TISSUE AND ORGAN CULTURE OF EUCALYPTUS

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ABSTRACT

Tissue culture of **Eucalyptus** stems and lignotubers is described, and the responses of calluses to various auxin-cytokinin modifications of the culture medium are summarised. Regeneration of plants from such calluses was not achieved. A cultural mixture of **Eucalyptus** callus and regenerating tobacco callus did not induce regeneration of **Eucalyptus**.

Organ culture of **E. grandis** is described. Axillary bud development and root development was most frequent in nodal explants at the basal end of seedlings, particularly with foliated explants. Successful root initiation and development of plants from nodal cultures has also been achieved with older (up to 7 month) **E. grandis** trees, using a culture medium with 5×10^{-6}M indole-butyric acid. These plants had more than 50 nodes, and these organ cultures were initiated from nodes considerably higher than the 15th node "barrier" experienced with classical methods of propagation.

INTRODUCTION

Tissue and organ culture techniques have been a part of experimental botany for some years. More recently, these techniques have been considered as being potentially suitable to overcome certain problems in plantation and horticultural crops. Organ culture of stem tips, for example, has been used to propagate specimens which are desirable but for the fact that they are carriers of viruses; since the apical tip region is relatively free of virus, virus-free clonal material can be obtained by the organ culture of excised stem tips (Walkey, 1968; Jones and Vine, 1968; Vine, 1968). Another use has been the clonal propagation of rare and valuable plants such as orchids (Morel, 1960, 1964; Wimber, 1963). The latest developments in this field have, in fact, caught the attention of many biologists because of their wide implications. These are the isolation and culture of protoplasts and the induction of microspores to develop beyond their normal 3-celled stage (pollen grain) to form haploid calluses and plants. The potential of protoplast culture lies in the possibility of hybridisation of selected strains in culture, i.e., without flowering and sexual union, a potential which should have considerable appeal to foresters faced as they are so often with long delays before trees

can be induced to flower. The potential of haploid callus induction is in the possibility of applying mutagenic agents to the callus, followed by diploidisation (e.g., with colchicine), and, if self-pollination can be done, the production of numerous homozygous clonal seeds. And, clearly, a marriage of protoplast hybridisation and haploid plant induction is another avenue worth exploring because, if successful, numerous clonal heterozygous mutant strains would be obtained with perhaps greater vegetative vigour. The potential of these techniques in forestry is so great, and the possibilities of achieving such results so agonisingly slow by traditional methods, that research in this area warrants a considerable increase in effort and resources.

Our work with the tissue and organ culture of *Eucalyptus* started with the more modest aim of achieving vegetative propagation of adult trees because of the failure of such classical methods as budding, grafting and layering, and/or the associated problems of incompatibility (delayed or otherwise) and/or a continuance of a plagiotropic habit. We set out to find the methods for achieving tissue and organ culture of various species of *Eucalyptus* and, in the case of tissue cultures, to find out how to regenerate plants from these undifferentiated cells. What we have achieved so far is a firm foundation not only in fulfilling our original aims but also in providing information useful to the more ambitious aims of achieving protoplast culture, haploid plant induction of *Eucalyptus* and the associated aims described in the first paragraph. Lest this appears to overstate our achievements to date, we must emphasise the progress report nature of this paper and stress that so far we have not been able to regenerate plants from tissue cultures, an achievement which must remain basic to further developments in this field.

EXPERIMENTAL

A. TISSUE CULTURE OF EUCALYPTUS

In essence, the tissue culture approach aims to isolate tissue from a selected tree, to induce this tissue to form a mass of dividing cells (a callus), and then to induce some of these cells, which are assumed to be clonal with the parent tree, to regenerate into complete plants.

Materials and Methods

1. *Isolation of tissue.* The essential problems in the isolation of tissue are to avoid contamination of the tissue with microbes and to find a culture medium which will favour growth of the tissue when removed from the tree. The surfaces of plants growing in the field carry a microbial flora which, although not harmful to the plants, multiplies rapidly on the nutritive culture medium, modifying the composition of the medium, and producing conditions that cannot be repeated; such microbes are also likely to over-run the excised tissue or kill it by their toxins. Our standard practice in tissue culture work is to treat the material with alcohol followed by flaming it, then immerse it in 7X-detergent for 5 min. followed by immersion for 20 min. in a freshly-prepared, filtered, saturated solution of chlorinated lime; several sterile water rinses follow this and finally small pieces of tissue are cut from the material and are placed on the surface of the culture medium in 10 × 2.5 cm pyrex tubes. Modifications of this

procedure depend on the material being used; for example, if tissue is to be taken from the stem of a mature tree, much of the bark is first removed with hammer and chisel, and the exposed area treated with alcohol, flame, detergent, and chlorinated lime. In the case of *E. bancroftii* lignotuber, the stem modification was used and was followed by sawing off a piece, approximately 10 × 10 × 1.2 cm, which was then dipped in molten wax and wrapped in an air-tight plastic bag; in the laboratory, the lignotuber piece was cut in 6.25 × 0.65 cm pieces using a band saw, and each piece was given a detergent-chlorinated lime treatment; finally the wax was melted and the pieces were given a further detergent-chlorinated lime treatment, and were then cut into smaller pieces with a dog nail-clipper; these pieces were placed on the surface of the culture medium. In other situations, less strenuous precautions are used, for example with tissue protected in some way such as anthers in pre-anthesis flowers. Using these various modifications, tissue cultures have been initiated from young and old trees, from main stems and small twigs, from lignotubers, and from anthers. The following species have been successfully tissue-cultured: *E. grandis, E. nicholii, E. laevopinea, E. bancroftii, E. melliodora* (de Fossard, 1974a), *E. urnigera.*

Species cultured by other workers are: *E. gunnii, E. tereticornis, E. cladocalyx, E. gomphocephala* (Jacquiot, 1964), *E. camaldulensis* (Jacquiot, 1964; Sussex, 1965), *E. citriodora* (Aneja and Atal, 1969).

2. *Culture medium.* The details of culture media used are listed in the Appendix. These media, which were found suitable for either initial growth of tissue or for the subculture of callus, should not be regarded as immutable since not all constituents were tested for essentiality or for optimal concentration. For example, in our early experiments, we tested *Eucalyptus* tissue on six media and calluses of various species were obtained on Media A, C and E. Later experimentation has indicated that various modifications of Medium A are likely to either induce callus formation from *Eucalyptus* tissue or be suitable for their sub-culture, e.g., Medium AB for *E. bancroftii.* Medium Alt is basically Medium A modified by attempting to duplicate the poorly described medium of Aneja and Atal (1969); it proved to be suitable for the initiation of lignotuber calluses from *E. bancroftii.* Medium B is suitable for rearing aseptic seedlings (seeds treated with detergent-chlorinated lime-sterile water are placed on the surface of the culture medium).

3. *Incubation.* Cultures of *Eucalyptus* grow best in the dark, and 8 hours and more of light per day have often led to complete growth inhibition. A constant temperature of 25°C is used, but other temperatures have not been tested.

RESULTS

1. *Auxin-cytokinin experiments.* Manipulation of auxin and cytokinin concentrations in the medium have often resulted in a range of responses in tissue culture work. For example, with tobacco tissue culture, undifferentiated cells form in calluses on a medium with 10^{-5}M IAA and 10^{-7}M kinetin, whereas calluses on medium with 10^{-5}M IAA and 10^{-5}M kinetin are compact and differentiate numerous tracheary cells, and calluses on IAA-free medium with 10^{-5}M kinetin become nodular and regenerate shoots (de Fossard, 1974b). These tobacco-auxin-cytokinin results were used as a model

for *Eucalyptus* tissue culture experiments and the following auxin-cytokinin combinations were tested with *E. bancroftii* calluses:

1.	IAA and kinetin	Auxins were tested at:
2.	2,4-D and kinetin	0, 0.04×10^{-5}, 0.2×10^{-5}, 10^{-5} and
3.	IAA and BAP	2×10^{-5}M
4.	2,4-D and BAP	Cytokinins were tested at:
5.	NAA and kinetin	0, 0.5×10^{-6}, 2×10^{-6}, 8×10^{-6}, and
6.	NOA and kinetin	16×10^{-6}M
7.	IAA	At 0.0625×10^{-5}, 0.25×10^{-5}, 10^{-5}, 4×10^{-5} and 8×10^{-5}M
	kinetin	At 0.5×10^{-6}, 2×10^{-6}, 8×10^{-6}, 3.2×10^{-5} and 6.4×10^{-5}M

Abbreviations: IAA, indole-acetic acid; 2,4-D, 2,4-dichlorophenoxy-acetic acid; NAA, α-naphthalene-acetic acid; NOA, 2-naphthoxy-acetic acid; BAP, N6 benzyl adenine.

The above 175 auxin-cytokinin combinations were tested on 1½-year-old subcultured *E. bancroftii* stem calluses (originally isolated from 5-week-old seedlings), and the final four experiments (involving 100 auxin-cytokinin combinations) were also tested with *E. bancroftii* lignotuber calluses in their second passage (sub-culture) ex-tree. The results of these experiments have been published (Lee and de Fossard, 1974), and will only be summarised here Fig. 1 shows the dose-response plane of stem calluses on media with NAA and kinetin which at the highest concentration of both hormones resulted in a ratio $\frac{\text{gain in fresh weight (G)}}{\text{initial fresh weight (I)}}$ of 105 and extrapolation indicates that higher ratios might be expected if higher concentrations of both hormones were tested. $\frac{G}{I}$ ratios of about 40 after six weeks incubation of stem calluses on Medium AB were found, and the highest ratio $\frac{G}{I}$ was found with stem calluses on 2×10^{-5}M 2-NOA and 16×10^{-6}M BAP. Lignotuber calluses generally did not grow so fast; the highest ratio $\frac{G}{I}$ was nearly 80 with calluses on 2×10^{-5}M 2,4-D and 2×10^{-6}M BAP.

The majority of auxin-cytokinin combinations which induced high $\frac{G}{I}$ ratios could be considered suitable for obtaining rapidly a large supply of callus. However, the soft watery texture of these calluses, and their complete lack of differentiation do not suggest that they would be suitable for regeneration of plants. A more realistic criterion for selecting auxin-cytokinin combinations for regeneration studies is a nodular appearance to the callus (Fig. 2); this was not achieved with sub-cultured stem calluses (which were already 1½ years old at the beginning of these experiments), but was found in many cultures of newly-isolated lignotuber and stem calluses.

2. *Mixed calluses.* The failure of the calluses exposed to various auxin-cytokinin combinations to regenerate plants led us to think that tobacco calluses might produce some regenerating factor when they are cultured on auxin-free 10^{-5}M kinetin Medium A.

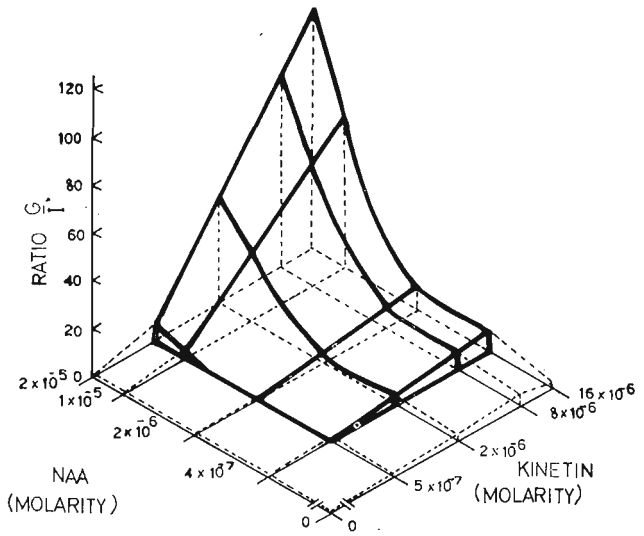

FIG. 1—The dose-response plane of **E. bancroftii** stem callus on medium AB using all combinations of NAA (0,0.04 × 10^{-5}, 0.2 × 10^{-5}, 1 × 10^{-5}, 2 × 10^{-5}M) and kinetin (0,0.5 × 10^{-6}, 2 × 10^{-6}, 8× 10^{-6}, 16 × 10^{-6}M; the plane was drawn from the regression equation: Y = 7570.7 + 1179.3 **** KIN + 2487.5 **** NAA + 204.8 **** NAA^2 + 387.5 **** KIN × NAA + 31.9 **** KIN × NAA^2 (r^2 = 0.943). **** = extremely significant $P < 0.01\%$

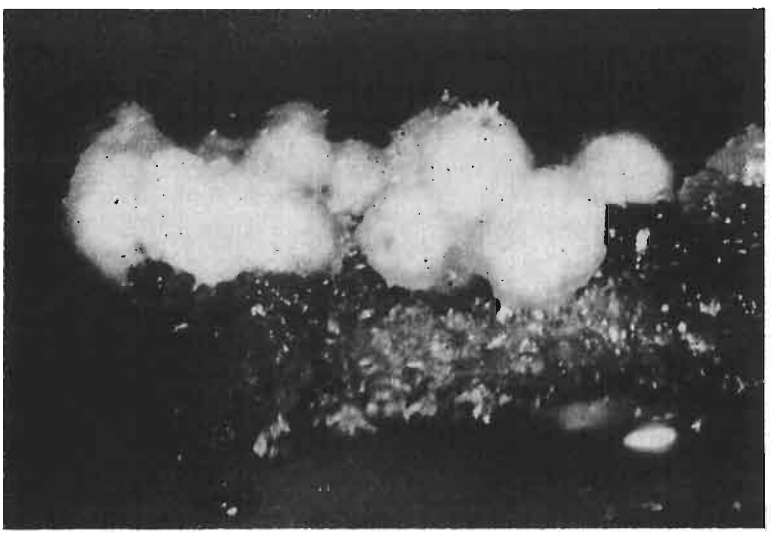

FIG. 2—Nodular appearance of freshly initiated callus on a 5-mm-long stem explant of **E. laevopinea**.

The hope was that if this did occur, the factor might be diffusible and not too specific and might induce calluses of *Eucalyptus* to regenerate. In consequence, mixed calluses of tobacco and, separately, of *E. bancroftii, E. grandis, E. nicholii* and *E. melliodora,* were prepared. In one treatment regenerating tobacco calluses were shaken in aqueous Medium A (auxin-free, 10^{-5}M kinetin) for 24 hr in the dark at 25°C and, without removing the immersed tobacco, *Eucalyptus* callus was added to a filter paper wick support over the medium. In another treatment with the aqueous medium, regenerating tobacco callus was placed on one filter paper support and *Eucalyptus* callus was added to a second support which was in contact with both the medium and the tobacco callus. Two other treatments used the same modification of Medium A but solidified with agar; one treatment had undifferentiated tobacco callus centrally placed on the medium, the other regenerating tobacco callus, and *Eucalyptus* calluses were placed in intimate contact on top of the tobacco calluses. In all cases, massive regeneration of tobacco occurred or continued to develop, but no shoots of *Eucalyptus* formed. However, some treatments developed massive roots (Fig. 3) unlike either those of tobacco-regenerated shoots or of *Eucalyptus* seedlings in culture.

Similar attempts with mixed organ cultures were made with *E. grandis* and a species of Labiatae whose stem tissues can easily be induced to form roots and buds in culture. A piece of *E. grandis* leaf was placed in contact with the cut longitudinal surface of labiate stem, and also by inserting a small stem of *E. grandis* in the pith of the labiate stem, in the hope that some bud-inducing factor of the labiate might be transmitted to the *E. grandis* explants. However, while buds and roots were formed on the labiate stem pieces within one week of culture, no buds were induced on the *E. grandis* material.

B. ORGAN CULTURE OF EUCALYPTUS

The organ culture approach to vegetative propagation differs from the tissue culture method by aiming to induce more or less directly the initiation and/or development of shoots and roots from small pieces of organs, e.g., nodes, stem or branch apices. If the failure of such methods as budding, cuttings, and layering is due to endogenous inhibitors acting against the initiation and/or development of roots and shoots, it is argued that the culture of excised organs might allow such initiation and development to occur because the excised organs have been separated from the endogenous inhibitors.

One of the main problems involved in attempting organ culture of field-grown trees is to obtain excised organs free of microbes. Harsh disinfection treatments have to be avoided because they might not only kill the microbes but also the organ to be cultured. Our early work (Cresswell and de Fossard, 1974) concentrated on finding media suitable for the organ culture of nodes and stem apices of *Eucalyptus* and, in order to avoid the microbial contamination-disinfection problem, organs were excised from aseptic seedlings. Later work has been aimed at overcoming the microbe-disinfection problem, and in extrapolating from results with seedings to adult trees.

MATERIALS AND METHODS

1. *Isolation of organs.* Seeds of *E. grandis* were partially separated from chaff by forced air in a baffled column, and were then treated with detergent and chlorinated lime, washed in sterile water and planted on Medium B. At various stages, 5-mm nodal explants were excised from the seedlings and placed on fresh Medium B.

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FIG. 3—Development of massive roots unlike either tobacco or **Eucalyptus** roots from a cultural mixture of tobacco and **E. bancroftii** calluses.

the experiments used tissue from plants up to 7 months old. The cut ends of the branches were paraffined before a disinfection treatment of: quick rinse in 70% ethanol containing several drops of Teepol, 5 min in 7% (w/v) chlorinated lime solution and three rinses in sterile distilled water. The explants were excised and planted onto Medium LPP (based on formula K in Nitsch and Nitsch, 1967).

2. *Incubation.* Seeds were exposed to continuous illumination at 25°C to 30°C, and an experiment with different light/dark regimes showed that, for cultures of seedling nodes, short days (8 hr light/16 hr dark) at 26° to 28°C gave the most suitable conditions.

RESULTS

1. *Effect of node level on root initiation of nodal cultures*

The seedlings used had developed four pairs of expanded leaves including the cotyledons, and each seedling was cut up to make five foliated nodal treatments, namely: 1 = cotyledons, 2 = 1st pair of true leaves, 3 = 2nd pair of leaves, 4 = 3rd pair

of leaves, 5 = apex. There were 36 replicates and incubation was for three weeks. Only four of the 36 seedlings failed to develop roots in any of the five nodal explants, and roots developed, in most cases, in two out of the five nodal cultures per seedling. Root development was most frequent at nodes 1 and 2, but declined in nodes nearer the apex. The development of axillary buds usually preceded root development but root development was not automatically assured by the development of axillary buds (Table 1); 49% of 180 nodal sites developed axillary buds whereas only 33% developed roots. However, axillary bud development was similar to root development in being most frequent at nodes at the basal end of the seedling.

TABLE 1—Development of roots and buds in nodal explants of **E. grandis** in relation to node number: 36 replicates

	Node Number				
	1	2	3	4	5
Axillary Buds	33	30	18	8	0
Accessory Buds	5	0	0	0	0
Root	21	21	11	6	0

2. *Effect of leaves on root development of nodal cultures*

Seedlings of two ages were used in this experiment, one group (S6) having six pairs of expanded leaves, the other group (S5) having five pairs of expanded leaves above the cotyledons. Two nodal sites from each seedling were used, one being the node above the cotyledon (N1), the other the node below the apex (N2). Four replicates were used per treatment, and the treatments were: (1) Two leaves present, (2) One leaf removed, (3) Half of each leaf removed, (4) Both leaves removed (but petioles left on the explants). The cultures were incubated for three weeks.

The N1 nodal cultures responded in a similar way irrespective of age of seedling, although there were a few minor variations. In this part of the experiment, most potential axillary buds developed, the exception being two that failed to develop on defoliated explants from S5 seedlings. The main trends were larger axillary buds, and more and longer roots with explants that had the most leaf. Explants with two half-leaves were clearly not so well developed as explants with one leaf, and complete defoliation resulted in no root development and substantially smaller axillary buds (Table 2).

Essentially the same trends were found with N2 nodal cultures from S5 seedlings, but the complete failure of foliated N2 nodal explants from S6 seedlings to grow in culture thwarts any attempt at an unqualified recommendation to use foliated explants in this type of work.

3. *Nodal cultures from older plants*

Nodes from 4-month-old plants, exposed to various phytotron environments, were placed on Medium LPP with 500 μg (2.7×10^{-6}M) NAA/1, and exposed to long days (18 hr light/6 hr dark) at 28°C. After 15 days, all cultures with signs of root initials

TABLE 2—Development of axillary buds, main roots and lateral roots in two types of nodal explants from two ages of seedlings as influenced by varying amounts of defoliation (coded: 0 = both leaves removed, 2 × ½ = half of each leaf removed, 1 = one leaf removed, 2 = two leaves present); total values for each treatment (4 replicates) have been tabulated

	S5 - seedlings Leaf treatments				S6 - seedlings Leaf treatments				All seedlings Leaf treatments			
	0	2x½	1	2	0	2x½	1	2	0	2x½	1	2
N1-nodal explants:												
No. axillary buds	6	8	8	8	8	8	8	8	14	16	16	16
Length (cm) axillary buds	6	14	18	31	4	27	31	37	10	41	49	68
No. explants with roots	0	2	3	3	0	3	4	3	0	5	7	6
No. roots	0	3	3	10	0	7	5	9	0	10	8	19
Length (mm) main roots	0	63	94	117	0	110	206	226	0	173	300	383
Lateral Root (Score)	0	1.5	2	5.5	0	4	15	5	0	5.5	17	10.5
N2-nodal explants:												
No. axillary buds	6	5	6	6	8	8	8	0	14	13	14	6
Length (cm) axillary buds	5	17	16	24	2	9	9	0	7	26	25	24
No. explants with roots	0	3	3	2	0	2	2	0	0	5	5	2
No. roots	0	4	10	7	0	4	2	0	0	8	12	7
Length (mm) main roots	0	143	176	287	0	88	70	0	0	231	246	287
Lateral Root (Score)	0	6	7	4	0	3.5	5	0	0	9.5	12	4

were transferred to Medium LPP without NAA. Roots formed on three out of 12 nodes from plants originally exposed to short days (9 hr light/15 hr dark at 32°C in light/27°C in dark) as well as on seven out of 11 from plants originally exposed to short days (9 hr light/15 hr dark) and on two out of 12 nodes from plants exposed to long days (16 hr light/8 hr dark) at 27°C in both light and dark periods; in addition two apical tips (one of four from plants originally in short days, and one of four from plants originally in long days, at 27°C), also produced roots. After two months in culture, when the roots and axillary buds were well established, these plantlets were transferred to the glasshouse. Only the two nodal cultures from plants originally exposed to long days at 27°C survived. These surviving plants differ from seedlings in that the axillary buds developed into branches, the internodes were long and the leaves alternate, whereas seedlings are markedly apically dominant, with shorter internodes and opposite leaves.

Successful root initiation and development of plants from nodal cultures of 7 month-old *E. grandis* trees (reared on 15 hr light/9 hr dark at 28°C/24°C) have also been obtained. The nodes, in this case, were placed on Medium LPP with indole-butyric acid (IBA), and incubated in the dark at 28°C for four weeks. Best root formation was on nodal cultures on medium supplemented with 1 mg (5×10^{-6}M) IBA/1; after roots had been initiated, the cultures were transferred to long days (18 hr light/6 hr dark at 28°C) for bud development. At the time of writing, some previously dormant axillary buds had developed but not on all specimens.

These experiments show that plantlets can be obtained by the organ culture of nodal

explants (Fig. 4), but as yet the success rate is not high. Experiments in progress indicate about 50% of explants initiate roots, but only 40% of these survive the transfer to glasshouse conditions.

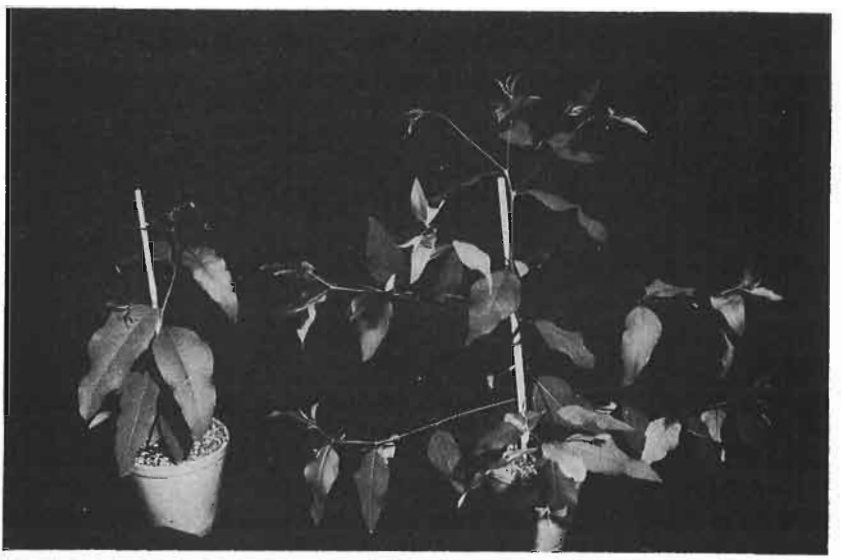

FIG. 4—Plants from nodal cultures of 7-month-old (left) and 4-month-old (right) **E. grandis** plants; note the large adult leaves (left) in comparison with juvenile leaves (right).

DISCUSSION

This paper describes our first attempt to obtain regeneration from tissue cultures of *E. bancroftii* as a method for clonal propagation. These attempts were essentially geared to finding out whether *E. bancroftii* calluses would respond to auxin-cytokinin manipulations of the culture medium in a way similar to that achieved with tobacco. Regeneration was not, in fact, obtained with the particular auxin-cytokinin combinations and concentrations tested in this investigation. This work has, nevertheless, helped to formulate the problem more precisely. The almost monotonous similarity in anatomy and morphology of most of the 1½-year-old stem calluses cultured on the 175 auxin-cytokinin combinations tested indicates a low level of responsiveness in this material. It may be that, since these calluses were descended from material used in our earliest experiments to obtain a chemically-defined medium, we have selected strains that are fast-growing but lack differentiating potential.

Regeneration of organs from lignotuber calluses appears to be a much more promising approach, not only because of the reported success with *E. citriodora* lignotuber tissue (Aneja and Atal, 1969), but also because of the nodular appearance of the calluses and the wider variety of cells making up these calluses. It should be pointed out that the majority of calluses initially formed from stem segments of *E. bancroftii, E. nicholii, E. laevopinea, E. melliodora* and *E. grandis* also had this nodular appearance, and contained a variety of cell types.

Our conclusions are that work should be continued using mainly nodular type calluses from newly initiated material, and the type of sequential exposure to different media recommended by Steward *et al. (1969)* should be attempted, i.e., sub-culturing from an auxin-containing medium to an auxin-free medium with coconut milk, with such newly isolated tissues.

Organ culture of *E. grandis* (a non-lignotuber-forming species) has progressed from our initial work with aseptic seedlings (to find a suitable medium, incubation treatment, and other basic factors involved in the organ culture of this species), to work with trees reared in a phytotron and in the field (to find a method of disinfecting the material without killing the tissue), and recently to the testing of a wide variety of media and pre- and post-excision environmental effects. The 7-month-old parent plant used in our most recent work was approximately six feet tall with adult foliage with more than 50 nodes. This experiment showed that *E. grandis* can be propagated by organ culture after the parent plant had developed more than 15 nodes regarded as the cut-off point for successful striking of cuttings (Paton *et al.* 1970).

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APPENDIX

CONSTITUENTS OF MEDIA (in molarity except where otherwise stated)

	A**	Alt	B	C	E	LPP*	KNOP'S*
$Ca(NO_3)_2$	$2x10^{-3}$	$2x10^{-3}$	$2x10^{-3}$	$2x10^{-3}$	$2x10^{-3}$	$2.1x10^{-3}$	$2.1x10^{-3}$
NH_4NO_3	$1.5x10^{-3}$	$1.5x10^{-3}$	-	-	$1x10^{-2}$	-	-
KNO_3	$16x10^{-4}$	$16x10^{-4}$	$1x10^{-3}$	$1x10^{-3}$	$1x10^{-2}$	$1.2x10^{-3}$	$1.2x10^{-3}$
KCl	$1x10^{-3}$	$1x10^{-3}$	-	-	$1x10^{-3}$	-	-
KH_2PO_4	$1x10^{-3}$	$1x10^{-3}$	$1x10^{-3}$	$1x10^{-3}$	$2x10^{-3}$	$9.2x10^{-4}$	$9.2x10^{-4}$
$MgSO_4$	$3x10^{-4}$	$3x10^{-4}$	$5x10^{-4}$	$5x10^{-4}$	$2x10^{-4}$	$5.1x10^{-4}$	$5.1x10^{-4}$
$FeSO_4$	$1x10^{-4}$	$1x10^{-4}$	$5x10^{-5}$	$5x10^{-4}$	$5x10^{-5}$	$1x10^{-4}$	-
Na_2EDTA	$1x10^{-4}$	$1x10^{-4}$	$5x10^{-5}$	$5x10^{-4}$	$5x10^{-5}$	$1x10^{-4}$	-
H_3BO_3	$1x10^{-4}$	$1x10^{-4}$	$1x10^{-5}$	$1x10^{-5}$	$3x10^{-5}$	$1.6x10^{-4}$	-
$MnSO_4$	$1x10^{-4}$	$1x10^{-4}$	$1x10^{-5}$	$1x10^{-5}$	$2x10^{-5}$	$1.1x10^{-4}$	-
$ZnSO_4$	$3x10^{-5}$	$3x10^{-5}$	$2x10^{-6}$	$2x10^{-6}$	$5x10^{-6}$	$4.3x10^{-5}$	-
$CuSO_4$	$1x10^{-7}$	$1x10^{-7}$	$1x10^{-7}$	$1x10^{-7}$	$1x10^{-6}$	$1x10^{-7}$	-
Na_2MoO_4	$1x10^{-6}$	$1x10^{-6}$	$1x10^{-7}$	$1x10^{-7}$	-	$1x10^{-6}$	-
$CoCl_2$	$1x10^{-7}$	$1x10^{-7}$	-	-	-	-	-
KI	$5x10^{-6}$	$5x10^{-6}$	-	-	-	-	-
H_2SO_4(36N)	-	-	0.5 µl/l	0.5 µl/l	-	-	-
D-Ca-Pantpthenate	-	$2x10^{-7}$	-	-	-	-	-
Inositol	$5x10^{-4}$	$3x10^{-2}$	-	-	$2x10^{-3}$	$5.6x10^{-4}$	-
Nicotinic Acid	$4x10^{-6}$	$4x10^{-6}$	-	-	-	$4.1x10^{-5}$	-
Pyridoxine HCl	$2.5x10^{-6}$	$2.5x10^{-6}$	-	-	-	$2.4x10^{-6}$	-
Thiamine HCl	$3x10^{-7}$	$3x10^{-7}$	-	$3x10^{-6}$	-	$1.5x10^{-6}$	-
Glycine	$2.5x10^{-5}$	$2.5x10^{-5}$	-	-	-	$2.7x10^{-5}$	-
L-Cysteine.HCl	-	-	-	$6x10^{-5}$	-	-	-
Biotin	-	$2x10^{-7}$	-	-	-	$2x10^{-7}$	-
Folic Acid	-	-	-	-	-	$1.1x10^{-6}$	-
2-NOA	-	$5x10^{-6}$	-	-	-	-	-
Kinetin	$1x10^{-6}$	-	-	-	$2x10^{-6}$	-	-
IAA	$1x10^{-5}$	-	-	-	$3x10^{-5}$	-	-
Coconut Milk	-	150 ml/l	-	150 ml/l	-	-	-
Casein Hydrolysate	1 g/l	1 g/l	-	-	-	-	-
Sucrose	$9x10^{-2}$	$6x10^{-2}$	$6x10^{-3}$	$15x10^{-2}$	$3x10^{-2}$	$6x10^{-2}$	-
Agar	8 g/l	4 or 8 g/l	8 g/l	8 g/l	10 g/l	10 g/l	-

* Amounts: Approximate Molarity

** Medium AB: Kinetin ($8x10^{-6}$M): IAA (10^{-5}M): No Casein Hydrolysate