

Effects of Sucrose and Myo-inositol on In Vitro Shoot Multiplication of Promising Interspecific F₁ Hybrid of Eucalyptus (*Eucalyptus Tereticornis* X *Eucalyptus Grandis*)

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Abstract – Micropropagation technique is standardized for its multiplication, using nodal segments of 25-30 years old trees i.e. promising interspecific F₁ hybrid of Eucalyptus (*Eucalyptus tereticornis* X *Eucalyptus grandis*). 0.1% Mercuric chloride solution for 10-15 minutes used for surface sterilization of nodal segments followed by 0.1% fungicide treatment for 1 minute and then washed 4-5 times with sterilized distilled water. These surface sterilized nodal segments were cultured on MS medium combination with auxin and cytokinin (NAA + BAP) for axillary bud proliferation. MS medium with combination of 1.5mg/l BAP + 0.1mg/l NAA gave optimum rate of axillary bud induction. The *in vitro* shoot were cultured on MS medium with different concentration of BAP (0.1–3.0 mg/l) alone or in combination with NAA (0.1-1.5mg/l) and supplemented with sucrose at 3% level was the best for the growth and development of shoots. These proliferated axillary shoots were excised and subcultured on MS + 1.0 mg/l BAP + 0.1mg/l NAA medium to proliferate *in vitro* shoots.

Keywords: Eucalyptus, MS medium and Proliferation

Introduction

Tissue culture, a major aspect of biotechnology

has a lot of potential for rapid mass multiplication and large-scale clonal plant development. The Forest Research Institute, Dehradun has generated promising interspecific F₁ hybrids of Eucalyptus that have shown a hybrid vigor in diameter, height, and wood quality. FRI- 6 is a hybrid of *E. tereticornis* and *E. grandis* that serves as a control (Venkatesh and Sharma, 1979). Because it contains *E. tereticornis* and *E. grandis* as parent species, this hybrid has a high economic value. The former has a rapid growth rate, best pulp quality, good stem form, and likes high rain fall places, whereas *E. tereticornis* is a drought tolerant species, hence this hybrid is quite likely to be suited for intermediate zones (Venkatesh and Sharma, 1979).

Material and Methods

Explants source and its culture: The source material for micropropagation was nodal segments with a single auxiliary bud. The nodal segments were rinsed in Certified (ICI Ltd. India) solution for 4-5 minutes before being surface sterilized with 0.1 percent Mercuric chloride solution (10-15 minutes) and then treated with 1.0 percent Bavistin for one minute. Other sterilants, such as NaOCl₂ (4%) and H₂O₂ (20%), were also investigated for nodal segment sterilization. Surface sterilized

nodal segments were washed with sterile distilled water three times. The axillary buds were surface sterilized and grown on Murashige and Skoog's (MS) medium combination with cytokinin (Kinetin and BAP). Before autoclaving the medium at 121° C for 15 minutes, the pH was adjusted to 5.8. Cultures were kept at 25 ± 2 ° C with 16 hours of illumination from white fluorescent tubes at a photon flux density of 2500 lux.

***In vitro* multiplication of shoot cultures** Proliferation of axillary shoots was seen in axillary buds cultivated on liquid and semi-solid MS media treated with cytokinin. These axillary shoots were removed and subcultured on semi-solid and liquid MS media containing BAP (0.1-3.0 mg/l) alone or in combination with NAA (0.1mg/l–1.5mg/l). The purpose of this study to increase the number of shoots to find the highest rate of shoot multiplication, a variety of studies were carried out. *In vitro* multiplied shoots were subcultured in a propagule of 6-8 shoots for this. A minimum of 12 repetitions were taken for each experiment. After a 5-week gap, observations were recorded. The shoots produced were excised in propagules and subcultured every 4-5 weeks once the ideal shoot multiplication medium was established. At 25±2°C, cultures were multiplied and kept under a photon flux density of 20-30 $\mu\text{EM}^{-2}\text{S}^{-1}$ during a 16-hour photoperiod. At the end of the experiment, the number of propagules cultivated and the number of propagules derived were compared.

Statistical Analysis All of the trials (experiments) were carried out three times. There are 12 duplicates in each treatment. For data from a completely randomized design, statistical tools Excel 2.0 and GenStat 8.0 were used to evaluate the data reflecting the means of three experiments. During the study, the data collected for various parameters was subjected to one and two way analysis of variance (ANOVA). The significance of the data

was determined using the F-test, and critical difference (C.D.) values of 5% were calculated for comparing differences in treatment means.

Results and Discussion

In vitro shoot multiplication

Effect of Plant Growth Regulators: The proliferated axillary *in vitro* shoots were excised from the mother explants and different hormonal concentration (0.1-3.0 mg/l BAP) used in MS medium for promoteshoot proliferation. These multiplied *in vitro* shoots were later dissected out into propagule (group of 6-7 shoots) and were subcultured on MS medium supplemented with different concentration of (0.1-3.0mg/l) BAP for further *in vitro* shoot multiplication. The best shoot multiplication rate was obtained in MS medium supplemented with 1.0 mg/l BAP + 0.1 mg/l NAA. On this optimal medium the shoot multiplication of 4-5 folds in every 5 weeks subculture duration was obtained (Table-1). Optimum rate obtained in MS medium for the establishment of shoot cultures in Eucalyptus hybrids. In earlier reports, MS medium has been successfully used for shoot initiation and establishment of Eucalyptus F₁ hybrids cultures (Gupta *et al.*, 1981, 1983; Kapoor and Chauhan 1992; Chang *et al.*, 1992; Bennett, 1994; Bisht *et al.*, 2000a and 2000b; Joshi *et al.*, 2003) and *Eucalyptus* F₁ hybrids (Arya *et al.*, 2009).

Table – 1 Hormonal interaction (BAP+NAA) on *in vitro* shoot multiplication in MS medium. Data recorded after 5 weeks.

Hormonal conc. (mg/l)	Average no. of shoots developed	Multiplication rate	Average no. of shoots length (cm)
0.1 NAA + 1.0 BAP	49.8 ± 1.9	7.12 ± 0.28	1.12 ± 0.03
0.5 NAA + 1.0 BAP	40.5 ± 1.8	5.79 ± 0.26	1.10 ± 0.04
1.0 NAA + 1.0 BAP	31.8 ± 2.00	4.55 ± 0.44	0.75 ± 0.08
1.5 NAA + 1.0 BAP	27.8 ± 1.7	3.98 ± 0.24	0.73 ± 0.09
Significance	***	***	***
CD	6.48	0.947	0.192

NS – non – Significant, *- Significance at 5%,

- Significance at 1%, *-Significance at 0.1%

Effect of sucrose: Because the photosynthetic power of cultures is restricted, carbon sources in the form of sugars must be introduced to the nutritional medium for development and proliferation of cultures *in vitro*. In this case, the growth and development of *in vitro* shoots were examined using varied concentrations of sucrose (1 percent - 6 percent) in MS media. Sucrose at 3% in MS medium produced the best results, with *in vitro* shoot multiplication of 6-7 folds (Table-2). The *in vitro* shoots did not multiply in a sucrose-free media, and the leaves and shoots turned pale green over time. The findings of this study are consistent with the reports of many researchers that used 3 percent sucrose as a carbohydrate source for shoot proliferation in several Eucalyptus species. (Gupta *et al.*, 1981; Gupta and Mascarenhas, 1983; Kapoor and Chauhan, 1992; Bisht 2000a and 2000b). However, there are many reports on successful multiplication of Bamboo shoots with 2% sucrose (Nadgire *et al.*, 1984; Nadgauda *et al.*, 1990; Saxena, 1990; Joshi and Nadgauda, 1997;

Yasodha *et al.*, 1997). Sharma *et al.*, 2013 had reported that increased level of sucrose at 3-4% did not effect shoot number but caused albinism. In this study, at high levels of sucrose (5-6%) no such instance of albinism were noted but the shoot multiplication rate declined. Similarly at 1% sucrose thin shoots and leaves were developed which were not suitable for further subculturing.

Effect of myo-inositol: The effect of myo-inositol on the rate of *in vitro* shoot multiplication was investigated. In comparison to the other concentrations tested, MS medium supplemented with 100mg/l gave the best multiplication rate. *In vitro* shoot multiplication was lowered in MS medium without myo-inositol. Excessive myo-inositol use in MS medium (150 mg/l and above) not only reduced *in vitro* shoot multiplication rate but also had a negative effect on *in vitro* shoots. As a result, 100mg/l myo-inositol was added to the medium in all trials as the optimal necessary for the growth and multiplication rate of *in vitro* shoots (Table-3).

Table – 2 Effect of different concentration of sucrose on *in vitro* shoot multiplication. Shoots cultured on 1.0 mg/l BAP + MS medium. Data recorded after 5 weeks.

Sucrose concentration	Multiplication rate	Average number of shoots produced	Average shoot length (cm)
0 %	2.57 ± 0.19	12.83 ± 0.94	2.00 ± 0.05
1 %	4.35 ± 0.33	21.75 ± 1.64	2.10 ± 0.05
2 %	7.03 ± 0.30	35.17 ± 1.52	2.40 ± 0.06
3 %	10.53 ± 0.42	52.67 ± 2.08	3.20 ± 0.06
4 %	9.35 ± 0.39	46.75 ± 1.95	2.80 ± 0.07
5 %	8.13 ± 0.38	40.67 ± 1.90	2.20 ± 0.06
6 %	6.55 ± 0.38	32.75 ± 1.87	1.80 ± 0.05
Significance	***	***	**
CD	0.97	4.88	0.174

NS – non – Significant, *- Significance at 5%, ** - Significance at 1% *** - Significance at 0.1% ± Values represent the Standard deviation

Table – 3 Effect of myo-inositol on shoot multiplication rate. Shoots inoculated on MS medium + 1.0 mg/l BAP. Data recorded after 5 weeks.

Myo-inositol concentrations	Multiplication rate	Shoots produced (Average number)	Average shoot length (cm)
Control	2.00 ± 0.20	10.00 ± 0.10	1.80 ± 0.07
50 mg /l	5.12 ± 0.27	25.58 ± 1.36	2.20 ± 0.07
100 mg/l	9.92 ± 0.43	49.58 ± 2.13	3.10 ± 0.05
150 mg /l	8.50 ± 0.37	42.50 ± 1.84	2.80 ± 0.06
200 mg/l	7.32 ± 0.42	36.58 ± 2.12	2.50 ± 0.06
Significance	***	**	***
CD	0.97	4.94	0.18

NS – non – Significant, *- Significance at 5%, ** - Significance at 1%, *** - Significance at 0.1% ± Values represent the Standard deviation

Conclusion

Tissue culture technique utilized to standardized *in vitro* shoot multiplication, using nodal segments of 25-30 years old trees of interspecific F₁ hybrids of Eucalyptus. The conclusion of this paper is used 3% sucrose with 10mg/l myo-inositol was added to the MS medium supplemented with BAP produced the best results for in vitro shoot multiplication.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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