Research Article

"Multiple Shoots Regeneration Through Nodal Segment In *Cochlospermum religiosum* (L.) Alston: A Medicinal Endangered Plant."

Apexa Pareek*, Alok Sharma, Payal Lodha
Plant Pathology Tissue Culture and Biotechnology Laboratory, Department of Botany, University of Rajasthan, Jaipur, India.
***Corresponding author's E-mail:** pareekapeksha34@gmail.com

Accepted on: 29-09-2014; **Finalized on:** 30-11-2014.

ABSTRACT

In vitro propagation method was used for mass multiplication of an important medicinal plant *Cochlospermum religiosum* using nodal segment as explants, obtained from mature plant. Nodal segment were cultured on different media- MS, B_5 and MSB_5 to obtain best culture media for its propagation. MS medium shows highest number of shoot buds (7.2±0.480). The MS medium containing 6-benzylaminopurine (BAP) and kinetin (kn) either alone or in combinations was tried. BAP alone (2mg/l) showed highest number of shoot bud (7.2±0.480) than in combination with kn (5.2±0.215). The medium containing cytokinin (BAP, 1.5mg/l) and auxins (NAA, 1.5mg/l) showed maximum number of shoot bud (3.2±0.257) than BAP+IAA/IBA. The phytohormone combination resulted proliferation of shoot bud healthy and sturdy with large leaves. The present investigation describes an efficient multiple shoot regeneration protocol for *C. religiosum*.

Keywords: Auxins, Cytokinins, *C. religiosum.*

INTRODUCTION

India is one of the richest floristic diversity zones having 16 agro climatic centers of world with about 50000 plant species of which 15000 are medicinally important. The country accounts for 8% of total global biodiversity with an estimated 49000 species of plant of which 4900 are endemic.[1] There are many medicinal plants found in south-eastern Rajasthan. Some medicinal important plants of this region are *Naringi crenulata*, *Pterocarpus marsupium*, *Chlorophytum boribilianum*, *Cochlospermum religiosum*. Among the medicinal plants of south-eastern Rajasthan *Cochlospermum religiosum* is a very important and critically endangered medicinal plant. *Cochlospermum religiosum* (L.) Alston belongs to family Cochlospermaceae and commonly known as cotton tree, yellow silk cotton tree and Kateera. It is native to India, Srilanka, Bangladesh and Burma. Various parts of the tree are used as food and medicine. Dried leaf and flowers are used as stimulants, to cure asthama and young leaves are used as cooling hair wash.[2] Gum Kateera an insoluble gum derived from the bark of *Cochlospermum religiosum*, has many important medicinal and commercial uses. It is used in leather dressing, paper making and as gelling agent in tissue culture media and has wide application in Pharmaceutical and food industries.[3-4] Mixture of gum powder and ghee works as an aphrodisiac.[5] It is also thermogenic and sedative and used in cough, diarrhoea, dysentery and gonorrhea. It is also used for its anti-inflammatory in Siddha Drug 'Kalnar Parpam'. This drug is used in dental diseases, arthritis and genital urinary disorders.[6] During jaundice powder of tree bark is used.[7] Due to its medicinal uses and poor vegetative means of propagation, this plant population has decline. In view of this, In vitro propagation can be used as an effective and frequent method for regeneration and conservation of this medicinal important plant.

MATERIALS AND METHODS

For *In vitro* propagation of *Cochlospermum religiosum* (L.) Alston, nodal shoot segments were used as explants. Explants were collected from plants grown in Kapoor Chandra Kulish Smriti Van in Jaipur, Rajasthan (India). Nodal Segment cut into smaller segment approx. size 2-3 cm and had one node per segment. Explants were soaked in Tween-20 (0.1% w/v) a commercial detergent for 5-10 minutes and then rinsed with distilled water for 4-6 times. Subsequently, the explants were surface sterilized with 0.1% $HgCl_2$ for 3-5 min, followed by 3-4 rinses with autoclaved distilled water. The sterile nodal segments were cultured on different culture media like MS Medium, B_5-Medium and MSB_5 Medium. According to plants response, MS-Medium was found suitable for culturing. The explants were cultured on MS-Medium supplemented with 3% (w/v) sucrose and containing phytohormone auxins (NAA, IAA, IBA) and cytokinins (BAP, Kn) either alone or in combinations of various concentration. All experimental manipulation was carried out under aseptic condition. The cultured explants were incubated for 4 weeks in culture chamber using 26±2°C, 16 hours photoperiod and 55±5 % relative humidity. Five replicates per treatment were taken and experiment was repeated thrice for confirmation of result.

RESULTS

A series of experiments were set up to obtain regeneration from nodal shoot segment of *Cochlospermum religiosum*. Shoot buds emerged after 2-3 weeks of incubation under controlled environment conditions. Various synthetic media viz., MS[8]; B_5 [9] and MSB_5 (Modified MS-Medium) were tried to evaluate their

regenerative potential, with the objective to find out the medium which supported sprouting of optimal number of shoot buds from single nodal segment. All these media contained sucrose (3% w/v) and BAP (2mg/l). The explants showed altered response in different media in vitro. (Table 1). Result showed that shoot buds were proliferated in maximum number without callus formation on MS-Medium. It was found that BAP (2mg/l) evoked proliferation of maximum number of shoot buds. Further increase or decrease in concentration of BAP led to decline in the number of shoot buds. Therefore this medium was designated as "Shoot bud induction, Proliferation medium" (Table 2). The combined effect of BAP and Kinetin (Kn) showed the synergistic effect on shoot bud proliferation (Table 2). The combined effect of BAP (0.5-3.0mg/l) with auxins NAA/IAA/IBA (0.5-3.0 mg/l) showed that NAA (1.5mg/l) + BAP (1.5 mg/l) in combination was better for shoot bud regeneration (Table 3). Result had shown in plate 1. This protocol will help in regeneration, in vitro propagation and restoration of this valuable woody plant *C. religiosum*.

Table 1: Effect of various basal media on shoot bud proliferation through nodal segment

Media	No of shoot buds per explant Mean ± S.E.	Callus at base
MS (Murashige and Skoog 1962)	7.2 ± 0.480	C^{+}
B_5 (Gamborg *et al.*,1968)	4 ± 0.117	C^{++}
MSB_5 (Modified MS medium)	2 ± 0.189	C^{+++}

Medium: MS/B_5/MSB_5+Sucrose(3.0%)+BAP(2.0mg/l); Explant : Nodal stem segment; Incubation: At 26±2°C in 16 hr photoperiod (1000-2000 lux) up to 4 weeks.

DISCUSSION

C. religiosum is a woody plant. Reproduction via seeds is difficult into this plant due to low seed viability, seed dormancy, hard seed coat and various environmental factors. Due to its high medicinal properties, it is exploited rapidly. In vitro multiplication is an important alternative to regenerate this plant. For the production of true to type plants, nodal explants were used and obtained multiple shoot regeneration. During establishment, the number of shoot buds was low due to its woody habit of the material. Similar observation was obtained by previous workers in some other woody plants viz., in jackfruit,3-4 shoots per explants[10] and in *Ginkgo biloba*.[11] Cytokinins are very important growth regulators for induction of shoot buds. In this experiment BAP was most effective among known cytokinins for stimulating shoot buds. Similar observation was observed.[12-13] Kinetin in increasing concentration did not show any response as that BAP. Similar report was found earlier.[14-15] Best shoot multiplication was observed in BAP in combination with NAA than singly or in combination with IAA/IBA. Similar report has been found earlier.[16-17] Our findings are consistent with these reports.

Table 2: Effect of BAP and Kn alone or in combination on shoot bud proliferation through nodal stem segment.

Growth regulators (mg/l)		No of shoot buds per explant Mean ± S.E.
Control: MS basal medium		Nil
BAP	Kn	
0.5	-	1.2±0.397
1.0	-	2.0±0.491
1.5	-	2.8±0.365
2.0	**-**	**7.2±0.480**
2.5	-	2.4±0.365
3.0	-	2.1±0.365
4.0	-	Nil
5.0	-	Nil
-	0.5	2.4±0.180
-	**1.0**	**4.0±0.217**
-	1.5	Nil
-	2.0	Nil
-	2.5	Nil
-	3.0	Nil
-	4.0	Nil
-	5.0	Nil
0.5	0.5	3.7±0.457
1.0	**0.5**	**5.2±0.125**
1.0	1.0	4.6±0.595
1.5	1.5	2.8±0.401
2.0	2.0	1.9±0.335
3.0	3.0	2.5±0.359

Medium: MS + Sucrose (3.0%) + BAP/Kn (0.5-5.0 mg/l); BAP (0.5-3.0 mg/l) + Kn (0.5-3.0 mg/l); Explant: Nodal Segment; Incubation: At 26±2°C in 16 hr photoperiod (1000-2000 lux) up to 4 weeks.

Figure A-B: induction of shoot buds; **Figure C-D:** Multiple shoot on BAP (2.0mg/l) after 2 weeks and 3 weeks.

Table 3: Effect of BAP in combination with auxins on shoot bud proliferation.

Growth regulators		No. of Shoot bud per explant Mean±S.E.	Callus at base
BAP (mg/l)	NAA (mg/l)		
0.5	0.5	2.2±0.602	C^{+}
1.0	1.0	3.0±0.602	C^{+}
1.5	**1.5**	**3.2±0.657**	**C^{+}**
2.0	2.0	2.8±0.557	C^{+}
2.5	2.5	2±0.567	C^{++}
3.0	3.0	Nil	-
BAP (mg/l)	IAA (mg/l)		
0.5	**0.5**	**2.2±0.402**	**C^{+}**
1.0	1.0	2.0±0.367	C^{++}
1.5	1.5	1.7±0.457	C^{++}
2.0	2.0	1.6±0.602	C^{+++}
2.5	2.5	Nil	-
3.0	3.0	Nil	-
BAP (mg/l)	IBA (mg/l)		
0.5	0.5	1.1±0.457	C^{+}
1.0	1.0	1.3±0.634	C^{++}
1.5	1.5	1.7±0.634	C^{+++}
2.0	2.0	Nil	
2.5	2.5	Nil	
3.0	3.0	Nil	
Control MS medium		Nil	Nil

C = callusing response, + =slight response, ++ = moderate response, +++ = Maximum response

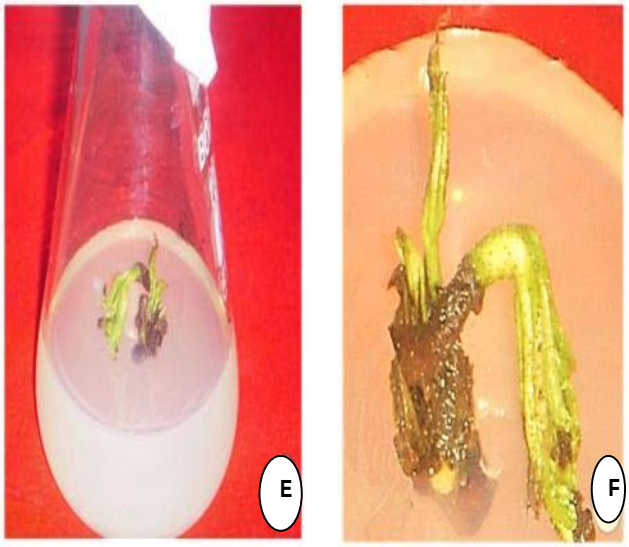
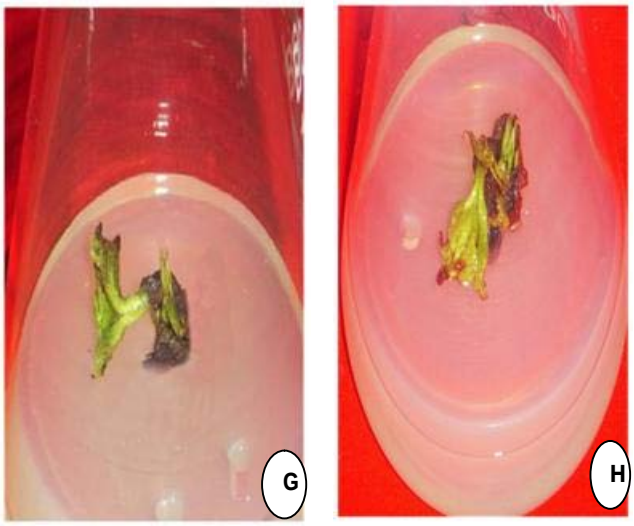


Figure E-F: Multiple shoot induction on Kn (1.0 mg/l)



Figures G-H: Effect of BAP in combination with auxin.

G: (BAP (0.5 mg/l) + IAA (0.5 mg/l) & **H:** (BAP (1.5 mg/l) + NAA (1.5 mg/l)

CONCLUSION

- Optimal shoot bud induction, proliferation and elongation were obtained on MS-medium, supplemented with BAP (2.0 mg/l). Therefore this medium was designated as “shoot bud induction and proliferation” medium.
- Addition of auxins (NAA/IAA/IBA, 0.5-3.0mg/l) to shoot bud induction and proliferation medium was inhibitory.

REFERENCES

1. Kumar V, Asija, Biodiversity conservation in: Biodiversity Principles and Conservation, Agrobiosis India, Jodhpur, 2000.
2. Khyade MS, Awasarkar UD, Deshmukh RR, Petkar AS, Ethnobotanical report about few important diseases from Akole Tehsil of Ahmednagar District (MS) India, Asian Journal of Experimental Biological Sciences, 2, 2010, 393-403.
3. Jain N, Babbar SB, Gum Katira, A cheap gelling agent for plant tissue culture media, Plant Cell, Tissue and Organ Culture, 71, 2002, 223-229.
4. Janki B, Sashidhar RB, Sub chronic (90 days) toxicity study in rats fed gum kondagogu (*C. gossypium)*, Food and Chemical Toxicology, 38, 2000, 523-534.
5. Savithramma N, LingaRao M. Suhrulatha D, Screening of Medicinal Plants for secondary metabolites Middle-East, Journal of Scientific Research, 8, 2011, 579-584.
6. Rajeswari RV, Paliwal K, *In Vitro* propagation of *Albizia odoratissima* L. F. (Benth.) from cotyledonary node and leaf nodal explants, In Vitro Cellular and Developmental Biology-Plant, 42, 2006, 399-404.
7. Dahare KD, Jain A, Ethnobotanical Studies on Plant Resources of Tahsil Multai, District Betul, Madhya Pradesh, India, Ethnobotanical Leaflets, 14, 2010, 694-705.

8. Murashige T, Skoog F, A revised medium for rapid growth and biodiversity with Tobacco tissue cultures, Physiologia Plantarum, 15, 1962, 473-497.

9. Gamborg OL, Miller, RA, Ojina K, Nutrient requirement of suspensions cultures of soyabean root cells, Exp. Cell Res., 50, 1968, 151-158.

10. Edson JL, Leegl-Brusven AD, Everett RL, Wenny DL, Minimizing Growth regulators in shoot culture of an endangered plant, *Hackeliavenusta* (Boraginaceae), In Vitro Cellular and Developmental Biology-Plant, 32, 1996, 267-271.

11. Mantovani NC, Grando MF, Xavier A, Otoni WC, *In vitro* shoot induction and multiplication from nodal segments of adult *Ginkgo biloba* plants, Horticultura Brasileira, 31, 2013, 184-189.

12. Bhojwani SS, *In Vitro* propagation of garlic by shoot proliferation, Sci. Hort., 13, 1980, 47-52.

13. Behra PR, Thirunavoukkarasu M, Chand PK, Adventitious plantlet regeneration from different explants of *Aegle marmelos* (L.) Corr, Journal of Medicinal Plant Research, 7, 2013, 2761-2768.

14. Sujhta M, Makkar HPS, Beeker Ks, Shoot Bud Proliferation from axillary node and leaf section of non-toxic *Jatropa curcas* (L.), Plant Growth Regulation, 47, 2005, 83-90.

15. Ali A, Ahmad T, Akhta A, Hafiz I A, Effect of Different Media and Growth regulators on *In Vitro* shoots proliferation of *Olive cultivar* 'Moraiolo', Pak. J. Bot., 41, 2009, 783-795.

16. Sahu B, Koche V, *In Vitro* Plantlets regeneration of *Terminalia bellirica* Roxb, An important medicinal tree, Int. J. Biological Sci., 2, 2013, 18-23.

17. Chandra I, Bhanja P, *In Vitro* Shoot Multiplication of *Flacourtia jangomas* (L.) Raeusch, Annals of Plant Sciences, 2013.

Source of Support: Nil, **Conflict of Interest:** None.