

***In vitro* callus induction of *Rosa hybrida* L. Cv. Babylon**

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ABSTRACT

In vitro study was carried out in the *Rosa hybrida* L.cv. *Babylon* to standardize the hormonal concentration for the callus induction using leaf bits as explant on Murashige and Skoog (MS) medium supplemented with different concentrations of auxins and cytokinins. MS medium with supplemented 0.5mg/L 2,4-D stimulated the callus initiation and the combination of 1.5mg/L 2,4-D and 0.1mg/L BAP concentrations was found to be suitable for maximum callus proliferation.

Key words: Callus, Leaf Bits, *Rosa hybrida*, Totipotency,
Indirect organogenesis, Auxins, Cytokinins.

INTRODUCTION

Roses have been for centuries, one of the most popular of ornamental plant. In terms of commercial flowering, roses are one of the top three in the world trade cut flowers. In India, roses are being grown for a long time and today are considered the principal cut-flowers crop grown in approximately 20% of the total area under ornamentals. Roses are propagated through direct and indirect organogenesis which enables substantial and fast multiplication, superior plant can be multiplied all the year round in large numbers and commercially exploited on large scale. This study was conducted to standardize the protocols for callus induction and somatic embryogenesis to further enhance the commercial value. (Lloyd, *et al.*, 1988) (Burger, *et al.*, 1988) reported that *in vitro* adventitious shoot differentiation in callus culture originated from leaf and stem bits and zygotic embryo segments of roses. Standardization of various auxins and cytokinins concentrations for callus initiation in rose using leaf bits as explants in the cultivar *Rosa hybrida* L.cv. *Babylon* were tried out .

MATERIAL AND METHODS

Rose plants were procured from Regional Agricultural research station, Kerala Agricultural University, Ambalavayal and maintained in the pots. Young Leaves were collected from the portion of shoots and were used as explants. They were washed with liquid soap followed by running water wash for 10 minutes. Then the plant material was subjected with 70% ethanol for about 30 seconds, and then treated with 0.1% mercuric chloride treatment for about 2- 3 minutes, followed by several washes with sterile distilled water to remove the traces of the surface sterilants. The explants were inoculated into MS medium (Murashige and Skoog 1962) with different concentrations of 2,4-D as plant growth regulator, ranging from 0.1 – 0.9 mg /L for callus initiation. For the rapid callus proliferation, the initiated callus was inoculated into the medium supplemented with the varying concentrations of 2,4-D (1.0 -3.0 mg/L) and BAP (0.1- 0.2 mg/L) respectively. Sucrose as carbon source and agar (phytoagar) as jelling agent. pH of the medium was maintained to be 5.8. The photo

period maintained was 18/6 hours dark /light at 1.0 ± 21°C for the callus initiation and for rapid callus proliferation.

RESULTS AND DISCUSSION

The hormonal concentration of 0.5mg/L 2,4-D was found to be the optimum hormone concentration for the callus initiation among the various concentrations tried out. Maximum percentage of callus initiation was achieved in this hormone concentration and the number of days was found to be 14.7 days and the percentage of initiation 83% respectively (Table 1). Low concentrations of 2,4-D resulted in delayed and decrease in percentage of callus initiation and even high concentration above the optimum level of the

hormone resulted in considerable reduction in callus initiation percentage. (Chi-ni Hsia and Schuyler S. Korban 1996) observed that pre incubation of somatic tissue of the cut rose '*care free beauty*' and miniature '*Red sunblaze*' and '*Babykati*' in 10,100 or 200 µM/ L 2,4-D induced rhizogenic callus.

The hormonal concentration of 1.5mg/L 2,4-D and 0.1mg/L BAP was found to be the optimum hormone concentration for rapid callus proliferation or multiplication among the various concentrations tried out. Number or amount of callus, No of days for callus proliferation and Percentage for the following parameters were recorded to confirm the above statement, was recorded as 3.6, 18 and 91% respectively (Table 2). (Rout, Debata and P.Das 1991) reported that callus was initiated from

Table 1: Various concentrations of the Auxins on callus initiation of *Rosa hybrida* L.cv. Babylon

S. No	Concentration of 2,4-D (mg/L)	No of days for callus initiation	Percentage of Callus initiation
1	0.1	33.2±1.07	45
2	0.2	25.7±1.07	58
3	0.3	21.8±0.9	65
4	0.4	18.9±0.8	70
5	0.5	14.7±0.48	83
6	0.6	15.75±0.68	75
7	0.7	15.9±0.72	72
8	0.8	16.1±0.8	69
9	0.9	16.2±0.85	65

Table 2: Various Concentration of the Auxins & cytokinins for Callus multiplication of *Rosa hybrida* L.cv. Babylon

S. No	Concentration of 2,4-D and BAP (mg/L)	Number or amount callus Proliferation	No of days for callus Proliferation	Percentage of callus Proliferation
1	1.0, 0.1	0.9± 0.08	20.7±0.74	70
2	1.5,0.1	3.6± 0.47	18±0.57	91
3	2.0, 0.1	1.8± 0.68	18.5±0.95	80
4	2.5, 0.1	1.6± 0.74	18.7±0.98	75
5	3.0, 0.2	1.85± 0.09	19.6±0.94	68



Fig. 1: MS medium with supplemented 0.5mg/L 2,4-D inoculated with Leaf Bit as explant for callus initiation

immature leaf and stem segments of *Rosa hybrida L. cv. Landora* and sub cultured every four weeks on basal half MS medium supplemented with 2.2-9.9 μM / L 2,4-D and 2.2 μM /L BAP and 5.4 μM /L NAA.

Further, the tubes with of low concentration of 2,4-D below the optimum level resulted in decreased percentage of callus. However, with the increase in concentration of 2,4-D also resulted in decrease of the above-mentioned parameters. Therefore, it becomes necessary to confirm that, though the concentration 2,4-D was increased above 1.5mg/L in combination with 0.1 mg/ L BAP the rapid proliferation of callus considerably reduction , with the above mentioned parameters, with the increasing concentration of 2,4-D and BAP. It has to be concluded that increased and decreased concentration of phytohormones above the optimum level inhibited the callus formation considerably.



Fig 2: MS medium with supplemented 1.5mg/L 2,4-D and 0.1mg/L BAP for rapid callus proliferation

CONCLUSION

MS media can be supplemented other chemical components for induction of somatic

embryogenesis. Auxin and cytokinin ratio has to be standardized for regeneration the callus into entire plantlet.

REFERENCES

1. Burger, D., Liu, W.L. Zary, K.W. and Lee, C. I. "Organogenesis and plant regeneration from immature embryos of *Rosa L. hybrida*." *Plant Cell Tissue Organ Culture*, **21**: 147-152 (1990).
2. Chi-ni Hsia and Schuyler S. Korban., "Organogenesis & Somatic Embryogenesis in callus cultures of *Rosa hybrida* and *Rosa Chinensis Minima*." *Plant Cell, Tissue Organ Culture*, **44** (1): 1-6 (1996).
3. Lloyd, D., Roberts, A.V. and Short, K.C. "The Induction of *in vitro* adventitious shoots in *Rosa*". *Euphytica*. **37**: 31-36 (1988).
4. Murashige, T. and Skoog, F. "A revised medium for rapid growth and bioassays with tobacco cultures." *Physiol. Plant.*, **15**: 473-97 (1962).
5. Rout, G.R. Debata, B.K. and Das, P. "Somatic embryogenesis in callus cultures of *Rosa hybrida L. cv. Landora*". *Plant Cell, Tissue Organ Culture*. **27**: 65-69 (1991).