Induction and Proliferation of Callus of Shorea Robusta In Vitro

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Abstract- *Callus of* ***Shorea robusta*** *G. was initiated and maintained from leaf explants, first soaked in MS liquid medium supplemented with BAP, Kn and 2, 4-D, then cultured subsequently on semi-solid medium of same composition. Browning of callus was observed due to phenolic accumulation after certain age. 2, 4-D + Kn was found suitable for long term callus culture and the growth rate revealed increasing growth indices with ageing of callus. Growth was found maximum in about 4 months duration.*

Keywords- Shorea robusta G., leaf, callus.

I. INTRODUCTION

During recent years tissue culture has been extensively tried in economically important tree species. Shorea robusta G. is one of the economic important trees which is known not only for timber but also for extensive tasar culture. It is the prime host plant of tasar silkworm Anthera mylitta D. One of the most economic ecorace of Antherea mylitta D "Raily" feeds on the foliage of Shorea robusta. The taxa is available world wide chiefly in the districts of Madhya Pradesh and Chattisgarh in India. Although Shorea robusta is a tall tree, however, we maintain it at human height level in order to do effective rearing of tasar silkworm. A part from Shorea robusta, different species of Terminalia i.e. T. arjuna, T. tomentosa, T. chebula, T. belerica are the chief host plant of different ecoraces of Antherea mylitta D. There are several other secondary and tertiary host plants of this silkworm.

Very scanty, almost nil, reports are available for tissue cluture of Shorea robusta G. An attempt has been made by Jain and Chaturvedi (20023) to induce tissue culture plants of Shorea robusta G., however, there was no follow-up afterwards. Other tree plants related to sericulture have been attempted successfully ie.., Morus alba (Mulberry) by Twewary and Oka (1999) to develop a suitable and reproducible single step protocol for regeneration of plant using liquid shake culture ; Terminalia arjuna (Arjun) and T. tomentosa (Asan) by Priya Ranjan et al. (1994) to induce callus ; T. arjuna (Arjun) by Rames et al. (2002) to regenerate plantlets; and T. belerica (Bahera) by Ramesh et al (2005) to induce somatic embryogenesis.

Under the present investigation, the authors for the first time have studies the repaid induction, proliferation and maintenance of callus of India Shorea robusta G. by in vitro technique for further scope of plant regeneration.

II. MATERIALS AND METHODS

Leaf explants of Shorea robusta G. after washing with 5% teepol for 10 minutes and rinsed several times in single distilled water, were surface strelized in 0.1% $HgCl_2$ solution for about 8 mintues followed by thorough washing with sterile distilled water.

Pieces of about 1 cm^2. of tender leaf explants were first soaked in liquid medium consisting of macro nutrients of Murashige and Skoog (1962); Gamborg's B5 vitamin (Gamborg et al., 1968) and 3% sucrose fortified with 6-benzyl aminopurine (BAP), Kineting (Kn), 2, 4-dichlorophenoxy acetic acid (2,4-D) of different concentrations (0.1-4 mg/I) and combinations, so as to allow the medium to penetrate into almot all the cells of explaints, The pH of the medium was adjusted between 5.6 – 5.8 with the help of KOH solution. After 24 hours, the explants were cultured on semi-solid media of the same cominations. All cultures were maintained at 25 ± 2^0C temperature exposed to photoperiod of 16/8 hours of light/dark duration (Fluorescent lamp, ca 3000 Lux).

The growth index of callus was calculated on every 21 days of growth. Growth index was calculated as follows :

$$\text{Growth index} = \frac{\text{F.W.} - \text{I.W.}}{\text{I.W.}}$$

F.W. = Final fresh weight I.W. = Initial fresh weight

III. RESULTS AND DISCUSSION

The explants cultured on semi-solid media with different concentrations of growth hormones, first enlarged

and then curled, Callusing was observed after about 4-5 weeks of culture. BAP and Kn alone did not provide any remarkable result. The maximum response of callus induction was found in the medium supplemented with 2, 4-D (3 mg / I) + Kn (0.1 mg/I) (Table I). Further, callus proliferation was also reported best in the same medium. Addition of kinetin at very low concentration to 2,4-D was found enhancing the growth of callus, Combination of BAP along with Kn did not provide any superiority over the use of 2,4-D + Kn. The callus formed was compact, nodular and creamish-yellow. During proliferation it turned greenish-yellow.

Table I. Callus response to different growth regulators supplemented media.

	2,4-D (mg/I)				
Kn (mg/I)	**0.5**	**1.0**	**2.0**	**3.0**	**4.0**
0.1	+	++	++	+++	++
0.5	+	++	+	+	+
1.0	+	+	++	+	+
2.0	+	++	+	+	+
3.0	+	++	+	++	+
4.0	+	+	+	+	+

+ Minimum ++ Moderate +++ Maximum

Ageing has considerable impact upon the callus, as during subsequent cultures callus turned soft, brown in colour. The callus ceased to grow due to accumulation of phenolic compounds which was also reported in other angiospermic taxa (Shah and Mehta, 1976; Singh and Rao, 1982).

Table II. Growth rate of callus in long term culture.

Days in culture	**21**	**42**	**63**	**84**	**105**	**126**	**147**	**168**
Growth index	0.20	0.50	1.40	2.50	3.85	4.75	5.20	5.45

The growth index showed slow increase in the beginning and gradually increases with the age of callus, culminating during 3-4 months of sub-culturing and slowing down almost to static after attending about 5-6 months of age (Table II).

In the present study, proliferation and maintenance of callus found responding to different growth hormones in different ways and the medium studied here is found most suitable for the purpose. In vitro technique for rapid callusing is possible, which in turn can be exploited for plant regeneration as and when required and for the study of biosynthetic products.

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