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Mass Propagation in *Eucalyptus camaldulensis* Dehn

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Abstract: Nodal segments of *Eucalyptus camaldulensis* were inoculated on MS medium containing various concentrations of growth regulators. Moderate amount of callus got induced explant on MS medium fortified with 0.5 mg L^{-1} BAP and 1.0 mg L^{-1} 2,4-D. When the explants were cultured on MS medium containing 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} Kn, excellent brownish-yellow callus got induced. Shoots formation occurred on the callus when cultured on MS medium containing 0.5 mg L^{-1} each of BAP and IAA. The results indicated that shoots regeneration frequency got increased by culturing callus to MS medium containing 1.5 mg L^{-1} BAP and 0.5 mg L^{-1} NAA. Roots were induced on regenerated shoots on MS supplemented with 0.5 mg L^{-1} IBA.

Key words: Nodal sagment, callus, shoot, mass propgation, *Eucalyptus camaldulensis*

Introduction

Species of *Eucalyptus* are important sources of fuel, pulp and essential oil. *Eucalyptus* produces some of the heaviest, hardest and most durable wood, which makes this genus the most valuable source of hardwood in the world.

Eucalyptus are fast growing than most of the hardwoods having a rotation of 8-15 years. Vegetative propagation through grafting of the *Eucalyptus* trees, like in many other wood species, present graft incompatibility problems. Propagation by rooting of cuttings has also presented problems because of low rooting frequency. Cultivation of some *Eucalypts* species by lignotuber is reported from India (Bhatnagar and Joshi, 1973). The use of tissue culture technique has been attempted as an alternative means to obtain the required propagules.

Initial tissue culture studies on *Eucalyptus* concentrated largely on callus culture from seedlings and organ regeneration from callus. Another early objective was to use nodes essentially as microcutting and to induce roots from nodes of trees higher than is possible with conventional cuttings (Cresswell and Defossard, 1974; Cresswell and Nitsch, 1975). This was achieved for *Eucalyptus grandis*.

Attention then turned to multiplication of shoots *in vitro* before rooting was attempted. Work done by Defossard and co-worker established basic media (Defossard and Bourne, 1976) and these have been subsequently simplified (Gupta *et al*., 1981; Lakshmi sita and Vaidyanathan, 1979). Rooting of *in vitro* raised shoots were obtained in *Eucalyptus tereticornis* by Ilahi and Jabeen (1987).

The main objective of the present investigation is to develop a methodology for mass propagation of *Eucalyptus camaldulensis* using tissue culture technology.

Materials and Methods

The experimental studies were carried out on *Eucalyptus camaldulensis*. Nodal segments were taken from two years old plants. The plant material was thoroughly washed with tap water, then surface sterilized with 1% $HgCl_2$ solution for 3 min. followed by several washings in sterilized distilled water and inoculated onto the MS medium. The basal medium used throughout these studies was Murashige and Skoog (1962), supplemented with 5% sucrose. Agar was used at 0.9% concentration and the pH of the medium adjusted to 5.6. The medium was then autoclaved at 15 lbs/square inch pressure for 15 min at 120°C. Cultures were kept in controlled 16 h cycled fluorescent light cooled incubators with temperature regulated at 25±1°C. Phytohormones used were 2,4-D, BAP, Kn, As, IAA, NAA, IBA.

Results

Callogenesis: It is evident from the literature on plant tissue culture that callus tissue usually initiated from organ explant under the influence of varying concentrations of growth regulators (Thomas and Davey, 1975).

In this investigation the nodal segments inoculated on plain medium, could not form any callus and the nodal segments later on died. Therefore, growth regulators were used for callus induction and its further proliferation.

Effect of 2,4-D and BAP: The nodal segments of *Eucalyptus camaldulensis* were inoculated onto the MS medium supplemented with different combinations of BAP and 2,4-D for callus formation and its further growth.

Table 1: Callus induction on *Eucalyptus camaldulensis* nodal explants, its proliferation and regeneration on MS medium supplemented with different combinations of pytohormones

	Pytohormones mg L^{-1}				
Nature explant	BAP	2,4-D		Culture period (weeks)	Remarks
Nodal segments	0.5	0.5		4	Yellow-green compact callus formed
	0.5	1.0		4	Moderate, compact callus formed
	BAP	Kn			
	0.5	1.0		6	Fair amount of yellowish-white callus got induced
	1.0	0.5		4	Brownish-yellow soft callus got induced
	BAP	Kn	AS		No callus was formed
	0.5	0.5	10.0	4	Small amount of callus was formed
	0.5	0.5	5.0	4	
Callus	BAP	IAA			Good callus induction along with shoot regeneration
	0.5	0.5		4	
	BAP	NAA			Good callus induction, some shoots bud also got induced
	1.0	0.5		4	
					Shoot buds developed into shoots
	1.5	0.5		4	
Shoots	IBA				Roots got induced on the regenerated shoots
	0.5			4	

When MS medium was supplemented with 0.5 mg L^{-1} of BAP and 0.5 mg L^{-1} of 2,4-D. After four weeks yellow-green, compact callus was formed at the cut ends of the explants. This callus was sub-cultured on MS medium supplemented with 0.5 mg L^{-1} BAP and 1.0 mg L^{-1} 2,4-D. A moderate callus formation took place after four weeks. This callus exhibited yellow-white colour with a hard texture (Table 1). As a whole the rate of callus formation in both of the above concentrations, was slow.

Effect of Bap and Kn: Since the callus formed previously on 0.5 mg L^{-1} each of BAP and 2,4-D did not exhibited prolific growth, therefore, it was further sub-cultured on MS medium supplemented with BAP at a level of 0.5 and 1.0 mg L^{-1} and Kn 0.5 mg L^{-1}. The calli transferred to medium supplemented with 0.5 mg L^{-1} BAP and 1.0 mg L^{-1} Kn exhibited good growth in six weeks. This callus was greenish-white and hard (Table 1). Some calli were sub-cultured on MS medium containing 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} Kn, after four weeks, a brownish-yellow somewhat soft callus was induced. The callus formation was good at these concentration of BAP and Kn.

Effect of Bap, Kn and As: In another set of experiments the nodal segments were inoculated onto the MS medium supplemented with a combination of BAP, Kn and AS. When the medium was supplemented with 0.5 mg L^{-1} each of BAP and Kn and 10.0 mg L^{-1} of AS. No callus was observed on the explant. Nonetheless, some swelling were observed at the cut ends of the explants (Table 1). These explants were sub-cultured on freshly prepared MS medium decreasing the level of AS to 5 mg L^{-1}, while BAP and Kn were supplemented at a level of 0.5 mg L^{-1} each. This time small amount of callus was induced on the explants alongwith axillary shoots formation. These axillary shoots attain a length of 3-5 cm after four weeks (Table 1).

Organogenesis: Many plant growth regulators included in the culture medium induce *in vitro* organogenesis. A large number of plants species respond to a suitable auxin/cytokinin balance, forming shoots and roots. Evans *et al.* (1981) found that for 75% of the species forming shoots either Kn or BAP was used in a concentration range of 0.05-40 mg L^{-1}. Auxins such as IAA and NAA were used in concentration of 0.06-20 mg L^{-1}.

In this investigation, different combinations of cytokinins and auxins were used to induce regeneration in *Eucalyptus camaldulensis*. To induce shoots regeneration, the yellowish-green callus previously cultured callus induced on MS medium containing 0.5 mg L^{-1} of BAP and 1.0 mg L^{-1} of Kn was further sub-cultured to the medium fortified with 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} BAP. At this concentration of BAP and NAA there was a good callus formation. Shoot buds were also induced on the callus. This callus was further inoculated on medium raising the concentration of BAP to 1.5 mg L^{-1}, while NAA concentration was kept

constant i.e. 0.5 mg L^{-1}. After four weeks, the adventitious buds exhibited prolific growth and developed into 3-4 node shoots. A moderate amount of callus was also induced (Table1).

Rizogenesis: To induced rooting and obtained whole plantlets, the *in vitro* raised shoots were cultured on MS medium supplemented with either 0.5 mg L^{-1} IBA, 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} IAA. After a culture period of one week, these shoots were consequently transferred to plain MS medium. After a culture period of four weeks, roots were got induced on the shoots treated with 0.5 mg L^{-1} IBA. The rooting percentage was about 20%. No root was induced in cultures supplemented either IAA and NAA. Nonetheless, the cut ends of the shoots show swelling after four weeks. The rooted shoots were kept on the same medium for another four weeks so as to develop a better root/shoot system.

Discussion

These studies were undertaken with a view to regenerate plantlets in *Eucalyptus camaldulensis*. From the results obtained in the preceding section, it is evident that callus induced on the stem nodal explant could be successfully and continuously sub-cultured for at least six months period. Further sub-culturing of the callus resulted in shoot regeneration and plantlet formation.

It is evident from the literature that BAP or 2,4-D alone has no effect on callus induction in *Eucalyptus* species, (Ameen *et al*., 1985), therefore, the nodal segments were inoculated on MS medium supplemented with 0.5 mg L^{-1} BAP and 0.5 mg L^{-1}, 1.0 mg L^{-1} 2,4-D. At both these concentrations, moderate amount of callus formation occurred, exhibited hard texture. Similarly Jamal (1985) obtained friable callus in *Eucalyptus tereticornis* using 2,4-D at a level of 0.5 mg L^{-1} in combination with 0.5 mg L^{-1} BAP. Thus it confirm our finding.

A combination of BAP and Kn. was also used for callus proliferation. The MS medium was supplied with 0.5 mg L^{-1} BAP and 0.5 mg L^{-1} Kn. At this concentration the callus shows prolific growth and exhibited greenish-white hard texture. Contrast to obtained findings, Ilahi and Jabbeen (1987) obtained high frequency of multiple shoots on nodal segments of *Eucalyptus tereticornis,* when MS medium was supplemented with 1.0 mg L^{-1} each of BAP and Kn.

When MS medium was supplemented with a combination of 0.5 mg L^{-1} each of BAP and Kn. and 5.0 mg L^{-1}, 10.0 mg L^{-1} of AS. Axillary buds sprouting were observed, when callus was cultured on medium containing 0.5 mg L^{-1} each of BAP, Kn and 5.0 mg L^{-1} AS. When the concentration of AS was increased to a level of 10.0 mg L^{-1}, 4-5 axillary shoots were obtained on the explant. Similarly Ilahi and Jabeen (1987) obtained high frequency axillary shoot percentage in *Eucalyptus tereticornis* on MS medium supplemented with 0.5 mg L^{-1} BAP, 0.1 mg L^{-1} Kn and 5.0 mg L^{-1} AS.

Shoot regeneration was obtained on callus inoculated on medium supplied with 0.5 mg L^{-1} each of BAP and IAA and 0.5 mg L^{-1} NAA and 0.1 mg L^{-1} BAP. Shoot regeneration was initiated on the callus on both the mediums with in a culture period of four weeks. Further sub-culturing on the same medium resulted vigorous growth of the shoots. Along with shoot regeneration, increase callus mass was also obtained. Islam and Joarder (1995) obtained similar results. They got vigorous shoots on *Eucalyptus* callus sub-cultured to a medium supplemented with NAA and BAP.

For root initiation IBA, NAA and IAA were used at a level of 0.5 mg L^{-1} each. Of the auxins tried for root initiation, only IBA induced extensive rooting with in four weeks. Lakshmi Sita and Shobha Rani (1985) in *Eucalyptus grandis* obtained similar results. They observed 30% rooting on 1/5 MS medium supplemented with 0.5 mg L^{-1} IBA

This study induce plantlets regeneration from explant of *Eucalyptus camaldulensis*. These regenerated plantlets could be further transferred to the natural coditions.

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