BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2011. Vol. **8**(2), p. 881-883

Optimization of Media and Micropopagation of *Eucalyptus citriodora*

Farhina Pasha* and Sufia Irfan

Department of Biology, University of Tabuk, Science Girls College. Tabuk (K.S.A).

(Received: August 10, 2011; Accepted: September 17, 2011)

Key words: Micro-propagation, *Eucalyptus*, Cytokinin, BAP.

Plant tissue culture is an emerging field of plant biotechnology and a potential tool for rapid propagation of some economically important crops. The technique involves sterilization, inoculation and regeneration of plant cell, tissue or organ under aseptic condition in culture vials containing synthesis under medium. Both the chemical composition of the medium and the controlled environmental conditions (temperature, light, humidity, aeration, etc) effectively control the expression of any genotype or phenotype potential in the explants. *Eucalyptus* often called "gums" are a valuable source of hardwood and eucalyptus oils which are of commercial value, can be grouped according to their uses into three classes, viz. medicinal, industrial and perfumery oils. The main chemical components of Eucalyptus are camphene, citronelleal, fenchene, phellandrene, cineole. Eucalyptus has a clear, sharp, fresh and very distinctive smell. It is a clear to yellow in color and is thin and watery in viscosity. The medicinal *Eucalyptus* Oil is probably the most powerful antiseptic, Oil is used as a stimulant and antiseptic gargle Its antiseptic properties confer some antimalarial action, though it cannot take the place of Cinchona.

* To whom all correspondence should be addressed.

MATERIAL AND METHODS

One liter of MS (1962) media, was prepared using macro and micronutrients, sucrose and plant growth hormones. pH was adjusted between 5.2 to 5.8 by adding 0.1N NaOH of 0.1N HCl drop wise. The media was then transferred in cultural vials, after plugging, labeled and autoclaved at 15lbs 121^0C for 15- 20 minutess. Fresh sprouted lateral branches (10-20cm length) containing performed apical and auxillary bud were collected from upper branches of mature fast growing selected "elite" trees of *Eucalyptus citrodora* and highly oil containing (3.5%) "elite" tree of *Eucalyptus citriodora* at a forest of MP. Branches were brought of the laboratory in plastic bags with wet cotton, thoroughly washed with running tap water, followed by 2-4 drops of surfactant (Tween 20 or any soft liquid soap for 10 mins) and rewashed with tap water. The media was used with Cytokinin i.e., BAP (0.1-1.0 mg/l), Kinetin (0.1-0.5 mg/l), alone or with combination of Auxin i.e., NAA (0.1-1.0 mg/l), IBA (0.1-1.0 mg/l).The

culture were incubated cool with fluorescent light (2000–3000Lux) of 16hrs photoperiod and 8hrs dark period at a temperature of 25-30^{0}C and relative humidity of 50-60%. The culture was observed after 1 week interval.The axillary & apical buds were surface sterilization with 0.1% $HgCL_2$ solution for 5-10 mins. The sterile explant were than inoculated on Ms-medium containing different growth hormones with 30% sucrose & 4.5g agar.

For the initiation of shoot from axillary & apical bud media which is used are-

- 1-MS +0 .1BAP mg/l
- MS + 0.5 BAP mg/l
- MS + 0.5 BAP mg/l +0.1Kn mg/l
- MS + 0.5 BAP mg/l + 0.2 Kn mg/l
- MS + 0.5 BAP mg/l + 0.5 Kn mg/l

The growth was taken as the percent of bud response, number of shoots development, length of the shoots & callus developed. The initiation of shoots were excited & transferred in the fresh medium containing different concentration & combination of growth hormone to maximize the development of multiple shoots. The mediums which is used for the multiplication of eucalyptus initiated shoots are-

- 0.5 BAP mg/l +0.5 Kn mg/l
- 0.5 BAP mg/l +0.2 Kn mg/l
- 0.2 BAP mg/l +0.2 Kn mg/l
- 1.0 BAP mg/l +0.5 Kn mg/l
- 0.2 BAP mg/l +0.1 Kn mg/l
- 0.2 BAP mg/l +0.1 Kn mg/l +.5 Biotin mg/l
- 0.5 BAP mg/l +0.2 Kn mg/l +.5 Biotin mg/l

RESULTS

In the priming example of initiation of shoots the explant which is very soft responding fast in comparison to hard buds. The explant sterile for 5-10mins with 0.1% $HgCl_2$ solution shows 70-80% sterile culture. For initiation the effect of cytokinin (BAP & Kn) alone or in-combination of low concentration (.1-0.1mg/l.) were tested maximum percent of buds break and initiation of shoots was reported in the medium containing BAP & Kn in the concentration of 0.5 to 0.2mg/l about 1-2 shoots were developed after 10-15 days of culture the medium containing BAP alone shows 25-30% of shoot initiation callus was developed at the lower end of explant in the medium containing higher concentration of cytokinin. The initiated

Table 1. Effect of growth regulators on shoot induction in eucalyptus citriodora

S. No.	Medium + growth hormones (ml/l)	Age % of shoot induction	No. of shoot per culture	Average of shoot length in cm.	Callusing
1	MS + 0.1BAP	20%	1	0.5	-
2	MS+0.5BAP	20%	1	0.5	-
3	MS + 0.5BAP + 0.1Kn	30-35%	1-2	0.5-1	-
4	MS + 0.5BAP + 0.2Kn	50-60%	1-2	0.5-1	+
5	MS + 0.5BAP +0.5Kn	45-50%	1-2	0.5-1	++

Table 2. Effect of growth regulators on multiplication of shoot in eucalyptus citriodora

S. No.	Medium +Growth hormones (mg/l.)	Age % of response	Average of shoots	Average shoot length	Callusing
1	MS + 0.5 BAP	40%	4-5	1-2	+
2	MS + 0.5 BAP+0.5Kn	40-50%	4-5	1-2	+
3	MS + 0.2 BAP+0.2Kn	40-50%	5-6	1-2	-
4	MS + 0.5 BAP+0.2Kn	50-53%	7-8	1-2	-
5	MS +1.0 BAP+0.5Kn	50-60%	5-8	1-2	-
6	MS + 0.2 BAP+0.1Kn	60-65%	8-15	2-3	-
7	MS+0.2BAP+0.1Kn+0.5Biotin	70-80%	20-25	2-3	-
8	MS+0.5BAP+0.2Kn+0.5Biotin	50-60%	10-12	2-3	-

shoot attend 0.5- 1cm. length in 20 days culture.In order to optimize a best medium for mass multiplication of shoots different medium has been studies. The highest number of shoots were observed in the medium containing cytokinin in the concentration of (BAP 0.2-0.5mg/l) & Kn (0.1-0.3mg/l) about 8-15 no. of shoots were produced when subculture in the multiplication culture within 15 days. The number of shoots increase in the medium containing additional vitamin like Biotin about 20-25 shoots of 2-3cm. were developed within 20days. Thus the medium containing BAP, Kn, & BAP at low concentration is standard as the best medium for mass production of eucalyptus citriodora.

REFERENCES

1. Atal C.K, and B.M., Cultivation and utilization of medicinal plants, Regional research Laboratory. Jummu-Tawi, India 877p (1982).
2. Bouriey M, unpublished (personal communication). R.(1989) invitro studies phD (1986).
3. Baker P.K, Rade Fossard, Rabourn, progress toward conal propagation of *Eucalyptus* species by tissue culture techniques. In plant propagation soccomb proc **27**: 546-556 (1997).
4. Campbell, JY, Smith, DM, *The effects of short-rotation monocultures of Eucalyptus on rural ecosystems of India, with particular reference to* E. tereticornis *plantations in social forestry.* Report to the World Bank, Washington (1987).
5. Gill R.I.S, Gill S.S, GOSAL S.S, Rapid propagation of Eucalyptus. Tereticornis sm. *Through tissue culture and Biotechnology* 142-145 (1996).