	No. of shoots per callus (M±SE)	Height of Shoots (cm) (M±SE)	Nature of callus
2,4-D						
0.5	12-16	46.6±2.4	-	-	-	CF
1.0	11-17	56.6±1.4	-	-	-	CF
1.5	12-15	73.3±3.1	-	-	-	CF
2.0	10-13	96.6±0.3	20.0±0.0	2.0±0.6	5.6±1.6	CF
2.5	13-15	76.6±1.2	13.3±0.4	1.6±0.4	4.8±1.9	CF
3.0	14-17	53.3±0.7	-	-	-	CF
2,4-D+Kn						
0.5+0.2	13-16	23.3±1.6	23.3±0.4	1.2±0.8	3.9±1.4	CF
1.0+0.2	12-18	43.3±2.1	26.6±1.2	2.0±1.0	4.6±0.4	CF
1.5+0.2	10-15	66.6±0.9	63.3±1.3	3.6±0.6	5.3±0.8	CF
2.0+0.2	10-12	90.0±0.0	90.0±0.5	5.0±1.2	6.0±1.0	CF
5+0.2	14-17	76.6±0.8	53.3±0.8	4.2±1.4	5.0±1.0	CF
3.0+0.2	13-18	53.3±1.6	26.6±1.2	2.5±0.9	4.7±1.6	CF

The experiments were repeated thrice, each experiment consisting of 10 replicates. CF = Creamish friable.

Table 2. Effect of IBA and NAA on root induction and elongation from callus derived shoots

Growth regulators ($mg1^{-1}$)	Days to root initiation	% of shoots rooted (M±SE)	No. of roots/shoot (M±SE)	Root length (cm) (M±SE)
IBA				
1.0	15-19	26.6±0.9	2.0±1.0	2.6±1.3
1.5	16-18	56.6±1.3	2.6±1.5	3.0±1.0
2.0	12-17	73.3±0.6	3.6±0.4	4.3±1.6
2.5	14-19	46.6±1.2	3.0±1.0	3.6±0.9
3.0	13-16	20.0±0.0	2.3±1.3	2.3±0.5
NAA				
1.0	12-15	26.6±0.3	2.3±1.3	2.3±0.6
1.5	10-16	50.0±0.0	5.0±1.0	4.0±1.0
2.0	10-14	80.0±0.0	6.0±0.4	5.0±1.0
2.5	12-17	66.6±0.9	5.3±1.4	4.3±0.9
3.0	14-17	36.6±1.4	3.6±1.2	2.9±0.7

Note: The experiments were repeated thrice, each experiment consisting of 10 replicates.

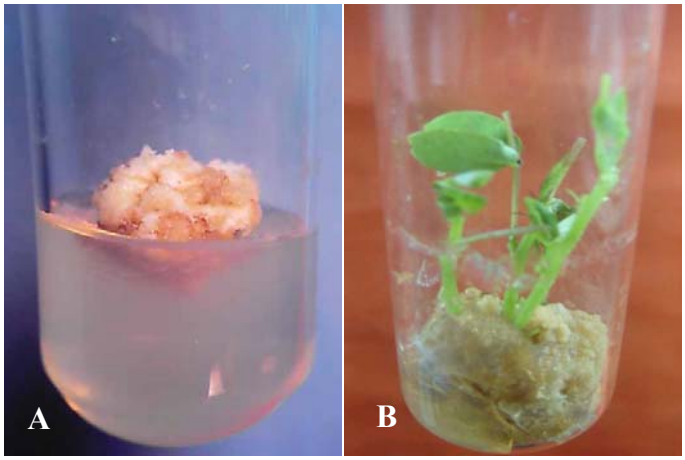

Figure 1. *In vitro* callus induction and shoot proliferation from shoot tips.
A. Callus induction, B. Shoot proliferation and elongation

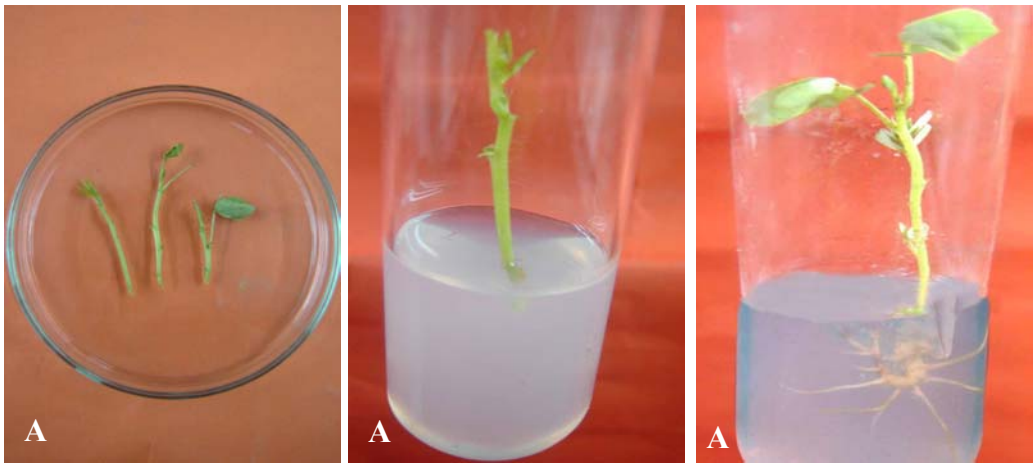

Figure 2.Root induction from callus derived shoots
A. Isolated single shoots,
B. Inoculated single shoot and
C. Root induction and elongation.

Figure 3. Acclimatization and establishment of *in vitro* grown chakunda
A. Plantlets in bagging condition and B. *In vitro* regenerated plantlets in field condition.

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