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TISSUE CULTURE OF *EUCALYPTUS*

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Abstract. Clones of several eucalypt species have been propagated *in vitro*, enabling the utilization of genotypes which have been selected as superior individuals. By this means it is possible to produce clonal populations showing superior growth rate, form, and adaptation to specific sites. Large numbers of hybrids exhibiting a marked degree of hybrid vigour can also be grown.

Close cooperation between researchers and the horticultural and forestry industries will be needed to fully exploit the commercial potential of this technology. The clonal propagation of high value horticultural specimens, such as *E. caesia* and *E. macrocarpa*, offers obvious and immediate commercial benefit. Extension of this practice to plantation forestry will require lower production costs but the large demand for plants will stimulate the development of improved and cheaper techniques.

INTRODUCTION

Eucalypts, like most forest trees, have long generation times (from years to decades), are very difficult to cross-pollinate to produce large quantities of seed of hybrids, and selection of genotypes for important characteristics like growth rate and form usually takes several years. All of the above features make tree breeding a slow and costly process.

The vegetative propagation of superior clones can assist in overcoming some of these problems as selected clones can be rapidly propagated for commercial plantations. Since vegetative propagation enables the cost of breeding and selection to be spread over a large number of clonal individuals then advanced breeding techniques (e.g. hybrids between inbred lines, interspecific hybrids, and back-crossing) could become as practical for tree breeding as they are with the breeding of annual crops.

There are several methods of vegetatively propagating eucalypts (35) but for forestry plantations stem cuttings and tis-

sue culture are the only practical methods. This paper mainly discusses micropropagation of eucalypts.

TECHNIQUES FOR MICROPROPAGATION OF THE EUCALYPTS

The techniques for micropropagation of the eucalypts are similar to those developed for other plants. Shoot explants (with the leaf laminae removed) from seedlings or from basal coppice shoots of older trees are surface sterilized in dilute sodium hypochlorite and placed onto a sterile medium consisting of ¼ strength Hoagland's solution (58) and 2% sucrose (Figure 1). After 1 month axillary shoots usually develop and these are subcultured to a shoot multiplication medium (Table 1) where the shoots grow rapidly producing many axillary shoots (Figure 2). Root formation occurs when the shoots are subcultured to a rooting medium containing an auxin, but no cytokinin (Figure 3 and Table 1).

Table 1. Composition of Media[1]

	Shoot Multiplication Medium	Rooting Medium
Murashige and Skoog salts	½ strength	¼ strength
Sucrose	2.0%	2.0%
Agar	0.8%	0.8%
Benzylaminopurine	1 μmol l^{-1}	Nil
Naphthaleneacetic acid	1 μmol l^{-1}	Nil
Indolebutyric acid	Nil	10 μmol l^{-1}

[1] The above media have worked well for a number of species but the optimal hormone concentration varies among clones.

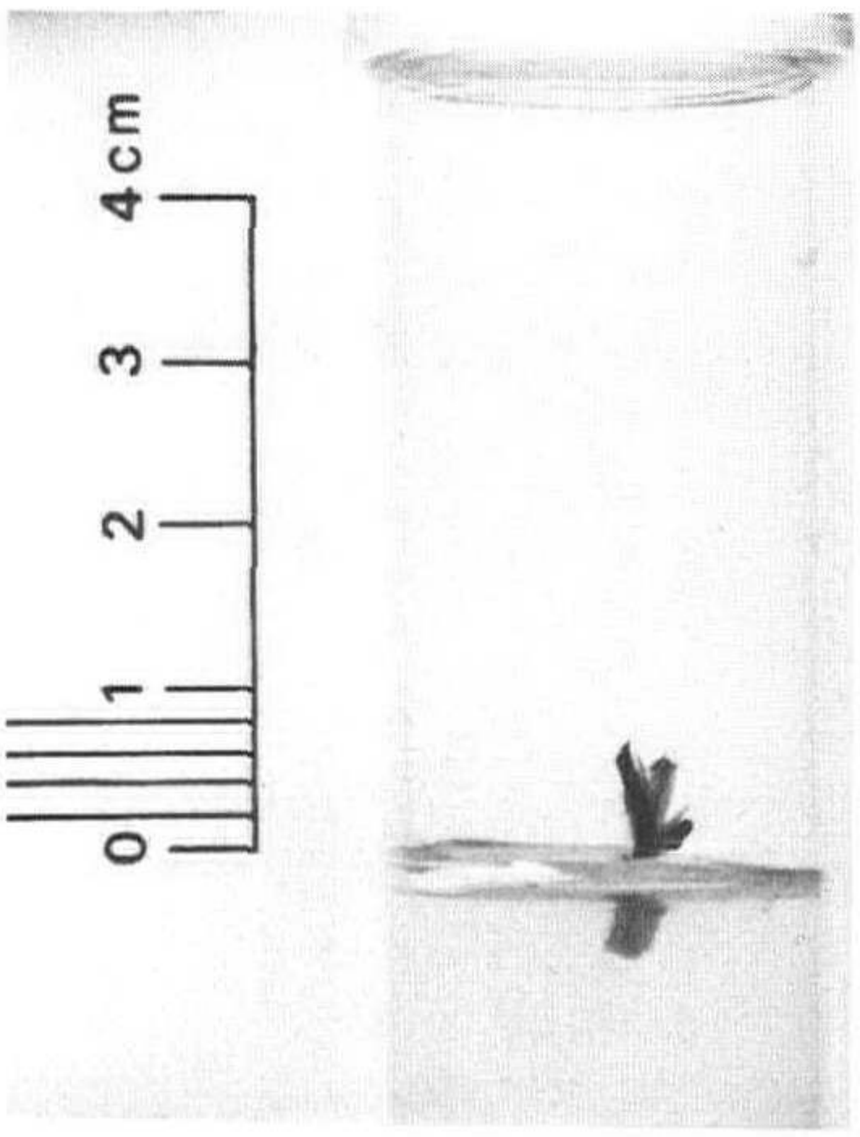

Figure 1. Initial shoot explants.

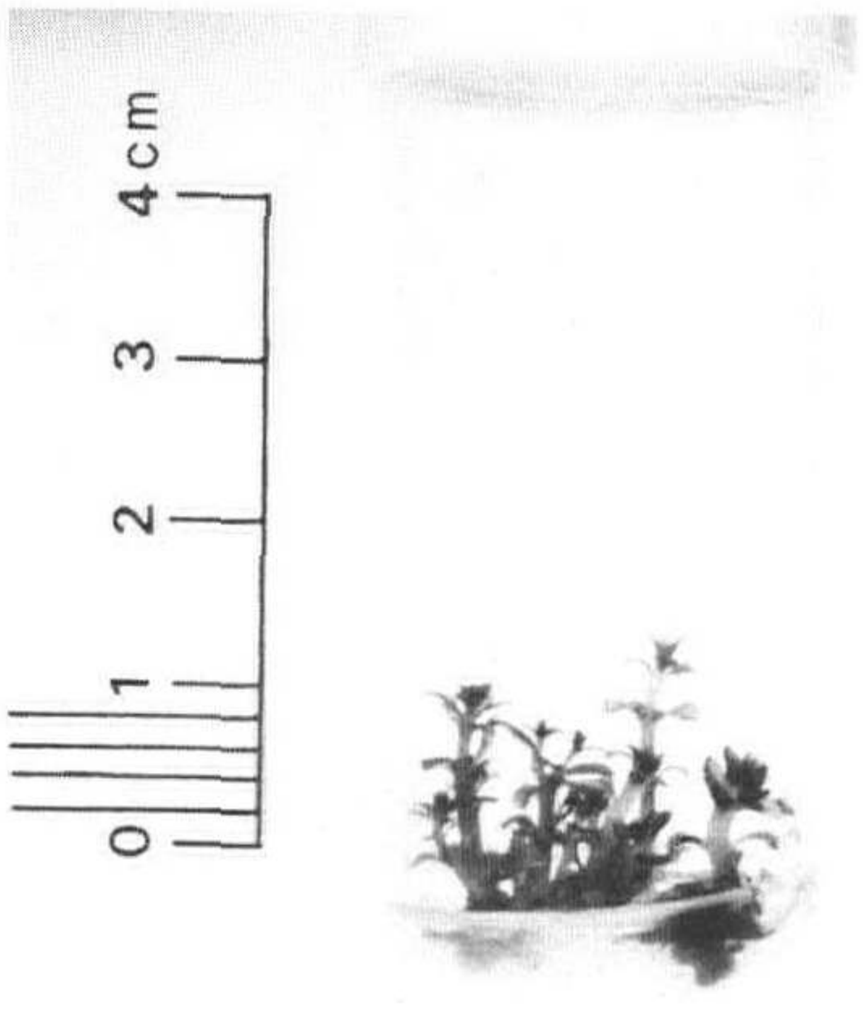

Figure 2. Shoot growth after 3 weeks on shoot multiplication medium.

Cultures are grown in a cabinet at a constant temperature of 25°C, a photoperiod of 8 h, and a light intensity by fluorescent tubes of 100 μE m^{-2} s^{-1}. Root development on some clones is enhanced by holding them in the dark for 7 days prior to placing them in the growth cabinet.

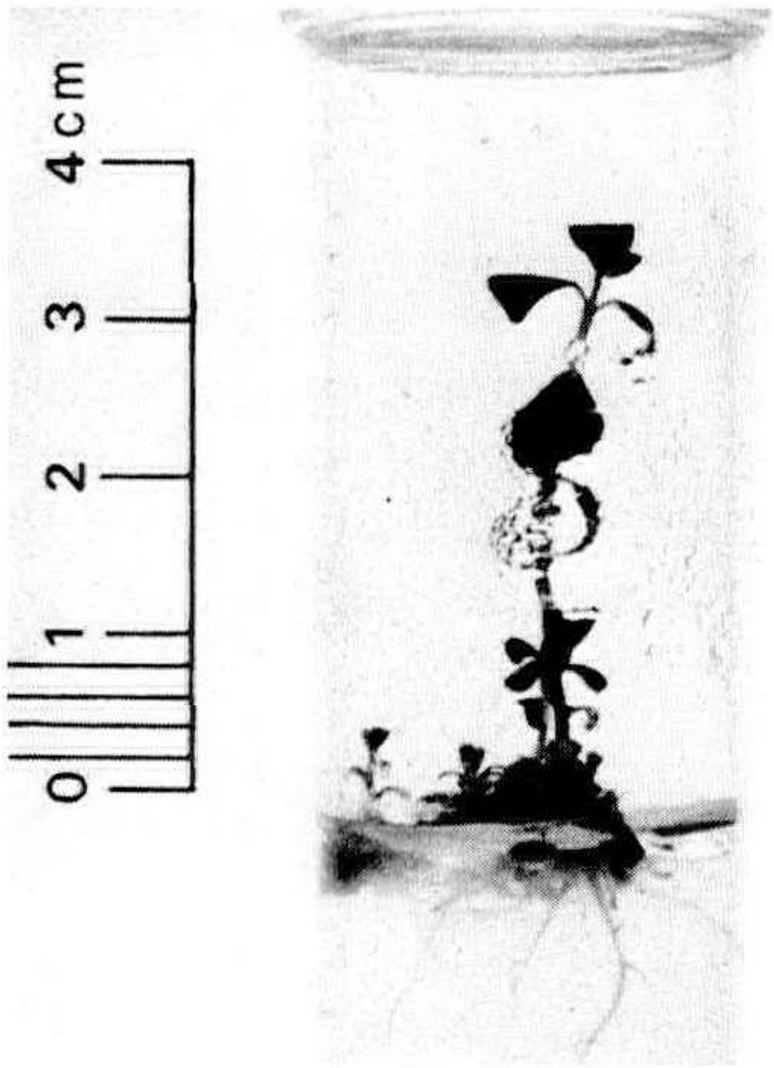

Figure 3. Root formation on shoots

The plants are hardened by removing them from their containers and placing them in a greenhouse under high humidity conditions (intermittent mist or a plastic cover) for two to three weeks (Figure 4).

The media described in Table 1 are very simple, consisting only of mineral salts, sucrose, and two hormones. No organic growth factors or amino acids are essential for propagation; in fact, some of them may be inhibitory (21,37).

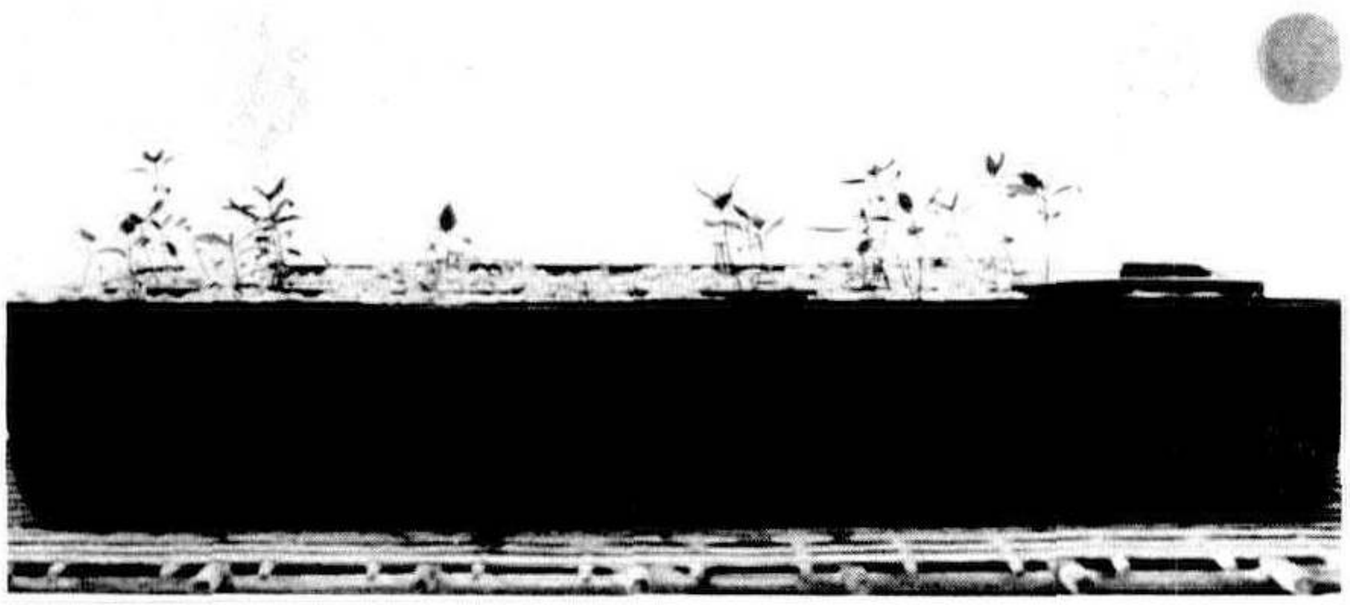

Figure 4. Hardening plants under high humidity conditions.

The three main advantages micropropagation has over propagation by stem cuttings are: a small requirement for space in order to maintain the parent plants, a higher multipli-

cation rate, and the absence of pests and diseases. One square metre of growing space can accommodate 1,000 parent plants in vials, but the same number of parent plants, maintained as hedges, would require about 1 ha in the field, or a large greenhouse if the hedges were grown in pots. The multiplication rate by micropropagation is at least double that of cuttings. After the clones in tissue culture are freed from diseases and insect pests (38) they can be held in this state indefinitely. On the other hand, it is very difficult to maintain hedges and stem cuttings free of diseases and insect pests. The absence of pests and diseases with axenic cultures simplifies long distance transport and quarantine procedures.

Several eucalypt species have now been propagated *in vitro* (Table 2). For all of the species we have propagated (36) the shoot multiplication rate is adequate (at least three-fold every 3 weeks), even for shoots from the crowns of adult eucalypts. However, some species (e.g. *E. regnans* and *E. globulus* subsp. *bicostata*) have a low and variable rooting percentage (less than 30%). Further research is necessary in order to find the optimal conditions and hormone concentration for the reliable propagation of these species.

Table 2. Eucalyptus species that have been propagated *in vitro*.

Species	Reference	Species	Reference
E. alba	41	*E. gunnii* × *E. cinerea*	25
E. camaldulensis	5,36,39	*E. gunnii* × *E. viminalis*	25
E. citriodora	3,32,33,44,45	*E. gunnii* × *E. dalrympleana*	25
E. curtisii	36	*E. macarthurii*	25
E. dalrympleana	24,25,26,27	*E. occidentalis*	5
E. delegatensis	25	*E. pauciflora*	25,26
E. ficifolia	4,16,17,18,20,21,36	*E. regnans*	36
E. globulus subsp. bicostata	36	*E. robusta*	5
E. grandis	11,12,15,27,36,39	*E. rudis*	36,52
E. grandis × *E. robusta*	39	*E. viminalis*	25
E. gunnii	24,25,26		

APPLICATIONS IN FORESTRY AND HORTICULTURE

Micropropagation of eucalypts selected as beautiful horticultural specimens, such as *E. caesia* and *E. macrocarpa*, offers immediate commercial benefit because of the high prices people are willing to pay for them.

Essential oil production from eucalypt clones is another potential application as individual trees are known that have both a high oil yield and desirable composition (54). Selected trees of *E. radiata* and *E. polybractea* (Syn.: *E. fructiceforum*?) are two oil producing species being studied.

In forestry, large economic gains are possible by growing clonal plantations. Vegetative propagation not only enables the outstanding individuals of a population to be grown, it also

enables hybrid vigour to be exploited. Clonal plantations of eucalypts are being established in the Congo and Brazil on a scale of millions of trees per year (8,23). The clones are propagated by cuttings taken from basal coppice shoots. Large scale plantations of eucalypt clones (produced by micropropagation) are planned in Florida for the production of methanol. Commercial plantations of clones of forest trees other than eucalypts are also being established (10,42,43,47,49,55,59).

The potential gains in growth rate from using clones in plantations are very large. Seedlings from selected trees of *E. grandis* had an average volume 54% greater than routine seedlots (1). If the best individuals within these selected seedlings were propagated as clones, volume gains in the order of 100% could be realized. Very large gains in wood volume, in the order of 30 to 100% greater than routine planting stock, have been recorded from plantations of hybrid eucalypts in several parts of the world (6,8,9,22,29,57).

In continental Europe and in the USA there is a demand for cold-tolerant eucalypts. Natural hybrids have been selected and vegetatively propagated in Florida, USA, and France (25).

Since all the individuals in a clone are genetically identical they enable certain experiments to be evaluated more efficiently. For example, genotype × environment interaction is determined more efficiently with clones than with seedlings (7,46,48). A clone of *E. marginata* is being used in our laboratory to examine genetic variation in the pathogenicity of *Phytophthora cinnamomi*, the cause of Jarrah dieback in Western Australia.

Eucalypts are one of the world's most important exotic hardwoods. Plantations now exist in over 58 countries on an area exceeding 4 million ha (28). Brazil alone plants 200,000 ha each year, which is six times greater than the annual establishment rate of all forest plantations in Australia (2).

The total area planted each year to eucalypts in Australia is relatively small (2,000 ha) (2) because we still have large natural stands. However, because of the demands placed on natural forests as a source of water, recreation, and conservation, and since many of our readily accessible forests have been harvested, an increasing area of eucalypt plantations are being grown (13).

Forestry entails not only the growing of trees for wood production, but also includes rehabilitation of mining sites, agroforestry (where trees are grown at a wide spacing in association with crops and livestock), and management of shelterbelts, woodlots, and amenity plantings. In many of these situations a special type of tree is required, e.g. salt tolerant trees

for planting on saline areas, trees tolerant of heavy metals on mining sites, and trees with good form, small branches and special wood properties for agroforestry. Clones with these characteristics will find a ready market as the annual demand is now very considerable. The Forestry Commission and the Natural Resources Conservation League in Victoria each produce about one million trees a year for farm and Shire plantings, and mining companies in Australia plant several million trees each year for the rehabilitation of mining sites. A large proportion of this demand is for eucalypts.

DANGERS OF CLONAL PLANTATIONS

Plantations consisting of one or a few clones lack genetic diversity and this increases the likelihood of disease and insect pest epidemics; poplar leaf rust in Australia is a recent example. The lack of genetic diversity is not restricted to plants grown as clones as many of our important crop plants have a restricted genetic base (51).

One method to reduce the problem of genetic uniformity in clonal plantations is to plant a large number of clones (preferably of known performance in relation to diseases and insect pests) either randomly or in small compartments throughout the plantation (43,47). As many of the clones to be planted in the future will be hybrids, a large number of new genotypes will be available for selection. Genetic variation in time can be produced by planting different clones in different years.

CLONES AS A METHOD OF GENETIC CONSERVATION

Clonal plantations may represent a loss of genetic diversity, but clones also offer a simple method of conserving genes for incorporation into future breeding programs. Genotypes selected for frost tolerance and disease resistance are obvious examples.

Clones maintained *in vitro* are particularly useful in this respect as a large number of disease-free individuals can be held in a small space with a minimum of maintenance. Shoot cultures of *E. camaldulensis* and *E. grandis* have been maintained on a simple medium in a domestic refrigerator for over 8 months. When the shoots were subcultured to a fresh medium and placed in a growth chamber they grew normally.

PROPAGATION OF ADULT EUCALYPTS

Shoots taken from the upper parts of adult trees cannot yet be routinely propagated by the above techniques. Many eucalypt breeders regard this as a major disadvantage for com-

mercial vegetative propagation. This is not so, since the majority of eucalypts can develop basal coppice shoots from mature trees and these can continue to serve as a source of material for vegetative propagation. Alternatively, parent plants can be maintained as hedges and serve as a source of material for vegetative propagation (35).

If it were possible to propagate shoots from the upper branches of adult trees one would have to determine that such propagules did not display any effects of cyclophysis (53), especially with regard to growth rate. On the other hand, if clones from the tops of adult trees flowered while they were small this would be a real advantage of marketing some ornamental eucalypts.

Several eucalypts have shown a limited potential for propagation from adult shoots; *E. ficifolia* (4,21), *E. citriodora* (33), *E. grandis* (12,27) and *E. dalrympleana* (27).

TRANSFERRING THE TECHNOLOGY OF MICROPROPAGATION FROM THE LABORATORY TO THE FOREST INDUSTRY

This year the Division of Forest Research and a collaborator will be undertaking a project to produce clonal trees for experimental plantations. Eucalypts to be propagated include highly salt tolerant clones of *E. camaldulensis* and several other species. Ramets of these clones will be provided to several organizations for field trials and for planting on saline areas on farms. Clones of *E. grandis*, *E. pilularis*, *E. cloeziana* and *E. regnans*, as well as several ornamental eucalypts, that have superior growth rate and form will also be studied.

In addition to eucalypts other tree species will be included in the project. These include hybrids of *Pinus caribaea* × *P. elliottii*, *Leucaena* hybrids, *P. radiata*, and *Santalum acuminatum*.

The aims of the project are to propagate selected clones, to examine the economics of micropropagation and, in association with others, to compare the growth of clones to that of seedlings from seed orchards. Our particular role will be to micropropagate clones that have not previously been propagated, to simplify the procedures so that the costs per plant can be reduced, and to make the clones available for long-term field trials.

If the project is to succeed cooperation must exist between many organizations and individuals. We have already received a great deal of cooperation as all of the selected clones have been made available to us by other research organizations; the salt-tolerant eucalypts from the University of Melbourne, *E.*

radiata from the New South Wales Museum of Applied Arts and Sciences, *E. ficifolia* from Mr. J.H. Browne, a botanist from Red Cliffs, NSW, the hybrid pines from the Queensland Department of Forestry, the *Leucaena* hybrids from the CSIRO Division of Tropical Crops and Pastures, and the *Santalum accuminatum* clones from the Division of Horticultural Research.

The field trials are an essential part of the project. For instance, the salt-tolerant eucalypts have been selected under laboratory conditions where they demonstrate a remarkable degree of tolerance to sodium chloride. However, they have yet to be thoroughly tested in the field for additional characteristics such as growth rate, frost resistance, tolerance to insect grazing, their ability to lower saline water tables, and how they can be incorporated into grazing and cropping programmes. Field trials of the salt tolerant clones will be undertaken by a number of organizations including the Victorian Forestry Commission, the Victorian Soil Conservation Authority, and the CSIRO Division of Land Resources Management in Western Australia. A number of other organizations, farmers, and Shire Councils have also made preliminary enquiries. For species with attributes other than salt tolerance, field trials are planned by APM Forests and the State Forest Services to compare growth of the selected clones to seedlings. Cost comparisons between micropropagation and cuttings will also be made.

Clones of many other eucalypts, their hybrids, and other forest species are worth propagating; *E. gomphocephala* is a species which is adapted to calcareous sites, and clones of *E. marginata* may exist which are tolerant to *Phytophthora cinnamomi*. This latter study is being undertaken by Dr. J. McComb and Ian Bennett at Murdoch University in Western Australia.

The cost of producing plants by tissue culture can be reduced from techniques practised in the laboratory (19,30). Disposable Petri dishes have been successfully used by us to grow and transport the salt tolerant clones within Australia (Figure 5). Labour costs can be reduced if root formation *in vitro* is unnecessary. Shoots of some eucalypt clones developed roots when they were pre-treated with a rooting hormone and set as miniature cuttings. Another approach that will be investigated is to examine whether shoot multiplication and root formation can take place on the one medium.

In the future cell cultures of eucalypts (31) could be used as a tool for genetic engineering, *in vitro* selection, somaclonal variation, and haploid plants (56). Some of these techniques are already starting to play an important role in the breeding and selection of our crop plants. There is no inherent reason

why they cannot also play a role in the breeding and selection of forest trees and other woody perennials (34,40,50).

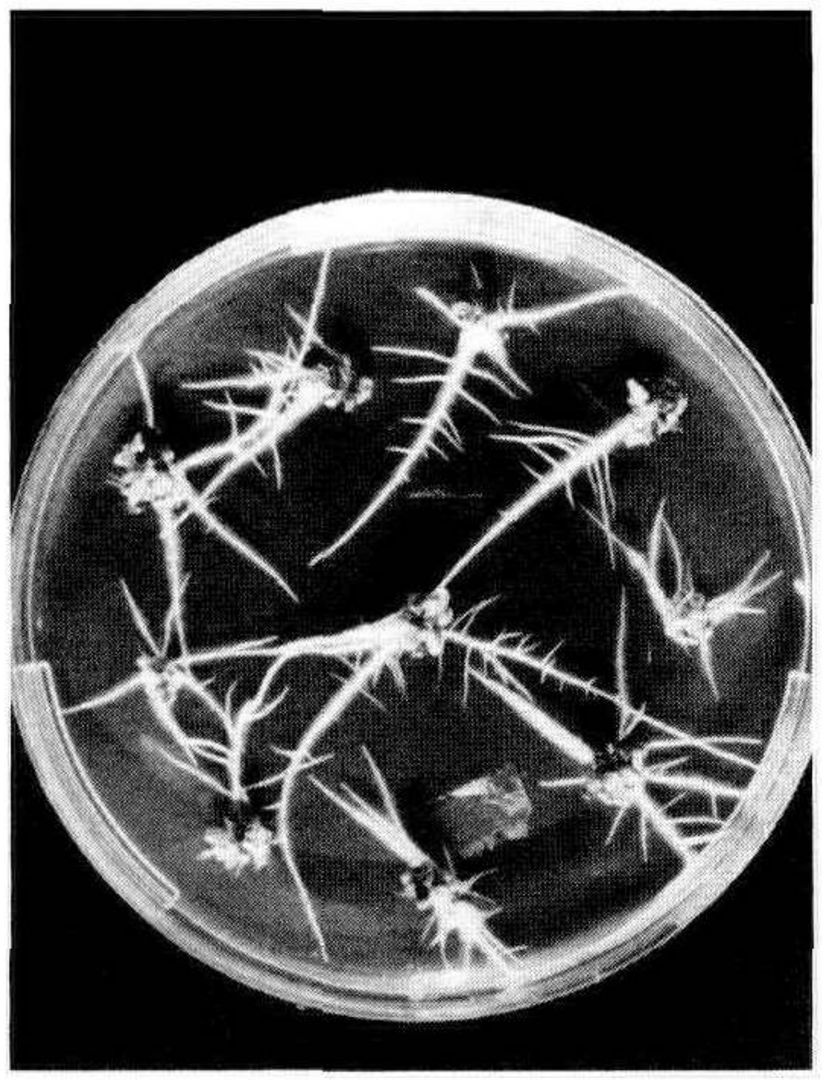

Figure 5. Plantlets of a salt tolerant eucalypt growing in Petri dishes.

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PRELIMINARY REPORT ON A TECHNIQUE WHICH PROVIDES A "MATURITY FACTOR" FOR TREES GROWN IN TISSUE CULTURE

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I would like to preface this paper with a quote from Dr. Ron de Fossard. He advises that:

"We should not lose sight of the advantage of tissue culture plants and propagation. It is not just to clonally propagate a cultivar. It is to produce a *far superior* product, free of virus, fungi, and bacteria, from a highly desirable horticultural specimen and, where yield is important, from the upper 0.1% or better of the normal curve of distribution of the species."

Good and timely advice, indeed. Anyone can produce a plant in tissue culture. Often, a little careful juggling with media can produce better yield results than those published in the literature — but, to what end? Many of the plants grown in culture originate from seed or spores. Frequently, too, tissue-grown plants are just that, and *no positive selection* has actively taken place. Consequently, these plants are of little or no value in improving the standards of that cultivar. I feel it is an essential feature of any commercial tissue culture lab to actively improve the quality of those plants chosen for culture.

Dr. de Fossard goes on to say:

"It (the tissue culture plant) should be able to outsell plants produced by other methods of propagation because it should yield a *more valuable plant* and thus sell for a higher price. It should permit all-year round propagation. It should permit the propagation of species that cannot be vegetatively propagated by any other means. It should lead to the exploitation of protoplast and haploid work. It should enable clean plants to be kept clean more easily than at present. It should enable the expedition of plants from one country to another. It should give us high multiplication rates."