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# IN VITRO STUDIES IN COCHLOSPERMUM RELIGIOSUM (LINN.)

**Jyoti Ghodke, Swati Shilwant, Narayan Pandhure and Sudhir Solanke**
Tissue Culture Laboratory, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad

## ABSTRACT
*Effect of different plant growth regulator viz. BAP, KIN, IBA, IAA, NAA, and 2, 4-D on in vitro multiplication and callus induction was studied in important medicinal plant Cochlospermum religiosum. Apical shoots and nodal explants have shown maximum multiple shoot formation using different combination of cytokinins and Auxins. 1.4 mg/l BAP along with 0.2 mg/l IBA given maximum 10 shoots and 1.0 mg/l KIN and 1.0 mg BAP and 1.2 mg/L2, 4-D was responsible for Callus induction.Cochlospermum contains important medicinal properties, secondary metabolites and gum katira which is important in human welfare. Therefore the present investigation was carried out to conserve the valuable deciduous tree Cochlospermum religiosum.*

*Keywords: In vitro: Cochlospermum religiosum, callus, multiplication.*

## INTRODUCTION
One of the rare flowering plant *Cochlospermum religiosum* is belongs to family Bixaceae from the tropical region of Southeast Asia and the Indian Subcontinent. In India it is commonly found in Andhra Pradesh, Maharashtra, Madhya Pradesh, Uttar Pradesh and Bihar (RCDC). It is a small tree growing to a height of 7.5 m usually found in dry deciduous forests. The name *religiosum* derived from the fact that the flowers are used as temple offerings. It is also known as Silk-Cotton Tree because the capsules containing the seeds have a fluffy cotton-like substance similar to kapok. It is very common and conspicuous tree, characteristic of the hottest, driest and stoniest slopes. It frequently founds in Telangana forest.

Plant can be identified by deeply furrowed bark, palmately 5-lobed leaves and bright golden yellow bisexual flowers. *Cochlospermum* is genus of trees and shrubs scattered throughout the tropics mostly xerophytes, deciduous in drier season. Capsules containing numerous seeds some species are medicinal, Ornamental, yielding floss, fibre, gum and timber. *Cochlospermum* quick growing tree yield a gum known as gum katira from a juice orange in colour exudes from the bark. This gum has been exported from India in increasing quantities in recent years and the fact that the price has been low and stable, as compared with tragacanth, has led to new uses and applications for it in industry and pharmacy. Katira is primarily used as a substitute of Tragacanth in most American countries. There are lot other uses of Katira like in calico-printing, polishing paper and leather dressing. It is also used for polishing tusser silk. It is exported to many countries of Latin America to use in cigar and Ice cream industries. As a laxative it is considered to be superior to other gums. As an emulsifying agent it is a good substitute for tragacanth.

This gum is used in medicinefor the treatment of cough, diarrhea and dysentery. The dried leaf and flowers are used as stimulants, antipyretic, laxative and sedative (Kirtikar and Basu, 1975). Root powder mixed with water applied to face reduce wrinkle (RLS sikarwar, et al., 2007). The oral gum powder about 20g mixed with ghee works as an aphrodisiac (SavithrammaN. et al., 2011). Katira used in cosmetics and for bookbinding the floss is used for stuffing pillows, mattresses, cushions, life jackets. Seed cakes are used as manure and cattle feed.Bark Powder of tree is used with water during jaundice (Dinesh K. and Aruna J., 2010).*Cochlospermum* is propagated by seed broadcast, seeds are broadcasted in primary beds in June and seedlings are pricked out to polythene bags when six months old. Therefore the aim of present work is to conserve important medicinal plant *Cochlospermum religiosum* through *in vitro* tissue culture techniques.

## MATERIAL AND METHOD
### Preparation of Explants
Seeds of *Cochlospermum religiosum* were collected from Buldhana district and grown in the green house, Botanical garden, Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Apical shoot, Axillary leaves and nodal explants of *Cochlospermum* were collected from 30 days old plants grown in the greenhouse of Departmental Botanical garden. All these explants were used from donor plants during present study. The explants were washed carefully in running tap water for 5 minute and followed by distilled water for 5 minutes. For surface sterilization, chemical such as 70% ethanol, $Hgcl_2$ (0.3 %) were used. Explants were surface sterilized for 5 minute by 0.3% mercuric chloride followed by three subsequent rinses with sterilized double distilled water in a laminar air flow. All these explants were dissected into small pieces and inoculated on culture vessel and test tube containing 25 ml MS medium.

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**Culture media**

MS medium (Murashige and Skoog 1962) was used for multiple shoot formation and apical shoot, Axillary bud and nodal parts were used as explants. MS medium was supplemented with various growth hormones viz.BA, KIN and for rooting, half strength MS medium was supplemented with various concentrations of auxins IAA, IBA, and NAA were examined. MS medium with 3% sucrose and gelled with 2.5% Clerigel, and the pH was adjusted to 5.8 after addition of growth regulators. The media were steam sterilized in an autoclave under 15 psi and 121° C. for 20 minutes. After the autoclave media was transfer to aseptic condition in laminar air flow for inoculation.

**Culture conditions**

After the inoculation culture tubes and culture vessels were transferred to culture room under a 10 h photoperiod supplied by cool white fluorescent tubes light and 25 ± 2 $^{0}$C temperatures. At least ten cultures were raised for each treatment.

**Data record**

Data were measured after 30days of five replicate for callus induction, shoot multiplication and shoot length Mean (μ) values with the standard error (S.E.).

**RESULTS AND DISCUSSION**

Surface sterilization of explants it is necessary to disinfect tissues with a minimum amount of cellular damage to the host tissue (Conger 1987). Therefore these sterilized explants outline of both ends were cut in proper size and shape and aseptically inoculated on MS medium. MS media in different concentrations with BAP 1.0, 1.5, 2.0, 2.5, 3.0, mg/l and IBA, NAA gives maximum average percentage of shoot multiplication. Combination of BAP and 2, 4-D given better result for callus induction as compare to any other combination of growth regulators. It is notice that *Cochlospermum* religiosum given maximum *in vitro* shoots at low temperature.

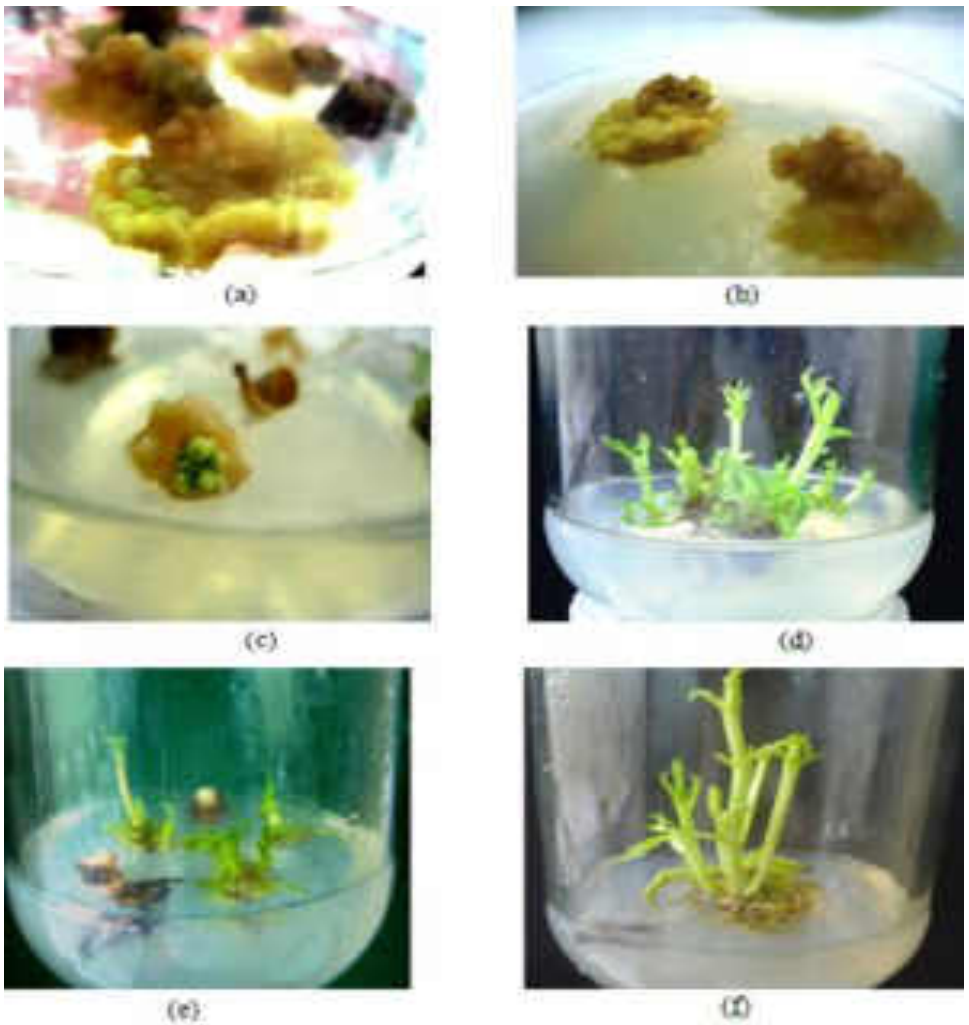


Photo plate: 1- showing fig. (a)Callus induction on axillary leaves explant, fig. (b)Callus induction on apical shoot explant, fig. (c)Callus with shoot initiation, fig. (d) Multiple shoots formation on apical shoot explant; fig. (e) Shoots formation onNodal explant, fig. (f) Well growing culture.

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Various explants were tried in MS medium supplement with 3% sucrose 2.5% clerigel in combination with growth regulators viz. 2, 4-D and BAP as shown in the (table 1.). Maximum callus induction was observed in 1.0 mg/l BAP and 1.5 mg/L, 2, 4-D using shoot tip as explants and followed by1.0 mg/l BAP in combination with 1.0 mg/l, 2, 4-D using Axillary leaves explants. Both these calli are compact and yellowish coloured. When concentration of growth regulators was increased (Table No. 1) subsequently there was decrees in the induction of callus formation in *Cochlospermum religiosum*. This callus subculture on BAP containing MS medium after 30 days shoot initiation (organogenesis) was observed. This callus was subcultured on MS along with BAP in combination with of different auxins but no effect on shoot induction was recorded.

**Table-1: Effect of BAP and 2, 4-D on callus induction using various explants**

| Source of explant | Growth regulators Mg/l | | Frequency Of callus formation | FreshWeight (gm) µ ±SE | Dry weight (gm) µ ±SE | No of shoots /callus |
|---|---|---|---|---|---|---|
| | 2,4 D | BA | | | | |
| Axillary Leaves | 0.5 | 1.0 | + | 0.94±0.150 | 0.44±0.051 | - |
| | 1.0 | 1.0 | ++ | 1.34±0.121 | 0.64±0.103 | - |
| | 1.5 | 1.0 | ++++ | 1.48±0.153 | 0.68±0.037 | - |
| | 2.0 | 1.0 | ++ | 1.36±0.129 | 0.64±0.051 | - |
| Apical Shoots | 0.5 | 1.0 | + | 1.02±0.256 | 0.54±0.136 | 2 |
| | 1.0 | 1.0 | +++ | 1.44±0.092 | 0.56±0.051 | - |
| | 1.5 | 1.0 | ++ | 1.54±0.132 | 0.58±0.073 | - |
| | 2.0 | 1.0 | ++ | 1.24±0.051 | 0.52±0.086 | - |

*After 30 days mean ± SE of 5 replicate

When apical shoot tip and nodal explant were inoculated on full strength MS medium supplemented with 3% sucrose, 0.3% clerigel and various combinations of growth hormones as shown in the table No. 2 average percentage of multiple shoot was highest i.e.8.05 shoots in 1.5 mg/L BAP combination with 0.2 mg/L IBA. These multiple shoots were subcultured on MS medium 1.0 mg/l BAP in combination with 0.2 mg/l IBA given maximum percentage of shoots heights. Increase in the concentration of BAP (cytokinins) lead to induction of callus and minimum shoots formation was observed. After the shoot formation and elongation and leaves developing quick abscission and senescence were observed. It was problematic to keeping healthy culture in *Cochlospermum religiosum*. It is considered that necrosis and abscission of leaves and shoots were due to the accumulation of ethylene but by adding $AgNo_3$ in the medium the problem was eliminated. *Cochlospermum religiosum* gives good response for low temperatures ($21\pm^0C$) and shoot multiplication rate was enhanced.

For the Rhizogenesis, in vitro grown multiple shoots were transferred in MS medium containing 0.5 mg/l NAA. After 15 days roots initiation was observed. After the shoot and roots formation these plantlets were successfully hardened and transferred to green house.

**Table-2: Effect of BAP and IBA on multiplication of nodal and shoot tip explants.**

| Source of Explant | Conc. of growth regulator (mg/L) | | Shoot length (cm) (Mean± SE) | % of shoot formation |
|---|---|---|---|---|
| | BAP | IBA | | |
| Shoot tip | 1.0 | 0.2 | 2.04±0.140 | 55 |
| | 1.5 | 0.2 | 8.05 ±0.165 | 65 |
| | 2.0 | 0.2 | 3.5±0.132 | 47 |
| | 2.5 | 0.2 | 1.86±0.067 | 40 |
| | 3.0 | 0.2 | 1.84±0.213 | 30 |
| Nodal | 1.0 | 0.2 | 2.52±0.162 | 50 |
| | 1.5 | 0.2 | 1.88±0.111 | 62 |
| | 2.0 | 0.2 | 1.92±0.162 | 58 |
| | 2.5 | 0.2 | 1.86±0.067 | 45 |
| | 3.0 | 0.2 | 1.84±0.213 | 32 |

*After 30 days mean ± SE of 5 replicate

Similar results were recorded for callus and shoot multiplication using various explants in different plants *in vitro*. Proliferating shoot cultures was established by repeatedly sub culturing the mother explants on the hormone free medium. Repeated sub-culturing was said to be one of the methods of maintaining juvenility (Franclet et al., 1987). In the present work highest number of shoot percentage was recorded in third sub

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culturing. Somatic embryos were developed into plantlets and subsequently grown to maturity. These results indicate that nodal explants have high competence for somatic embryogenesis in *Eclipta alba* (Devendra et al 2011). In the present study nodal explants have shown direct multiple shoot formation.

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