

Callus culture from apical bud of *Bombax ceiba* L. (semul)

Dr. Kanchan Vaidya*

*Asst. Professor (Botany) Govt. Sanjay Gandhi Smriti PG College, Ganjbasoda (M.P.) INDIA

Abstract - Callus of *Bombax ceiba* L. Regenerated from apical bud explant. WPM. supplemented with different concentration and combination of plant growth regulators with NAA, KN, BAP maximum callus induction occurred in high concentration of NAA with low to high concentration of BAP

Keywords- Callus, Plant growth hormones, NAA, BAP, KN.

Introduction - The major micro propagation system can be recognised by axillary shoot proliferation, adventitious shoot formation directly from organ and indirectly by callus and somatic embryogenesis directly from organ or from callus. (Jones 1983, Ammirato and styer 1985, Bonga and Aderkas 1992, Finer 1995). In these system callus also play an important role in Plant Tissue Culture Technique. The main advantage of propagation from callus, and especially from cell suspension, are that propagation can potential be achieved in large number per unit time, that handling procedure are simpler and that experimental genetic modification is easier to achieve (Krikorian 1982). Semul *Bombax ceiba* L. is an important tree species used in match would and insulation industries (Venkatesh 1988). The young fruits are reported to be employed as expectorant, stimulant and diuretic. The oil from the seeds is edible and can be used as a substitute for cotton seed oil. It can also be used for soap making and as an illuminant (Chatterjee and Prakash 1994). Besides it possesses high medicinal value. Its propagation through conventional vegetative method is very poor and survival rate meagre. (Ghate et al 1988). Besides naturally propagated through seeds with long generation period, improvement possibilities of the tree are limited since callus induction in semul (*Bombax ceiba* L.) proceeds for adventitious shoot formation and somatic embryogenesis. In present investigation has been taken up apical bud for callus culture

Material and method: Various vegetative mature and juvenile explant were used for callus culture. explants were collected from the nature grown selected plus tree 0.4 -0.7 cm long apical bud

Mature apical but were thoroughly washed in running tap water 2 - 3 hour immersed in depol 0.01% (v/v) for 10 minutes and then rinsed with tap water 2 -3 times and finally wash with distilled water 2-3 times they were then immersed

in 70% (v/v) ethanol 5-7 minutes this was followed by mercuric chloride 0.1% (w/v) treatment for 10 minutes. These explant work right between presterilized filter papers and inoculated on the sterile culture media

The medium was supplementary with different concentration of growth regulators viz. cytokinin BAP (Benzyl Amino Purine) and KN (6 Furfuryl Amino Purine). Or in combination with an auxin viz. NAA (Naphthalene Acetic Acid) the pH of the medium was adjusted 5.6—5.8 before autoclaving at 15 lbs pressure and 121°C for 20 minutes. The medium was solidify using 8 g/l agar (Difco Bacto) at least 15 culture were raised in each experiment and all experiments were repeated at least twice. Culture maintained at 25 ±2°C and relative humidity (RH) of 70% with 16-hour photoperiod (approx. 1500 lux) and 8 hr dark and sub-cultured every 4-6 weeks

Result and Discussion: In present work initially all experiments were carried out on both WPM (woody plant medium) and MS medium. But in case of callus induction WPM was found to be better than MS media. WPM supplemented. With various auxin NAA, 2,4 -D, IAA and cytokinin with BAP, KN were observed. In aseptic culture of apical bud in the present study the main hazards was exudation of phenolic substance in the medium due to which apical bud is necrosed and provide no in vitro response as reported in the past (Cresswell et al 1982). Phenolic exudation observed in mature plant was for higher than Juvenile once.

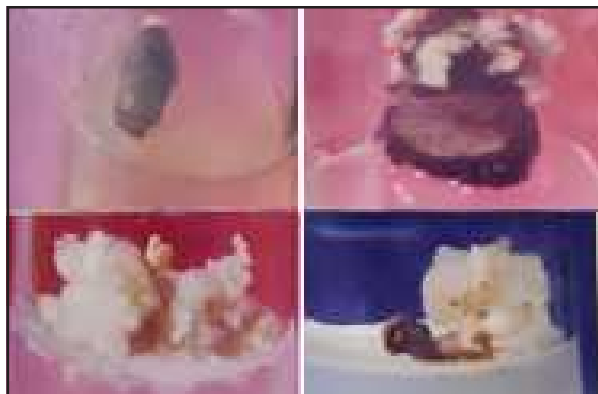
Apical bud of semul cultured in WPM (Woody plant medium). Containing high concentration of NAA and low to high concentration of BAP is best for callus proliferation. callus observed in 100% apical bud explant within 10 — 12 days, in NAA 2.68 µm + BAP 2.21µm, NAA 5.37 µm + BAP 2.21µm, NAA 5.37µm + BAP 44.39µm, NAA 26.35 + BAP 0.44µm. The callus obtained was white friable

in texture and fast growing and also used for further regeneration. Whereas 2,4-D is also produced 80% callus but this callus is dull brown, soft and slow growing. In IAA only lowest concentration supported callusing. Callus was soft slow growing. Regeneration of plants from callus or cell suspension has been achieved with several tree species viz. *Santalum* (Laxmi Sita et. al. 1980, Bapat and Rao 1984) *Hevea* (Carron and Enjalric 1982) *Mangifera indica* (Litz 1984) *Cocos nucifera* (Branton and Blake 1983). In this way callus induction by apical bud of *Bombax ceiba* L. may be used for further regeneration by in vitro method.

Callus Culture From Apical Bud of *Bombax Ceiba* After 10-12 Days of Culture (Data Are Mean±Se)

S.	Concentrations (μm)	Nature Of Response	Frequency Of Callus
	NAA+ BAP	-	-
1	0.53+0.44	-	-
2	0.53+2.21	C ⁺	98.65+0.6
3	0.53+4.43	C ⁺	87.96+1.2
4	0.53+22.19	C ⁺	55.34+1.1
5	0.53+44.39	C ⁺	73.48+1.4
6	2.68+0.44	C ⁺	100.0+0.0
7	2.68+2.21	C ⁺	80.00+1.9
8	2.68+4.43	C ⁺	-
9	2.68+22.19	-	42.06+0.0
10	2.68+44.39	C ⁺⁺⁺	100.0+0.0
11	5.37+0.44	C ⁺⁺⁺	100.0+0.0
12	5.37+2.21	C ⁺⁺⁺	73.67+8.16
13	5.37+4.43	C ⁺⁺⁺	58.55+1.8
14	5.37+22.19	C ⁺⁺⁺	100+0.00
15	5.37+44.39	C ⁺⁺⁺	100+0.00
16	26.35+0.44	C ⁺⁺⁺	97.22+2.8
17	26.35+2.21	C ⁺⁺⁺	73.61+1.4
18	26.35+4.43	C ⁺⁺⁺	61.11+1.1
19	26.35+22.19	C ⁺	-
20	26.35+44.39	-	77.7+2.7
21	53.70+0.44	C ⁺⁺	72.5.1.4
22	53.70+.2.21	C ⁺⁺	53.7+1.9
23	53.70+4.43	C ⁺⁺	-
24	53.70+22.19	-	-
25	53.70+44.39	-	-

C Callus ++ Localised callus - No response
+ scanty Callus +++ profuse callus



Callus induction form Apical bud of *Bombax ceiba* L. (Semul).

Conclusion: The above results emphasizes that callus culture of *Bombax ceiba* L is feasible from apical bud explant. It is suggested that further study of in vitro regeneration should be carried out from the apical but derived callus in order to enhance the propagation efficiency of *Bombax ceiba* L as a high multiplication rate could be achieved further experiment can be carried out to produce secondary metabolite from the apical bud derived of *Bombax ceiba* L

References:-

1. Ammirato, P.V. and Styer, D.J. (1985), Strategies for large scale multiplication of somatic embryo's in suspension culture in: Biotechnology in plant science. Zaitin, M. Day P. and Hollender, A. (eds) Acad. press New York. PP-161-178.
2. Bapat, V.A. and Rao, P.S. (1984). Regulatory factor for in vitro multiplication of sandalwood tree *Santalum album* I shoot but regeneration and somatic embryogenesis in hypocotyle cultures Proc. Ind. Acad. Sci. (Plant Sci) 93 (1):19-27
3. Bonga, J.M. and Aderkas, P.V. (1992), Clonal propagation, In vitro culture of Trees Kluwer Acad. pub. Dordrecht. The Netherlands
4. Branton, R.L., and Blake, J. (1983). Development of organised structure in callus derived from explant of *cocos nucifera* L. Ann. Bot 52: 673—678
5. Carron, M.P., and Enjalric, F. (1982). Studies on vegetative micropropagation of *Hevea brasiliensis* by somatic embryogenesis and in vitro microcutting. In: Plant Tissue Culture, fojiwara, A. (ed) Maruzen. Co. Tokoya, PP 111—112.
6. Chatterjee, A. and Pakrashi, S.C. (1994). The treatise on Indian Medicinal Plant Vol. 3 publication and information Directorate, New Delhi, PP 36—71.
7. Finer, J.J. (1995). Direct somatic embryogenesis in plant cell tissue and organ culture-Fundal Methods Gamborg, O.I. and Philips, C.G. (eds) Springer—Verlag, Barlin Heidelberg. PP 91—102.
8. Cresswell, D., Boulay, M. and Franclet, A. (1982). Vegetative propagation of Eucalyptus. In Tissue Culture in Forestry. Bonga, J.M. and Durzan, D.J. (eds), Martinus Nijhoff/Dr. W. Junk Publisher, Dordrecht, PP 150—181
9. Ghate, V.S., Agte, V. and Vartak, V.D. (1988). Promising economic potential of Semul (*Bombax ceiba* L.) as a Tuber Crop. Ind. J. For 11(2):158-159.
10. Jones, O.P. (1983). In vitro propagation of tree crop. In Plant Biotechnology, Mantell S.H. and Smith, H. (eds), Cambridge Univ. Press PP:139-159.
11. Krikorian, A.D. (1982). Cloning higher Plants form aseptically Cultured tissue and cells. Biol. Rev, 57:151-218.
12. Laxmi Sita, G. Raghva Ram, N.V. and Vaidyanathan, C.S. (1980). Triploid plants from endosperm culture of

- Sandal Wood by experimental embryogenesis. Plant Sci.Lett. 20:63 -69.
13. Litz,R.E.(1984). In vitro somatic embryogenesis from nuclear cell of monoembryonic *Mangifera indica* L .Hort.Sci.19:1962-1964.
14. Venkatesh,C.S.(1988). Genetic importance of multipurpose tree species. The International Tree Crop.Jour, 5:109-124.
