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MASS PROPAGATION OF *EUCALYPTUS TERETICORNIS* SMITH

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Abstract

Eucalyptus tereticornis seeds germinated on 5% sucrose agar medium. A massive callus was induced on 6 week old seedlings when cultured on MS containing 0.5 mg/l each of 2, 4-D and BAP. Multiple shoots resulted when MS was supplemented with 0.1 mg/l each of K and BAP and 5.0 mg/l of AS. Although massive callus was induced on six month old nursery raised seedlings, no organogenesis was detected on MS with 1.0 mg/l 2, 4-D. Addition of various cytokinins and CM with or without NAA had no effect on organogenesis. NAA @ 0.1 or 0.5 mg/l with 0.5 mg/l BAP and 500 mg/l CH induced a massive callus which gave rise to numerous bud primordia.

Introduction

The world demand for wood production is expected to rise over the next few decades from 130 million metric tonnes in 1970 to twice that in 1983 and five times more by the turn of the century (Wayman, 1972). To meet this growing demand there will be an increasing need for large number of trees of improved quality and shortened rotation. The present day tree improvement practices offer only limited possibilities to achieve this goal. One new technique that shows promise is tissue culture and its use in forestry should be considered since haploid plants raised *in vitro* will provide homozygous lines for breeding purposes. Furthermore, polypoloidy is a rare phenomenon in tree species but in a few cases success has been achieved as polyploid *Populus* sp. exhibited higher growth rates compared to the diploids (Gustasson, 1960; Muhle, 1970).

Eucalyptus belonging to the family Myrtaceae is an economically important plant. It ranges from straight-trunked forest trees upto 90 m tall to multiple-stemmed, shrubby mallees. Some of the species are salt tolerant and can help alleviate the problem by lowering the water table with a consequent leaching of the salts. Eucalypts grow in a variety of climates and are finding their importance in paper industry, as shade, windbreak and ornamental trees. The essential oils of the foliage are long chain or cyclic hydrocarbon and used for medicinal, industrial or perfumery purposes. Furthermore, the oils are also being considered as possible components of liquid fuel (Calvin, 1980). For vegetative propagation of *Eucalyptus* sp., tissue culture techniques have been utilized for a few species (Lakshami Sita, 1981; McComb & Bennet, 1986). Methods used for mass propagation of *Eucalyptus tereticornis* are presented here.

Material and Methods

The experimental studies were carried out on *Eucalyptus tereticornis* of the family Myrtaceae. Two types of explants were utilized (a) *in vitro* raised seedlings and (b) young stem pieces obtained from 6 month old seedlings. The seeds and seedlings were obtained from the Silviculture Farm, Pakistan Forest Institute, Peshawar. Seeds of *E. tereticornis*, thoroughly washed in tap water, were surface sterilized with 1.0% solution of mercuric chloride for 2 min., then treated with 75% alcohol for 2 min., and rinsed three times with sterile distilled water. The seeds were inoculated on plain agar containing 5.0% sucrose. The seeds started germination after about 4 weeks and were then inoculated on the test media. Apical portions of the tender stem from about 6 month old nursery raised seedlings were surface sterilized as above and used as explants.

The basal medium consisted of MS salt solution prepared according to Gamborg & Wetter (1975) with 0.9% Difco-Bacto agar, 5% sucrose in distilled water and adjusted to pH 5.8. Fifty ml of the medium poured into 100 ml Erlenmeyer flasks were plugged with cotton wool and their mouth covered with aluminium foil. The medium was autoclaved at 15 psi for 15 min.

Coconut milk (CM) was prepared according to Jamal (1985). Coconut milk, other organic addenda and growth substances were added to the medium before sterilization. The explants were inoculated onto the medium under aseptic conditions and kept under 16 h cycled fluorescent light cooled biotrons at 25 ± 1°C with 65% RH.

Results

a) *Micropogation by in vitro raised seedlings:* Seeds inoculated on 5% sucrose agar started germination after about 4 weeks. Small seedlings produced were transferred after 6 week to MS medium containing 2, 4-D 1.0 mg/l. Callus was induced on seedling tips which multiplied further and after about one month a massive callus was produced. Cytokinins viz., 6 furfurylaminopurine (K), Benzylaminopurine (BAP) and Adenine sulphate (AS) used alone had no effect on callus induction. A massive callus was obtained when 2, 4-D @ 0.5 mg/l and BAP at 0.5 was added to the MS medium.

In the presence of 0.1 mg/l each of K and BAP and 5 mg/l of AS, the seedlings exhibited prolific vegetative growth compared to those growing on MS alone (Table 1). Little callus formation was also observed on leaves of seedlings growing on MS supplemented with BAP (0.1 mg/l) and AS (5 mg/l). When the three cytokinins were combined in different ratios i.e. either 0.1 or 0.5 mg/l of K, 0.5 of BAP and 5 of AS, multiple shoots were produced on the inoculated seedlings. These shoots were formed by the division of the apical meristem after 6 weeks of inoculation. Usually 5-6 such daughter shoots resulted on each seedlings (Fig. 1). The leaves in contact with the medium also

Table 1. The effects of K. BAP and AS on *Eucalyptus tereticornis* seedlings raised *in vitro.*

Growth regulators mg/l K	BAP	AS	Callus	Remarks	Weeks of culture
0.1	0.1	–	–	Seedling showed good vegetative growth	10
0.1	0.1	5.0	+	Good vegetative growth with whitish soft callus formation near the midribs	10
0.1	0.5	5.0	+	Seedlings formed multiple shoots. Whitish soft callus formed on leaves	10

Legends used: No callus –, Little callus +

produced vigorous callus which after initiation from the midrib proceeded to the whole leaf. These daughter shoots could be easily separated from each other and grown independently on MS medium without the supplementation of any growth regulating substance. However, only one daughter shoot contained the mother root system. Therefore, further studies are being carried out to induce roots in these plantlets. Another advantage of these regenerated shoots was their vigour compared to parent seedlings.

b) *Micropropagation by stem explants:* Stem explants, about 1 cm long, obtained from the upper tender portion, were inoculated on MS containing various growth hormones at various concentrations (Table 2). Cell division was very rapid at 1.0 mg/l of 2, 4-D and a yellowish friable callus mass of a large size with patches of reddish pigments was obtained after 6 weeks of culture (Fig. 2). This callus remained healthy but later exhibited browning with prolongation in the culture period. On further subculture, although the callus increased further, no organogenesis could be noticed in this callus

Fig. 1. Multiple shoot formation on 6 week old seedlings cultured on MS medium supplemented with 0.1 mg/l of K, 0.5 BAP and 5 of AS.

Table 2. Effects of 2, 4-D, BAP and CM on callus induction on *Eucalyptus tereticornis* stem explants after 6 weeks of culture.

Growth regulator mg/l			Callus	Remarks
2, 4-D	BAP	CM		
0.1	0.1	–	+	Yellowish-green, soft and friable callus
0.1	0.5	–	+	Greenish friable callus
1.0	0.1	–	++	Yellowish-green friable callus
1.0	0.5	–	++	Yellowish-green friable callus
1.0	–	–	+++	Green friable and nodulated callus
1.0	–	10%	++	Compact callus

Legends used: No callus –, Little callus +, Moderate callus ++, Copious callus +++

mass. At 1.0 mg/l of 2, 4-D and 10% CM, somewhat compact and hard callus of yellowish colour was obtained (Fig. 3). At different concentrations of 2, 4-D and BAP, similar type of calli without exhibiting any organogenesis were obtained. The callus mass was also reduced in these treatments (Table 2).

A friable type of callus resulted on stem explants when naphthalene acetic acid (NAA) at 0.1 or 0.5 mg/l was added to the MS (Table 3, Fig. 4). This callus turned brown within 4 weeks and was difficult for further subculture. The callus induced on stem explants was large and a compact type of callus resulted when MS contained 0.5 mg/l each of NAA and BAP (Fig. 5).

Fig. 2. Induction of yellowish friable callus on stem explants when cultured for 6 weeks on MS supplemented with 1.0 mg/l 2, 4-D.

Fig. 3. Formation of compact callus on stem explants after 6 weeks on MS fortified with 1.0 mg/l 2, 4-D and 10% v/v CM.

Table 3. Effect of NAA, BAP, CM and CH on callo- and organo-genesis in *Eucalyptus tereticornis* stem explants after 6 weeks of culture

Growth regulators mg/l		Organic addenda mg/l		Callus	Remarks
NAA	BAP	CM	CH		
0.1	–	–	–	+	Yellowish brown callus
0.5	0.5	–	–	++	Yellowsh beaded callus
0.1	0.5	–	–	++	Compact, nodulated callus
0.5	0.5	10%	–	++	Whitish-yellow friable callus
0.1	0.1	–	500	++	Soft callus with shoot primordia
0.1	0.5	–	500	+++	Fairly large number of shoots
0.5	0.5	–	500	+++	Green nodulated callus
0.5	0.5	–	1000	++	Green nodulated callus

Legends used: No callus –, Little callus +, Moderate callus ++, Copious callus +++

Addition of CM to the above treatments was without any effect on organogenesis. However, when NAA at 0.1 or 0.5 mg/l alongwith 0.5 of BAP and 500 mg/l of casein hydrolysate (CH) was added to the MS, the callus induced on the explants was massive (Table 3). It gave rise to numerous bud primordia on further subculture on the same medium after about 6 weeks (Fig. 6). The number of these bud primordia further increased when the callus was further subdivided and inoculated on the same medium. This callus mass gave rise to more plantlets which assumed a healthy growth within 4 weeks of culture (Fig. 7). The individual plants are being treated with root-inducing substances so that these can be transferred to field conditions. Surprisingly when the CH level was

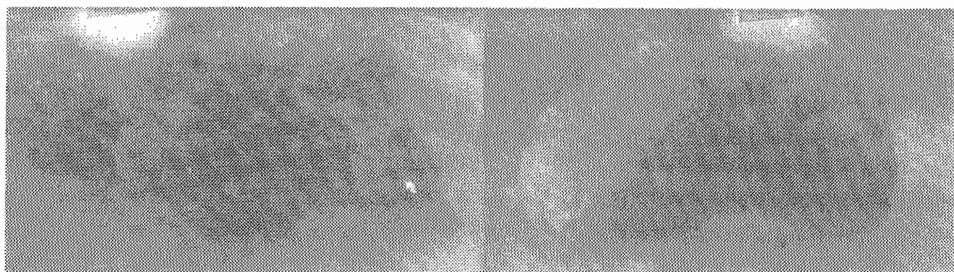

Fig. 4. Friable callus formation on stem explants raised on MS containing 0.5 mg/l NAA.

Fig. 5. Compact callus formation on MS fortified with 0.5 mg/l each on NAA and BAP.

Fig. 6. Numerous bud primordia were induced on the callus fromed on MS fortified with 0.1 mg/l NAA, 0.5 of BAP and 500 of CH.

Fig. 7. Plantlet formation from bud primordia of Fig. 6.

raised to 1 g/l, only callus was induced on the explants which exhibited no organogenesis after any number of subcultures. Similarly when NAA was substituted with Indoleacetic acid (IAA), the callus induced exhibited no organogenesis. Moreover, addition of gibberellic acid (GA_3), biotin, yeast extract was without any added advantage as no organogenesis resulted in the callus nurtured on MS containing these organic addenda.

Discussion

Although 2, 4-D was without any effect on seedling growth, it induced a massive callus on stem explants at 1.0 mg/l level. This effect is in contrast to another tree species like peach, where both an auxin and cytokinin were required for callus induction (Rehman, 1983). Use of a cytokinin alone was without any effect on callus induction although it supported vegetative growth of the *in vitro* raised seedlings.

In *E. tereticornis,* however, plantlets were induced directly on the static callus mass and only in the presence of NAA (0.1 or 0.5 mg/l), BAP (0.5 mg/l) and CH (500 mg/l). All the other combinations and variety of growth regulating substances were without any effect on organogenesis. Mascarenhas *et al* (1981, 1982) were further able to induce plantlet formation in *E. citriodora* on MS containing K (0.2 mg/l), BAP (0.3), calcium pantothenate (0.1) and biotin (0.1). Although calcium pantothenate was not used during the present investigations, biotin was found to be without any effect on organogenesis in combination with K and BAP. This difference might be due to the hereditary make up of both the species. The review of literature also indicated difference in response of the explant regarding its origin from the parent plant and its age. Nonetheless, our results offer good opportunity for the mass propagation of this important eucalypt species.

Tissue culture techniques further offer methodology for rapid cloning and vegetative propagation of a desired tree species which by conventional methods of cutting, grafting and other silvicultural or horticultural practices may take years (Bajaj, 1986). This faster rate of multiplication enables rapid production of phytomass energy. During the next decade this biotechnology would be utilized for the improvement and production of novel trees. The methodology developed for *E. tereticornis* would help for rapid production of seedlings on large scale cultivation to acclaim the waste land and improvement of the trees for rapid growth. Eucalypts are also helpful in apiculture, thus their cultivation might have enormous benefits for our country.

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