In Vitro Clonal Propagation of Mature Eucalyptus F1 Hybrid (E. torelliana F. V. Muell x E. citriodora Hook)

By M. L. KAPOOR[1]) and J. M. S. CHAUHAN[2])

Radio Isotope Laboratory, Division of Genetics and
Tree Propagation, Forest Research Institute,
Dehra Dun — 248006, India

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Abstract

Multiple shoots from mature nodal segments of 8-yr-old *Eucalyptus* F1 hybrid *(E. torelliana* X *E. citriodora)* were obtained through tissue culture on MURASHIGE and SKOOG's medium supplemented with BAP, NAA and/or GA_3. Rooting of these shoots was obtained in one-half and one-fifth MS media each supplemented with 0.1 and 1.0 mg/l IBA, but the shoots were healthier in the latter medium. The rooted shoots (plantlets) were transferred to the field. Forty subcultures have already been carried out without any reduction in the capacity for shoot or root formation. It can be estimated that about 2,00000 plantlets can be obtained from a nodal segment in a year by this process. The method helps in retaining the vigour of F1 hybrid of *Eucalyptus* which declines in successive generations through seed.

Key words: Tissue culture, *Eucalyptus* F1 hybrid, mature nodal segment, mass clonal multiplication.

Introduction

F1 *Eucalyptus* hybrid (*E. torelliana* X *E. citriodora*) is 3 to 5 time superior in growth (KAPOOR and SHARMA, 1984). When F2 population was raised through seed a lot of segregation was observed and only a few good recombinants were obtained. Segregation reduced the average yield per unit area, per unit time. To overcome this problem, propagation through tissue culture was considered economically advantageous. In general, it is believed that tissue culture can be used as a potential adjunct to accelerate tree improvement achievements (BAJAJ, 1986; BONGA, 1977; DURZAN and CAMPBELL, 1974; KAPOOR *et al.*, 1991). Success has already been achieved for several tree species by tissue culture as an alternative method for rapid multiplication (BHOJWANI *et al.*, 1986; BONGA, 1982; HU and WANG, 1983). Tissue culture work has already been done in *Eucalyptus* species (MC COMB and BENNETT, 1986), however, there are only few reports available in the literature on the multiplication of mature hybrids of *Eucalyptus* through tissue culture (LUBRANO, 1984, 1988; POISSONIER *et al.*, 1984). The main objective of these studies was to increase productivity of *Eucalyptus* F1 hybrid by producing clonally uniform plantlets on mass scale in a shorter duration of time through tissue culture.

This paper describes the successful *in vitro* multiplication and transplantation of *Eucalyptus* F1 hybrid (*E. torelliana* X *E. citriodora*) with high rate of multiplication and field survival.

Materials and Methods

(i) Explant

Young shoots were collected from 8-yr-old tree of *Eucalyptus* F1 hybrid (*E. torelliana* X *E. citriodora*) growing in New Forest campus of this Institute (altitude 610m, latitude 30° N, longitude 78° E, annual Rain fall 216 cm) during September to November. Shoots were washed in running tap water to remove the dust particles. Nodal segments measuring 5 mm to 8 mm in length were cut, washed with teepol solution (5 to 10 drops in 100 ml water) and surface sterilized in 2% solution of Sodium hypochlorite for 30 minutes. To remove the sterilent nodal segments were again washed with sterile distilled water and were then inoculated in different media combinations.

(ii) Culture media

MS basal medium (MURASHIGE and SKOOG, 1962) containing 3.0% sucrose, solidified with 0.6% bacteriological agar (Ranbaxy) and supplemented with BAP, NAA and/or GA_3 at different concentrations was used for multiple shoot induction, solid MS medium supplemented with BAP at 1.0 mg/l for shoot multiplication and for rooting solid one-half and one-fifth MS media each supplemented with IBA at 0.1 mg/l and 1.0 mg/l were used. The pH of all media were adjusted to 5.8. All the glasswares were of Borosil-India brand and chemicals were of analytical grade (Ranbaxy, British durg house, E. Merck or Sigma).

(iii) Culture Conditions

Cultures were incubated at 23° C for 16 hours in light (illuminated by 40 watt fluorescant tubes, 1200 lux) and for 8 hours in dark.

Sixteen replicates were used for shoot initiation and 96 replicates for rooting studies.

Results and Discussion

Multiple shoot initiation and multiplication

Of the different media combinations tested, 72.72% of the nodal segments were found to develop multiple shoots (4.5 ± 0.18) (*Table 1*) within 20 to 25 days on MS + BAP (2.0 mg/l) + NAA (1.0 mg/l) medium. The multiple shoots were separated and subcultured on MS + BAP (1.0 mg/l) medium. By this procedure, 40 subcultures have already been carried out at intervals of 30 days. The rate of shoot formation increased to 20 to 25 shoots per culture after sixth subculture *(Figure 1).*

Rooting

Details of rooting results are provided in *table 1.* 95.83% rooting was obtained in 1/5 MS + IBA (0.1 mg/l) medium followed by 50.00% in 1/2 MS + IBA (0.1 mg/l) medium but the plantlets had better shoot growth in the latter medium (*Figure 2*). Callus formation was also observed at the base of few shoots in both 1/2 and 1/5 MS media each supplemented with IBA at 1.0 mg/l which inhibited root development in those shoots.

[1]) Head, Division of Genetics and Tree Propagation
[2]) Research Assistant, Grade — 1

Table 1. — Multiple shoot initiation and rooting of shoots.

MULTIPLE SHOOT INITIATION								ROOTING OF SHOOTS				
Plant growth regulators in addition to MS medium. (conc. in mg/l)			No. of explant used.	No. of explant contaminated.	No. of explant produced multiple shoots.	Percentage of cultures with multiple shoots.	No. of shoot per culture/ explant (Mean±SE)	Medium/Treatment. (Conc. in mg/l)	No. of shoots kept for rooting.	No. of shoots rooted.	Rooting percentage.	Remarks
BAP	NAA	GA_3										
0.5	0.01	-	16	2	2	14.28	1					
0.5	0.1	-	16	1	4	26.66	2.5±0.25					
0.5	0.5	-	16	0	4	25.00	2.3±0.22	1/2MS+IBA(0.1)	96	48	50.00	Plantlets had good shoot growth.
0.5	1.0	-	16	5	4	36.36	3.3±0.22					
1.0	0.01	-	16	4	2	16.66	1.5±0.35					
1.0	0.1	-	16	0	6	37.50	3.3±0.19					
1.0	0.5	-	16	1	2	13.33	1					
1.0	1.0	-	16	3	4	30.76	2.7±0.22	1/2MS+IBA(1.0)	96	32	33.33	
2.0	0.01	-	16	3	1	7.69	1					
2.0	0.1	-	16	0	8	50.00	3.6±0.17					
2.0	0.5	-	16	0	2	12.50	1					
2.0	1.0	-	16	5	8	72.72	4.5±0.18					
5.0	0.01	-	16	1	1	6.66	1					
5.0	0.1	-	16	2	2	14.28	1					
5.0	0.5	-	16	2	2	14.28	1	1/5MS+IBA(0.1)	96	92	95.83	Plantlets had poor shoot growth.
5.0	1.0	-	16	5	1	9.09	1					
0.5	0.01	0.5	16	4	1	8.33	1					
0.5	0.1	0.5	16	1	10	66.66	4.4±0.15					
0.5	0.5	0.5	16	2	10	71.42	4.5±0.16					
0.5	1.0	0.5	16	0	2	12.50	1	1/5MS+IBA(1.0)	96	56	58.33	
1.0	0.01	0.5	16	2	1	7.14	1					
1.0	0.1	0.5	16	2	7	50.00	3.4±0.18					
1.0	0.5	0.5	16	1	6	40.00	3.5±0.20					
1.0	1.0	0.5	16	2	2	14.28	1					

Acclimatization of the rooted shoots

When the rooted shoots had attained a height of about 2.5 cm to 3.0 cm they were transferred to pots containing a sterile mixture of soil : sand : manure (1:1:1) and covered with polythene bags. The polythene bags were periodically withdrawn to acclimatize the plantlets and when new leaves had emerged the polythene bags were completely withdrawn. After 10 months when the plants had attained a height of 1.15 m (*Figure 3*) they were planted out in the field (*Figure 4*). The survival rate during transfer to soil mixture in pots and in the field was recorded 60% and 80% respectively. By this procedure, it can be estimated that more than 2,00000 plantlets can be obtained from a nodal segment in a year.

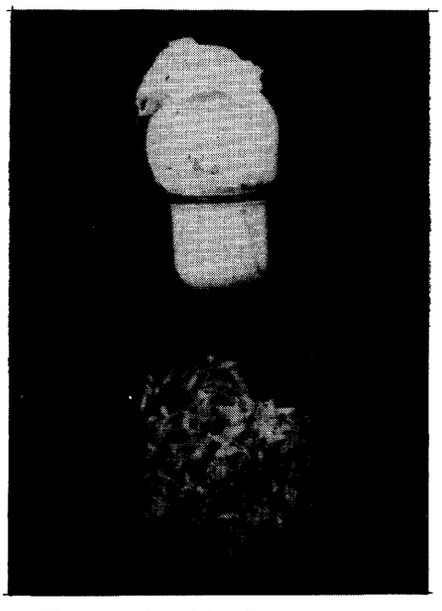

Figure 1. — Axillary shoot-proliferation on MS + BAP (1.0 mg/l) medium.

Figure 2. — A rooted shoot on 1/2 MS + IBA (0.1 mg/l) medium.

Direct regeneration of plantlets from the explants such as buds, meristems ensures the cloning of genetic stocks (Bonga, 1977; Bajaj, 1986). So, by adopting this *in vitro* technique and taking mature nodal segments as explants, the hybrid vigour can be stabilized and novel hybrids can be multiplied on mass scale.

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Figure 3. — A ten-month-old potted plant.

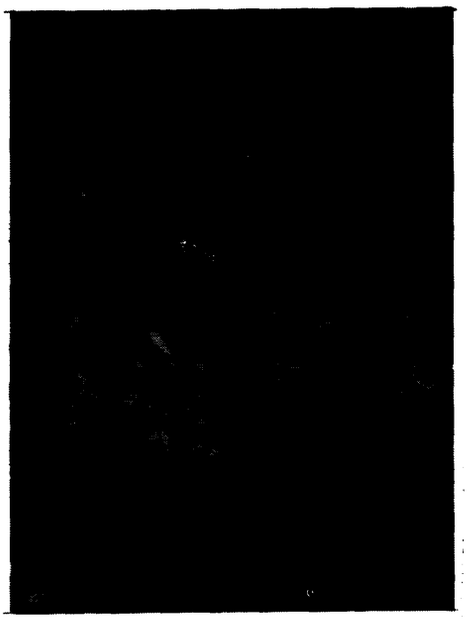

Figure 4. — An eleven-month-old field established plant.

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Will Traditional Conifer Tree Breeding for Enhanced Stem Production Reduce Wind Stability?

Genetic Variation in Allocation of Biomass to Root Classes and Stem

By C. Ch. N. Nielsen[1])

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Abstract

The productivity of different root groups are to a considerable extend influenced by other genes than stem production, which is the basis for a genetic variation in different root/stem-ratios. Traditional selections in conifer breeding programmes for enhanced stem production might reduce the ratio between below and above ground biomass compartments and might as such decrease the wind stability of genetic "improved material".

Key words: root, root/shoot-ratio, stability, selection, breeding.

Contents

[1]) Dr. agro, ass. Prof. at the Danish Forest and Landscape Research Institute, Skovbrynet 16, DK-2800 Lyngby, Denmark