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Callus induction of *Adeniumobesum* through Leaf Explant – An Ornamental Tree of Medicinal Value

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ABSTRACT: *Adeniumo be sum is an important medicinal plant belonging to family Apocynaceae valued for containing cardio toxic glycosides. A root or bark extract of Adeniumo be sum is used as a bath or lotion to treat skin diseases and to kill lice. Latex of Adeniumo be sum is used to treat decaying teeth and septic wounds. A root decoction of Adeniumo be sum is used as nose drops and prescribed for rhinitis. The secondary metabolites, present in plants are known to play a major role in the adaptation of plants to their environment and also represent an important source of drugs which are used in the treatment of different diseases. Adeniumo be sum is the very important source of cardiac glycosides which is used in anticancer drugs. In order to obtain high yields suitable for pharmaceutical industry, efforts have been focused on the cells which produce the bioactive compounds. Plant tissue culture techniques hold great promise for the production of large number of useful secondary metabolites.Callus induction of Adeniumo be sum was tried with different explants, medium, phytohormone concentration.The best suitable callus culture was obtained in MS medium supplemented with 8.0 mg/lt IAA + 2.0 mg/l 2,4-D from leaf explants of Adeniumo be sum.*

Key words: *Adeniumobesum, glycosides, anticancer drugs, Callus induction*

INTRODUCTION

Adeniumo be sum belongs to the family Apocynaceae. It is an evergreen shrub of 1-3 mt height with thick roots. *Adeniumo be sum* is a very important medicinal plant of Africa and South Asia. The toxic sap of its roots and stems is used as arrow poison for hunting large game throughout much of Africa. The latex is used to treat boils, septic wounds and tooth cavities. It has many uses as a decoction from the roots with the combination of other plants extract, is used to treat venereal diseases. A root or bark extract of *Adeniumo be sum* is used as a bath or lotion to treat skin diseases and to kill lice. Latex of *Adeniumo be sum* is used to treat decaying teeth and septic wounds.A number of alkaloids, glycosides, flavonoids, steroids and triterpenes have been reported frommedicinal species [7]. Dimmit and Hanson [2] has reported the presence of several cardiotonic glycosides in addition to anthocyanidin glycosides and [10]. Some ofthese bioactive compounds have found application in health care. Many natural products from plants have been identified to exert anticancer activity. Almehdar *et al.*, [3] reported the anticancerous property of Adeniumo be sum while studying in vitro screening of medicinal plants. *Adeniumobesum* is the very important source of cardiac glycosides which is used in anticancer drugs. *A. obesum* is known as desert rose Cardiotonic glycoside from *Adeniumobesum* shows the anti-influenza virus activity was investigated by Kiyohara H *et al.*, [6]. The roots and stems of *Adeniumo be sum* contain the same and different glycosides in similar or different amounts. Oleandrigenin and some of the glycosides derived from *Adeniumo be sum* is having cytotoxic effects and are being used as potential components of anticancer drugs.

Plant tissue culture techniques hold great promise for the production of large number of useful secondary metabolites. In order to obtain high yields suitable for pharmaceutical industry, efforts have been focused on the cells which produce the bioactive compounds. these cells are cultured by providing the optimum cultural conditions, selecting the strains which produce high amount of bioactive compounds and Tissue engineering, transformation methods, elicitation process and immobilization techniques [1]. As a promising alternative for the enhancement in

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the production of plant secondary metabolites, plant cell culture technology has many advantages over traditional field cultivation, particularly for many natural compounds that are either derived from slow-growing plants or difficult to be synthesized with chemical methods [11, 12]. In vitro studies through callus induction were also done in order to develop regeneration system of important medicinal plants and for secondary metabolite production. *In vitro* micro propagation of *Daturametel* L. through callus induction from leaf and anther culture was done by Jha M and Pandey RK [4]. *In* vitro regeneration of an endangered ornamental *Adenium multiflorum* was reported by Talukdar T [9]. Juvenile leaves were used as explants for *in vitro* organogenesis through callusing and cultured on three different basal media-MS, B-5 and N6 supplemented with phytohormones -α-naphthaleneacetic acid (NAA), kinetin and Gibberelic Acid (GA).

Present study for callus induction of *Adeniumo be sum* was carried out for in vitro studies keeping in view production secondary metabolite level through tissue culture techniques.

MATERIAL AND METHODS

Plants Material

The plants of *Adeniumo be sum* were bought from the nurseries of Noida.

Explants Materials

Leaves of *Adeniumo be sum* were cutwith the help of sterilized needle and sharp scalpel in the laminar air flow after sterilization.

Sterilization of Glasswares

All the glass wares were sterilized by washing with labolene followed by dipping in chromic acid (7%) overnight. After rinsing with distilled water glasswares were kept in hot air oven till dry.

Sterilization of Leaves

The freshly taken explants (leaves) from *Adeniumo be sum* were treated with 1% tween-20 for 10 minutes followed by washing with tap water. After washing with tap water, the explants were treated with sodium hypochlorite (1%) solution for 10 minutes followed by mercuric chloride for 3 minutes with thereafter rinsing 4-5 times with sterile distilled water inside the laminar air flow.

Medium Preparation

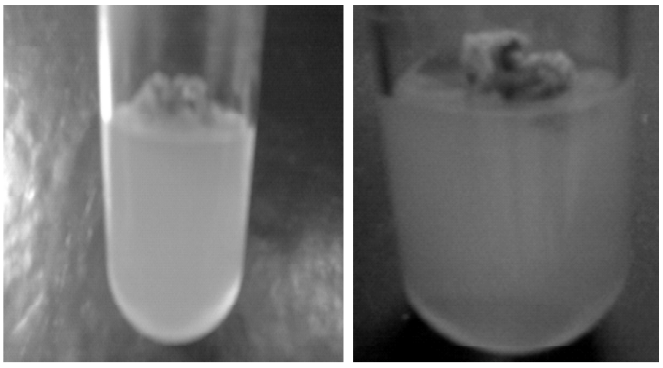
Fresh leaf explants were used for callus induction experiments. Leaf explants were sterilized and were cut about 0.5-1 cm. These explants (1 cm long) were excised aseptically and cultured on MS basal medium supplemented with different concentrations of phytohormones and adjuvants.A culture cycle was for 4-6 weeks. Following this period, the cultures were trans-ferred to a fresh medium or used for callus induction.

Culture Conditions

All media were supplemented with 30 g l-1 sucrose and 8.0 g l-1ofagar. The pH of all media was ad-justed to 5.7 with 1 N NaOH or 1 N HCl prior to auto-claving at 15lbs pressure, 121ÚC for 20 min. Cultures were maintained at 25±1°C air temperatures in a culture room with a 16/8 h light/dark photoperiod under an illumina-tion provided by cool-white fluorescent light. Plant materials were stored in culture tubes each containing 10 ml of medium.

RESULT AND DISCUSSION

The effect of different concentration of 2, 4 - D and IAA were studied for callus induction of *Adeniumo be sum* from the leaves explants on MS medium. Out of the all concentration of IAA (1mg/lt, 2 mg/lt, 4 mg/lt, 8 mg/lt), the best result was seen in IAA (8 mg/lt). (4mg/lt) (Fig. 1). When IAA was supplemented with the combination of 2,4 D, the best results was observed in 2,4 D (2 mg/lt) + IAA (8 mg/lt) and 2,4 D (4 mg/lt) + IAA (8 mg/lt) (Fig. 2, table 1).



MS+IAA(8 mg/lt) MS+2, 4 D(2 mg/lt)+IAA(8 m

Figure 1 & 2

DISCUSSION

For callus induction in *Adeniumo be sum,* different hormonal concentration of IAA and with the combinations of 2,4 D was tried. When the different concentration of IAA was used, the best result was

Table 1
Effects of different concentration of 2, 4 D and IAA on callus induction

Medium : MS+ IAA+2,4 D

Explants : Leaves

Incubation : 16 hrs of light and 8 hrs of dark

No. of explants	*Concentration of 2,4 D+IAA(mg/lt)*	*Response*					*Remarks*
		Week 1	*Week 2*	*Week 3*	*Week 4*	*Week 5*	
1		++	++	++	++	++	Good response
2		-	++	+++	+++	+++	
3	2,4 D (2 mg/lt) +	++	++	++	++	++	
4	IAA (8 mg/lt)	+++	+++	+++	+++	+++	
5		-	-	++	++	++	
1		++	++	++	++	++	Good response
2	2,4 D (4 mg/lt) +	-	-	-	++	++	
3	IAA (8 mg/lt)	-	+++	+++	+++	+++	
4		++	++	++	++	++	
5		+++	+++	+++	+++	+++	
1		-	++	++	++	++	Poor Response
2	2,4 D (6 mg/lt) +	-	-	-	-	-	
3	IAA (8 mg/lt)	-	-	-	-	-	
4		-	-	-	-	-	
5		-	-	-	-	-	
1		-	-	-	-	-	
2	2,4 D (8 mg/lt) +	-	-	-	-	-	
3	IAA (8 mg/lt)	-	-	-	-	-	No response
4		-	-	-	-	-	
5		-	-	-	-	-	

- : No response, + : Good response, ++ : Very good response, +++ : Excellent response

found in MS + IAA (8 mg/lt). The callus obtained was of green and brown in colour. However, the rate of response was not found in MS + IAA (1 mg/lt) and MS + IAA (2 mg/lt). When the IAA was used with different combinations with 2,4 D the best result was found in MS + IAA (8 mg/lt) + 2,4 D (2 mg/lt). Response was not observed in MS + IAA (8 mg/lt) + 2,4 D (8 mg/lt) (Fig. 3).

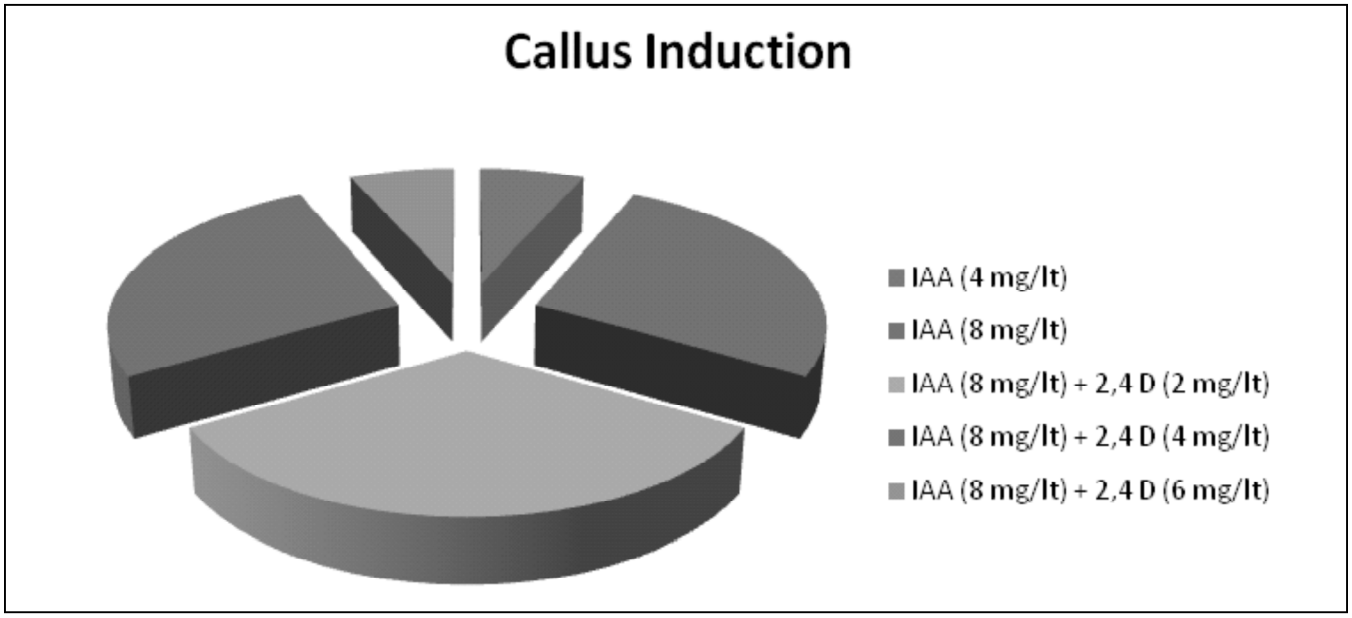


Figure 3: Callus induction with different concentration of IAA and 2,4 D in MS medium

When the leaves explants were inoculated in MS medium, it was found that response also occurred in the medium containing IAA (8 mg/lt). When the combination of 2,4 D also used very good response also occurs in the medium supplemented with IAA (8 mg/lt) + 2,4 D (4 mg/lt) followed by 2,4 D (6 mg/lt) + IAA (8 mg/lt). This work is in accordance with the work done by Kanchanapoom *et al.,* [5] similar work has also been carried out by Shukla [8]by showing the effect of phytohormone on aromatic tree species. Leaf explants of *Adeniumobesum* inoculated in MS medium supplemented with IAA (8 mg/lt) and in combination with 2,4 D (2 mg/lt) found to be most suitable for callus induction.

CONCLUSION

Callus induction throughleaf explants of*Adeniumobesum*was successfully developed.The best suitable result was obtained on MS medium fortified by IAA (8 mg/lt) + 2,4 D (2 mg/lt).The callus developed can be further used as a source of metabolite production.

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