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Morphogenic Callus Induction From Shoot Tips Of *Cochlospermum Religiosum* (L.) Alston- A Medicinal Tree Taxon

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ABSTRACT

Cochlospermum religiosum is an important medicinal plant which needs to be conserved through *in vitro* propagation. Indirect shoot regeneration through callus is encouraged to conserve the plant material. Callus induction in *Cochlospermum religiosum* was achieved from shoot tip explants on Murashige and Skoog's (MS) medium fortified with different concentrations of growth regulators. The callus was sub cultured for multiple shoot induction. The shoot tip derived callus is proved to be best in indirect shoot regeneration. Callus induction was necessary for the organogenesis and propagation of plants, which have been genetically transformed.

KEY WORDS

Cochlospermum religiosum, shoot tip, callus, indirect shoot regeneration

INTRODUCTION

Callus is defined as vigorous growth of cells or irregularly arranged plant cell cluster produced from a single permanent cell that is totipotent and can propagate the entire plant. In fact the main difference is its plasticity (Maria et al., 2021). From a practical point of view, the most important feature of callus is that this growth has meristematic activity at specific regions and primitive cambial area with zones of stelar tissue differentiation that can produce normal shoots, roots and embryoids that later can develop (Dodds and Roberts, 1982). Callus can be produced *in vitro* by balancing two types of plant hormones, auxin and cytokinin, which regulate

differentiation and dedifferentiation (Nisansala *et al*., 2024). Shoot organogenesis by callus culture has been shown to be an effective method for mass propagation of medicinal plants (Azad *et al*., 2005). Shortly after the discovery of kinetin in the 1950s, it was found that the callus tissue from tobacco pith sections differentiate in to root or shoot organs depending on the ratio of auxin to kinetin in the culture medium (Joon Sang, 2023). The cell culture widely used for *in vitro* secondary metabolite production is obtained from the callus tissue of cell suspension culture.

Cochlospermum religiosum (L) Alsten is a small tree of Cochlospermaceae family which is a very beneficial plant. It has been used in many ailments since ancient times from various places in India. The gum of this tree is used for stomach ache, as sedative, to cure gonorrhea, syphilis and asthma. Application of the paste of stem bark on the bone fractured areas can be observed at some regions (Lenin and venkataratnam 2009). Herbalists sell the bark under the name of Jalajamini as a medicine against diabetes. The bark is used for therapeutic purposes together with kalimirch. In case of jaundice bark powder is used with water (Dinesh *et al*. 2010). Seed germination rate is very low (8.%) (Andhra Pradesh Forest Department – Silviculture of species). To overcome this problem it will be better to propagate the plant *in vitro* through tissue culture processes.

MATERIALS AND METHODS

Plant Material

Healthy shoot tips of *Cochlospermum religiosum* are collected from Tirumala Hills, Chittoor District, Andhra Pradesh, India and stored in plastic containers.

Washing and Storage of Glassware

The glassware was placed in chromic acid for 24 hours and cleaned thoroughly with teepol cleaner. Then, it is washed with running tap water jet, rinsed with distilled water and dried. The vessels were decontaminated by autoclaving them at 15 lbs/in^2 for 20 min, then washed with soap, tap water and distilled water and finally dried at 180^{0}C for 2 hours. Now they are cooled and stored in dust free place.

Preparation and Sterilization of Nutrient Media

The media used in this study were Murashige and Skoog (MS) medium (1962) woody plant medium (WPM) and B5 medium (Gamborg *et al*., 1968).

Various levels of sucrose were added to the culture medium and plant growth regulators were added at different levels. Then the pH of the medium was adjusted to 5.6 to 5.8 with 0.1 N HCl and 0.1N NaOH, and finally the medium was brought to a known volume. Agar (1% w/v) was added to the culture medium and dispensed into the containers (25 ml for each container) which were autoclaved at 121^{0}C for 15 min at 15 lbs/in^2. After sterilization, removed them from the autoclave and cooled to room temperature.

Explant sterilization

All explants were first washed with tap water for 15 minutes to remove dust from the surface of explants, then washed with 1% teepol detergent for 5 minutes, and then washed again in running tap water until all traces of detergent disappeared. Then rinsed with distilled water for 4-5 times. Seeds are then kept in 70% alcohol for surface sterilization for 15 seconds and rinsed 3 times with sterile water. Finally the explants were treated with 0.1% $HgCl_2$ (w/v) for 2 minutes. After surface sterilization explants is rinsed with sterile distilled water 5-6 times. The explants were then blotted on sterile media.

Inoculation and incubation

Before inoculation, the laminar airflow chamber is cleaned with ethyl alcohol. Transfer all the required equipment for inoculation in to the chamber. Switch on the UV lamp for half an hour before inoculation to sterilize the chamber. During inoculation, instruments such as scissors, forceps, needles etc., are dipped in ethyl alcohol and ignited both before and after their use to sterilize. Use ethanol on hands periodically to prevent contamination. Inoculation was done in the presence of a spirit lamp in the chamber.

Culture Conditions

All cultures were incubated in a culture room at 25 ± 2^0C with a relative humidity of 50-60% and 16 hours photoperiod at a photon flux density of 15-20 μE m^2/S^{-1} from white cool fluorescent tubes.

Subculture

Sub culturing was carried out after inoculation at regular intervals of 30 days. Cultures were observed at every transfer during sub culturing, and the effects of different treatments were investigated according to their response.

RESULTS AND DISCUSSION

The growth and development of callus are influenced by the interaction between the cultured tissue, medium and environment. Successful callus cultures can be produced from almost any part of the plant. It is important to choose the right explant for callus induction. In general young explants respond more sensitively to callus proliferation than explants from mature trees (Thorpe *et al*., 1991). The site of explants and its functional state are important factors for the induction of organogenic callus (Harms *et al*., 1983 and Varghese and Kaur, 1991). Auxins alone cannot trigger the organogenisis in different explants. As reported by Skoog and Miller (1957) and Torry (1961) the presence of cytokinin and auxin is required for indirect adventitious bud induction.

Exogenously supplied growth regulators regulate the callus induction. Callus induction is usually associated with a high auxin to low cytokinin ratio (Centeno *et al*., 1996). *Cochlospermum religiosum* explants from *in vitro* raised shoot tips were inoculated on MS medium containing different concentrations of auxin and cytokinin for callus induction. Shoot tips are found to be better at callus formation. The combination of BAP with IBA in MS medium was found to be most effective in producing more calli. On MS medium supplemented with 1.0 mg l^{-1} BAP in combination with 0.5 mg l^{-1} /1.0 mg l^{-1} IBA, green compact highly morphogenic calli were produced from the shoot tips. The results showed that IBA had the most significant effect on callus formation followed by IAA and NAA, while 2,4-D at low concentrations could not induce callus formation and produced brown dense calli at 1.5 mg l^{-1} and 2.0 mg l^{-1} concentrations.

During indirect shoot regeneration 2 mg l^{-1} BAP produced highest response (67%) with an average of 4 multiple shoots from shoot tip derived callus. KN was less effective at 2 mg l^{-1} concentration with 45% of response and 2.5 numbers of multiple shoots. Shoot tip explants are the best source for callus formation and plant let regeneration in the regeneration of *Allium cepa* also (Khar *et al*., 2005).

The capacity for morphogenesis was largely determined by the growth regulators supplied during callus induction. The extent and nature of indirect organogenesis can be modified by the medium and growth regulators provided during indirect shoot regeneration. It has been reported that callus compactness plays an important role in the growth process. Generally dense callus has high organogenic potential due to its rigid cell wall. Depending on the cell wall proteins, the cells will be active in cell division (Dey *et al*., 1998).

CONCLUSION

The present study shows that shoot tips of *Cochlospermum religiosum* are a good source of callus formation and produce green, highly morphogenic callus.

TABLES

Table1: Effect of different plant growth regulators on callus induction from shoot tip explants of *Cochlospermum religiosum*

Explant	Plant growth regulators in mg l^{-1}					Nature of response and morphogenic ability	
	BAP	NAA	IAA	24D	IBA		
Shoot tips	1.0	0.5	-	-	-	Light green coloured soft calli	++
	1.0	1.0	-	-	-	cream coloured soft calli	+
	1.0	-	0.5	-	-	Light green coloured fragile calli	++
	1.0	-	1.0	-	-	Green coloured compact calli	++
	1.0	-	-	-	0.5	Dark green friable calli	+++
	1.0	-	-	-	1.0	Dark green compact calli	+++

+' represents organogenetic ability of callus, ' -' non organogenetic ability of callus, '++' indicates moderately organogenetic, '+++' highly organogenetic

Table2:Effect of different PGR on indirect shoot regeneration from the callus derived from shoot tip explants of *Cochlospermum religiosum*

Callus source	Plant growth regulators in mg l^{-1}		Percentage of shoot regeneration	Mean no. of shoots / explant	Mean length of shoot in cms
	BAP	KN			
Shoot tips	2.0	-	67	4.0±0.02	4.6±0.113
	2.5	-	50	3.5±1.19	4.2±1.71
	-	2.0	45	3.2±0.05	4.13±0.02
	-	2.5	30	2.5±0.13	3.5±0.16
	2.0	2.0	53	3.0±2.13	4.34±0.28
	2.5	2.5	40	2.8±1.87	4.0±0.36

Values are means of 12 replicates. Observations after 8 weeks of inoculation '±' indicates the standard error.

FIGURES

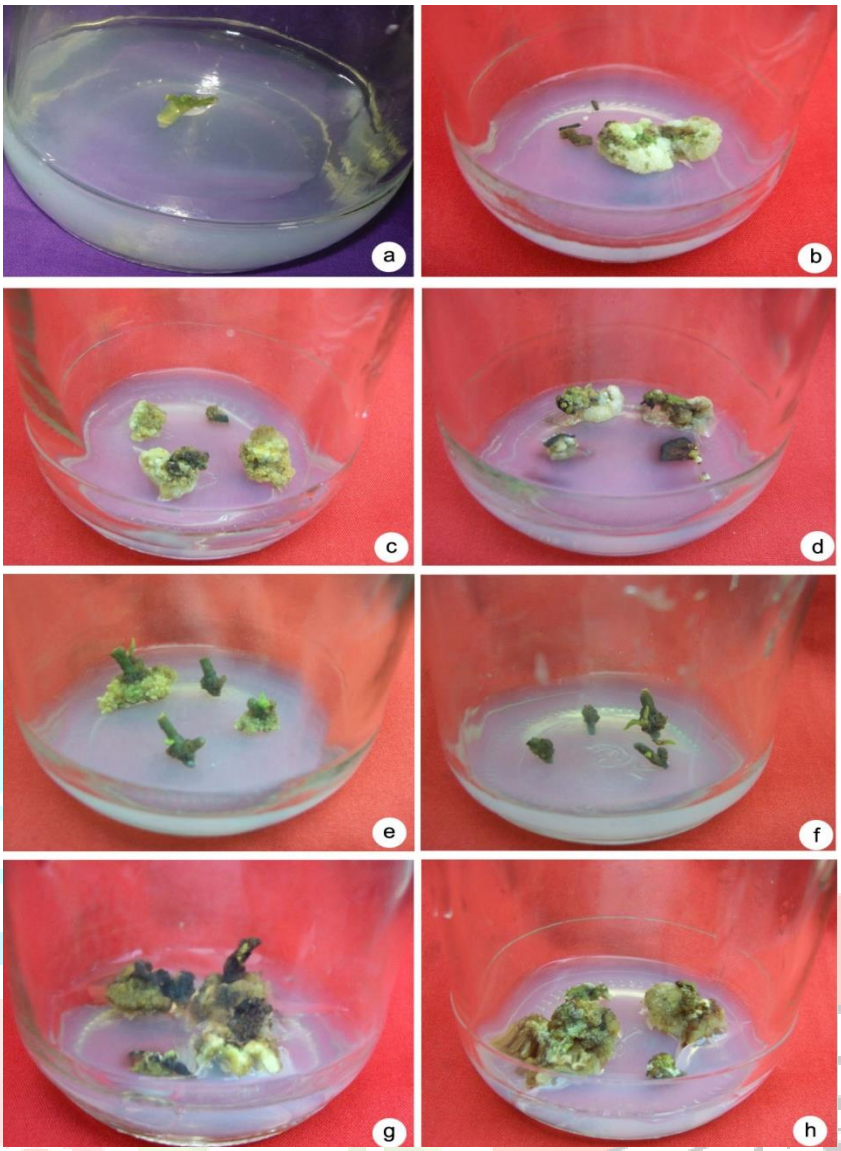

Fig-1: Effect of different plant growth regulators on callus induction from shoot tip explants of *Cochlospermum religiosum*

a. Callus induction in MS medium with 1.0 mg l^{-1} BAP and 0.5 mg l^{-1} NAA
b. Callus induction in MS medium with 1.0 mg l^{-1} BAP and 1 mg l^{-1} NAA
c & d. Callus induction in MS medium with 1.0 mg l^{-1} BAP and 0.5 mg l^{-1} IAA
e. Callus induction in MS medium with 1.0 mg l^{-1} BAP and 0.5 mg l^{-1} NAA
f. Callus induction in MS medium with 1.0 mg l^{-1} BAP and 1 mg l^{-1} NAA
g. Callus induction in MS medium with 1.0 mg l^{-1} BAP and 0.5 mg l^{-} IBA
h. Callus induction in MS medium with 1.0 mg l^{-1} BAP and 1 mg l^{-} IBA

Fig-2: Effect of different PGR on indirect shoot regeneration from the callus derived from shoot tips explants of *Cochlospermum religiosum*

a & f. Shoot regeneration in MS medium with 2 mg l^{-1} BAP and 2.5 mg l^{-1} BAP respectively

b & c. Shoot regeneration in MS medium with 2.5 mg l^{-1} BAP and 2.5 mg l^{-1} KN respectively

d & e. Shoot regeneration in MS medium with 2.5 mg l^{-1} BAP and the combination of 2 mg l^{-1} BAP and 2 mg l^{-1} KN

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